CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

761148Orig1s000

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 MEMORANDUM

Application Number*:	761148
Supporting Document Number/s:	65
CDER Receipt Date:	3/11/2022
Sponsor:	Spectrum Pharmaceuticals, Inc 157 Technology Drive Irvine, CA 92618
Product:	ROLVEDON (eflapegrastim-xnst)
Pharmacologic Class:	Leukocyte growth factor
Indication:	Decrease the incidence of infection, manifested as febrile neutropenia, in patients with non-myeloid malignancies receiving anti-cancer drugs associated with clinically significant incidence of febrile neutropenia
Therapeutic area:	Hematology
Clinical Review Division:	Division of Non-Malignant Hematology (DNH)
Pharm/Tox Division	Division of Pharm/Tox for Cardiology, Hematology, Endocrinology, and Nephrology (DPT-CHEN)
Reviewer:	Anthony Parola, PhD
Supervisor/Team Leader:	Pedro DelValle, PhD, FATS / Natalie Simpson, PhD
Project Manager:	May Zuwannin, BS
Purpose of Review:	Other
	Resubmitted BLA with no previously identified nonclinical complete response issues, no new nonclinical information, no changes to nonclinical information in the proposed label, and no nonclinical PMRs
Reviewer Completion Date:	July 25, 2022

Template Version: Sep 11, 2020

Summary

In October 2019, Spectrum Pharmaceuticals submitted BLA 761148 via the 351(a) regulatory pathway for eflapegrastim-xnst injection (ROLONTIS), a novel granulocyte-colony stimulating factor (G-CSF) consisting of a human G-CSF variant (serine substitutions at positions 17 and 65 without an additional N-terminal methionine, 18.6 kDa) coupled to the Fc fragment of human IgG4 (49.8 kDa) by a polyethylene glycol linker (3.4 kDa). Eflapegrastim (~72 kDa) is a leukocyte growth factor intended to decrease the incidence of infection, manifested by febrile neutropenia, in patients with non-myeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a clinically significant incidence of febrile neutropenia. Eflapegrastim injection is a drug/device combination consisting of a prefilled syringe delivering 0.6 mL of a clear, colorless solution of 13.2 mg (22 mg/mL) eflapegrastim, 4.2 mg/mL citric acid monohydrate, 50 mg/mL mannitol, 1.2 mg polysorbate 80, and 8.8 mg sodium chloride at pH 5.5 in water for injection. A 25 June 2020 nonclinical review of BLA 761148 by Dr. Huiqing (Karen) Hao recommended the nonclinical data support approval of eflapegrastim and provided nonclinical labeling recommendations. During the first review cycle, the applicant submitted several revisions of the proposed US prescribing information (USPI) in response to recommendations from the FDA. There were no proposed modifications to nonclinical information in an 8 October 2020 general advice letter from the FDA to the applicant regarding revised labeling for eflapegrastim injection, and during the first review cycle, the last version of the USPI was submitted on 13 October 2020 (supporting document 60). No nonclinical postmarketing requirements (PMRs) were recommended.

A 3 August 2021 complete response letter notified the applicant BLA 761148 could not be approved because it required resolving manufacturing facility deficiencies and the Agency reserved comment on the proposed labeling until the application was considered adequate, but there were no nonclinical complete response issues.

A class 2 resubmission for ROLVEDON (eflapegrastim-xnst injection) BLA 761148 was received 11 March 2022 (supporting document 65). No new nonclinical information was included in the resubmission. Based on a tracked changes version of the proposed USPI in the resubmission, there were no changes to nonclinical information in the following sections of the USPI (except for the brand name was changed from ROLONTIS to ROLVEDON because ROLONTIS may not have been acceptable):

8.1 Pregnancy
12.1 Mechanism of Action
13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Nonclinical information in the proposed label is consistent with nonclinical recommendations in Dr. Hao's 25 June 2020 nonclinical review. No new nonclinical issues were identified in resubmitted ROLVEDON BLA 761148.

1.2 Relevant INDs, NDAs, BLAs or DMFs

INDs

103461 for SPI-2012 (eflapegrastim) from Spectrum Pharmaceuticals for the treatment of neutropenia, active since October 2009

DMFs

(b) (4)

1.3 Previous Reviews Referenced

Application	Reviewer / Supervisor	Date in DARRTS	Notes
BLA 761148	Huiqing Hao / Calvin L Elmore	12/19/2019	Filing review
	Huiqing Hao / Federica Basso	6/25/2020	Review, no nonclinical complete response issues or PMRs

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/s/

ANTHONY L PAROLA
07/25/2022 01:28:19 PM

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07/25/2022 02:59:49 PM

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:	761148
Supporting document/s:	SDN1
Applicant's letter date:	10/23/2019
CDER stamp date:	10/24/2019
Product:	Rolontis® (Eflapegrastim)
Indication:	Febrile neutropenia in patients with nonmyeloid malignancies receiving myelosuppressive anti-cancer drugs
Applicant:	Spectrum Pharmaceuticals
Review Division:	Division of Hematology Products
Reviewer:	Huiqing Hao, PhD
Supervisor/Team Leader:	Federica Basso, PhD
Division Director:	Ann Farrell, MD
Project Manager:	Elizabeth Godwin

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of BLA 761148 are owned by Spectrum Pharmaceuticals or are data for which Spectrum Pharmaceuticals has obtained a written right of reference. Any information or data necessary for approval of BLA761148 that Spectrum Pharmaceuticals does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of BLA761148.

TABLE OF CONTENTS

1 EXECUTIVE SUMMARY 7

1.1 INTRODUCTION 7
1.2 BRIEF DISCUSSION OF NONCLINICAL FINDINGS 7
1.3 RECOMMENDATIONS 8

2 DRUG INFORMATION 9

2.1 DRUG 9
2.2 RELEVANT INDS, NDAS, BLAS AND DMFS 10
2.3 DRUG FORMULATION 11
2.4 COMMENTS ON NOVEL EXCIPIENTS 11
2.5 COMMENTS ON IMPURITIES/DEGRADANTS OF CONCERN 11
2.6 PROPOSED CLINICAL POPULATION AND DOSING REGIMEN 12
2.7 REGULATORY BACKGROUND 12

3 STUDIES SUBMITTED 12

3.1 STUDIES REVIEWED 12
3.2 STUDIES NOT REVIEWED 14
3.3 PREVIOUS REVIEWS REFERENCED 14

4 PHARMACOLOGY 15

4.1 PRIMARY PHARMACOLOGY 15
4.2 SECONDARY PHARMACOLOGY 18
4.3 SAFETY PHARMACOLOGY 19

5 PHARMACOKINETICS/ADME/TOXICOKINETICS 19

5.1 PK/ADME 19

6 GENERAL TOXICOLOGY 23

6.1 SINGLE-DOSE TOXICITY 23
6.2 REPEAT-DOSE TOXICITY 24

7 GENETIC TOXICOLOGY 45

7.1 *IN VITRO* REVERSE MUTATION ASSAY IN BACTERIAL CELLS (AMES) 45
7.2 *IN VITRO* ASSAYS IN MAMMALIAN CELLS 46
7.3 *IN VIVO* CLASTOGENICITY ASSAY IN RODENT (MICRONUCLEUS ASSAY) 47

8 CARCINOGENICITY 48

9 REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY 49

9.1 FERTILITY AND EARLY EMBRYONIC DEVELOPMENT 49
9.2 EMBRYONIC FETAL DEVELOPMENT 51
9.3 PRENATAL AND POSTNATAL DEVELOPMENT 63

10 SPECIAL TOXICOLOGY STUDIES 65

TOXICOLOGY STUDIES ON HUMAN IGG4 FC FRAGMENT (HMC001) 65

TOXICOLOGY STUDIES ON IMPURITIES.....74
11 INTEGRATED SUMMARY AND SAFETY EVALUATION.....75

Table of Tables

Table 1. Quantitative Composition of Eflapegrastim Drug Product, 13.2 mg/0.6 mL 11
Table 2. HM10460A receptor binding affinity and activities of stimulating mouse bone marrow cell proliferation and differentiation 15
Table 3. PK parameters of eflapegrastim after IV and SC administration in rats 20
Table 4. Pharmacokinetic data following a single SC dose in monkeys 21
Table 5. Incidence of hind leg/paw swelling on Days 10-12, Rat 4-week study 26
Table 6. WBC and Neutrophil counts at end of treatment, 4-week rat study 27
Table 7. Histopathology finding incidences (severity), 4-week rat study 28
Table 8. Toxicokinetic parameters on Day 1, 4-week rat study 29
Table 9. Incidence of antibodies (Tab) and neutralizing antibodies (Nab), 4-week rat study 29
Table 10. Hematology findings, 4-week monkey study 33
Table 11. Clinical chemistry at end of treatment, 4-week monkey study 33
Table 12. organ weight findings, 4-week monkey study 34
Table 13. Histopathology incidence, 4-week monkey study 35
Table 14. Toxicokinetics parameters, 4-week monkey study 36
Table 15. Incidence of antibodies (Tab) and neutralizing antibodies (Nab), 4-week monkey study 36
Table 16. Neutrophil counts -cohort 1, 26-week monkey study 40
Table 17. Incidence of histopathological findings -cohort 1, 26-week monkey study 42
Table 18. Myeloid:Erythroid ratio, 26-week monkey study 43
Table 19. Toxicokinetics parameters, 26-week monkey study 43
Table 20. Incidence of TAb and NAb findings, 26-week monkey study 44
Table 21. WBC and neutrophil counts, rat FED study 50
Table 22. Fertility Parameters, rat FED study 51
Table 23. Toxicokinetic parameter, rat EFD study 53
Table 24. Dosing solution analysis results, rat EFD study 54
Table 25. Actual dose levels, rat EFD study 54
Table 26. Incidences of antibody and neutralizing antibody, rat EFD study 55
Table 27. Hematology findings on GD29, rabbit EFD study 59
Table 28. Toxicokinetic parameter, rabbit EFD study 59
Table 29. Dosing solution analysis results, rabbit EFD study 60
Table 30. Actual dose levels, rabbit EFD study 61
Table 31. ADA incidence and mean titer, rabbit EFD study 61
Table 32. Uterine and net body weight (g), rabbit EFD study 62
Table 33. Corpora lutea and implantation data, Rabbit EFD study 62
Table 34. Post-implantation data, Rabbit EFD study 63
Table 35. Mean fetal weight, rabbit EFD study 63
Table 36. TK parameters for HMC001 in rat serum 69
Table 37. Incidence of anti-HMC001 antibodies in rat serum 70
Table 38. Salivary gland weight (g), 4-w monkey study with HMC001 72
Table 39. incidence of histopathology findings at end of treatment, 4-week monkey study with HMC001 73
Table 40. Incidence of anti-HMC001 positive animals 74

Table 41. Study design, 2-w rat study with truncated HM10460A.....75
Table 42. Treatment related findings in rats, 2-w rat study with truncated HM10460A..75
Table 43. Safety margins in rodents and monkeys 78

Table of Figures

Figure 1. Duration of Neutropenia (DN) following sequential administration of cyclophosphamide and HM10460A in neutropenic rats 17
Figure 2. DN following co-administration of cyclophosphamide and HMA10460A in neutropenic rats 18
Figure 3. ANC levels in monkeys.....19
Figure 4. Expected products generated from HM10460A chain exchange in vivo and ex vivo 23
Figure 5. Mean maternal body weights during gestation, rabbit EFD study 59

1 Executive Summary

1.1 Introduction

Eflapegrastim (Rolontis®, SPI-2012, HM10460A) is a long-acting granulocyte-colony stimulating factor (G-CSF) indicated to reduce the incidence of infection, as manifested by febrile neutropenia, in patients with non-myeloid malignancies receiving myelosuppressive anticancer drugs. Eflapegrastim is produced by covalent coupling of a human G-CSF analog (HM10411, ef-G-CSF) and human immunoglobulin G4 (IgG4) Fc fragment (HMC001), via a polyethylene glycol (PEG) linker. The inclusion of human IgG4 Fc fragment increases the circulating half-life of G-CSF without inducing Fc-mediated effector functions.

1.2 Brief Discussion of Nonclinical Findings

Pharmacology, pharmacokinetics, general toxicology, reproductive toxicology, and genotoxicity studies were conducted with eflapegrastim administered by subcutaneous injection, the clinical route of administration. Safety margins relative to the maximum recommended human dose (MRHD) of 13.2 mg were calculated based Day 1 AUCs.

General toxicology studies were conducted in rats and monkeys with treatment durations of a single dose and 4 weeks in both species, and 26 weeks in monkeys. Treatment-related findings in both rats and monkeys were consistent with the known pharmacological effects of G-CSF, mainly increases in blood neutrophil counts, granulopoiesis in the bone marrow, and increased extramedullary granulopoiesis in the spleen, liver, kidney, and lymph nodes. In the 4-week studies, high doses were associated with mortality and severe toxicities including joint inflammation, reduced bone area, marrow necrosis in rats (≥11-fold MRHD) and hemorrhage in the lung and brain in monkeys (212-fold MRHD) in the early sacrificed animals. A 26-week study in monkeys did not reveal additional toxicities other than neutrophil count reduction (-78% by Week 26, -85% by end of the recovery) after a transient increase during the first 4 weeks at all doses tested. The neutrophil counts reduction was likely attributable to the presence of neutralizing anti-drug antibodies. Safety margins to the NOAEL were 0.2-, 39- and 82-fold the MRHD in the 4-week rat study, 4-week monkey study and 26-week monkey study, respectively.

Eflapegrastim did not affect the CNS and respiratory system in rats, or the cardiovascular system in monkeys.

Eflapegrastim did not bind to C1q, FcγRI, FcγRIIB, and FcγRIIIA suggesting low potential to induce Fc-mediated effector functions such as complement dependent cytotoxicity (CDC) and antibody dependent cell mediate cytotoxicity (ADCC).

Eflapegrastim was not mutagenic or clastogenic in a standard battery of genotoxicity studies.

Eflapegrastim did not affect fertility, embryofetal development, and pre- and post-natal development in rats at doses up to 7-fold the MRHD. Pregnant rabbits given eflapegrastim during organogenesis (GD7-GD19) exhibited increased post-implantation loss (mostly early resorption) and related reduction in litter size, increased incidence of dams with no viable fetuses, as well as decreased fetal weights at ≥6-fold MRHD). NOAEL for fetal development was 2-fold MRHD.

1.3 Recommendations

1.3.1 Approvability

The nonclinical data support market approval of eflapegrastim.

1.3.2 Additional Non-Clinical Recommendations

None

1.3.3 Labeling

The proposed text for the nonclinical sections of the label is provided below:

(b) (4)

2 Drug Information

2.1 Drug

CAS Registry Number (Optional)
1384099-30-2

Generic Name
Eflapegrastim

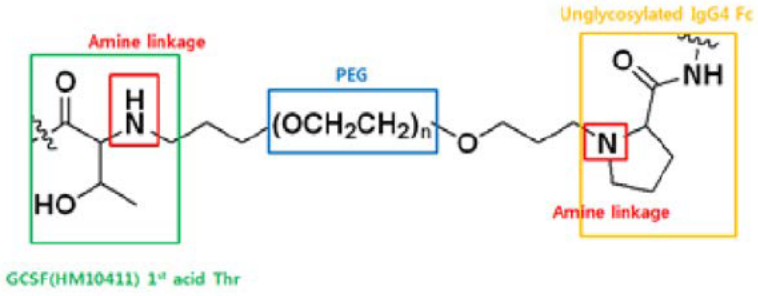
Code Name
HM10460A, HNK460, LAPS-rhG-CSF

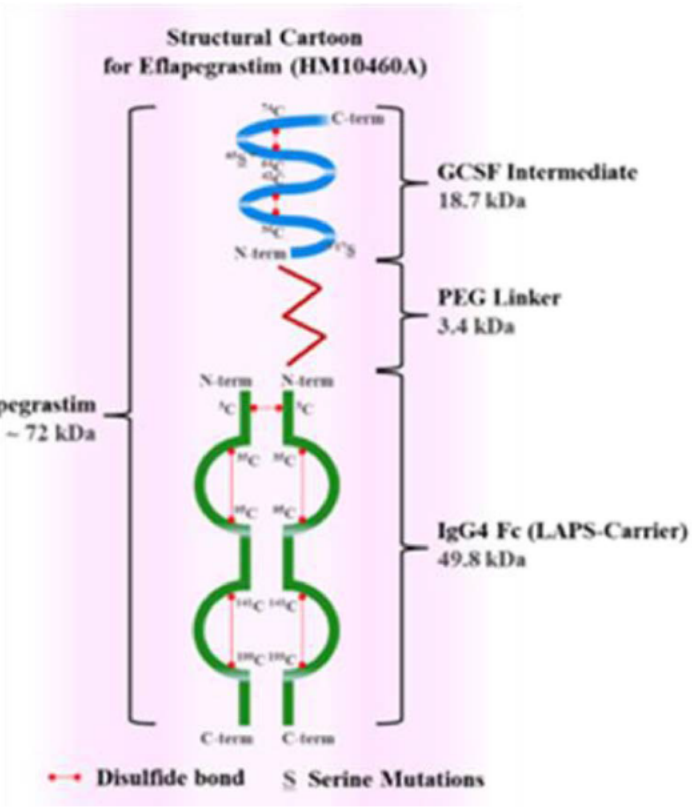
Chemical Name
Poly(oxy-1,2-ethanediyl), a-hydro-w-hydroxy-1-ether with immunoglobulin G4 [1-[1-(3-hydroxypropyl)proline]] (human Fc fragment), (3,3')-disulfide with immunoglobulin G4 (human Fc fragment), 1'-ether with granulocyte colony-stimulating factor [N-(3-hydroxypropyl),17,65-serine] (human).

Molecular Formula/Molecular Weight
$C_{3070}H_{4764}N_{806}O_{927}S_{23}\cdot[C_2H_4O]_{n,}$ ~72 kDa

Structure or Biochemical Description
Eflapegrastim (HM10460A) consists of a recombinant human G-CSF analog (ef-G-CSF or HM10411) and a recombinant fragment of the Fc region of human IgG4 (HMC001),

linked via a bimodal polyethylene glycol linker. Molecular weight of each component is presented below.





Pharmacologic Class
Leukocyte growth factor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND103461: eflapegrastim

BLA103353: filgrastim (recombinant methionyl human G-CSF, Amgen)
BLA125031: pegfilgrastim (pegylated filgrastim, Amgen).

DMFs: (b) (4)

2.3 Drug Formulation

Table 1. Quantitative Composition of Eflapegrastim Drug Product, 13.2 mg/0.6 mL

Ingredient	Reference to Standards	Quantity Per Syringe (mg/0.6 mL)	Primary Function
Eflapegrastim	In-house	13.2 mg (b) (4)	Active ingredient
Citric acid monohydrate	USP	2.52 mg	(b) (4)
Sodium chloride	USP	5.26 mg	
Polysorbate 80	EP	0.72 mg	
Mannitol	USP	30 mg	
Sodium hydroxide	NF	*q.s.*	pH adjustment
Water for injection	USP	*q.s.*	(b) (4)

q.s.. = quantity sufficient

1. (b) (4)

Table excerpted from applicant submission

2.4 Comments on Novel Excipients

There is no novel excipient. The proposed excipient levels are within approved range for subcutaneous administration as listed in the FDA inactive ingredient database.

2.5 Comments on Impurities/Degradants of Concern

1. Impurities: product-related impurities were identified as products of aggregation (identified by SE-HPLC), oxidation (identified by RP-HPLC), deamidation (identified by IE-HPLC) and fragmentation (identified by RP-HPLC). These impurities have low effect on bioactivity at low levels, except oxidation products that are reported having moderate effect. As per the CMC reviewer, the proposed impurity specifications are acceptable. Also, these impurities were present in nonclinical batches at similar or higher levels than the proposed product specification levels ((b) (4) analysis; LMW impurity NMT (b) (4)% and HMW impurities NMT (b) (4)%) and adequate safety margins in the toxicology studies qualified these impurities.

2. Leachables from the container closure system: The applicant conducted extractable and leachable screening assays. Per the CMC review team, the methods used in these studies are acceptable. No leachables were detected with reporting threshold of (b) (4) ug/mL in Liquid Chromatography/Diode Array Detector/Quadrupole Time of Flight (LC/DAD/QTOF) or (b) (4)ug/mL in GC/MS analysis. According to the applicant, these thresholds represent human exposure of (b) (4)and (b) (4) ug/dose, respectively. The applicant identified the following major extractables and provided toxicity information as per below. This reviewer considers that these compounds at exposure levels up to 3 ug/dose are of no safety concerns.

Potential Leachable (b) (4)	Toxicity assignment (from E2L Risk Assessment)	Reasons for assignment
	Medium	Not mutagenic, not carcinogenic, not a reproductive hazard, possibly more toxic in injection routes
	Low	Not considered to be a significant toxin in humans
	Low	Oral and injection dosing showed a low toxicity profile in humans
	Medium	Low acute oral toxicity in rats, related compounds showed low to moderate repeated dose toxicity
	Low	DEREK, *in silico*, assessment showed no structural alerts, resulting in indication of potential low toxicity
	High	Considered high due to lack of sufficient data, structure not completely known
	High	Considered high due to lack of sufficient data, structure not completely known

2.6 Proposed Clinical Population and Dosing Regimen

Eflapegrastim 13.2 mg/0.6 mL solution for injection is intended to be administered once per chemotherapy cycle in patients with cancer receiving myelosuppressive chemotherapy.

2.7 Regulatory Background

This product was previously submitted under BLA (b) (4) on 12/21/2018 but withdrawn on 3/14/2019 due to CMC related deficiencies.

No carcinogenicity studies of eflapegrastim have been conducted, as this product is indicated for treatment of patients with advanced cancer and FDA agreed that carcinogenicity studies were not warranted (Meeting Minutes for End-of-Phase 2 Meeting on 12 Dec 2014).

3 Studies Submitted

3.1 Studies Reviewed

	Stud Title	Study No.
Pharmacology	Efficacy Study of HM10460A by Different Dosing Regimen in Docetaxel / Cyclophosphamide induced Neutropenia Rats	Dv-8028
	Determination of Effector Function Activities of HM10460A *in vitro*	ME060102
	Identification of FcRn-mediated Transcytosis	ME100716

	in HM10460A	
Safety Pharmacology	Central Nervous System Safety Pharmacology Evaluation of HM10460A (a long acting G-CSF) Administered by Subcutaneous Injection to Male Rats	7805-168
	Respiratory Safety Pharmacology Evaluation Using Head-Out Plethysmography following Subcutaneous Injection of HM10460A (a long-acting G-CSF) to Male Rats	7805-149
PK	Investigation of PEG Bond Stability in HM10460A	ME080926
	Identification of chain exchange mechanism in HM10460A	ME090105
	Investigation of the interaction between HM10460A and human IgG4	ME090110
	Identification of the metabolites generated by HM10460A chain exchange mechanism	ME090128
Toxicology	4-week subcutaneous injection toxicity and toxicokinetic study with HM10460A, a long acting G-CSF, in cynomolgus monkeys with a 4-week recovery period	7805-159
	4-week subcutaneous injection toxicity and toxicokinetic study with HM10460A, a long acting G-CSF, in rats with a 4-week recovery period.	7805-158
	26-Week Subcutaneous Injection Chronic Toxicity and Toxicokinetic Study with HM10460A, a Long Acting G-CSF, in Cynomolgus Monkeys with 4-Week Recovery Phase	8203-497
Genotoxicity	*Salmonella-Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay	7805-162
	Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells	7805-163
	In Vivo Rat Bone Marrow Micronucleus Assay	7805-164
Reproductive toxicology	Subcutaneous Injection Study of Fertility and Early Embryonic Development to Implantation with HM10460A in Rats	8302-914
	Subcutaneous Injection Study for Effects on Embryo-fetal Development and Toxicokinetic with HM10460A in Rats	8222-311
	Subcutaneous Injection Study for Effects on Embryo-fetal Development and Toxicokinetic with HM10460A in Rabbits	8222-307
	Pre/Post-natal Development and Maternal Function Study by Subcutaneous Administration in Sprague-Dawley Rats	G117129
Other studies	A Single-Dose Subcutaneous Injection Toxicity and Toxicokinetic Study with HMC001 in Rats	7805-128

3.2 Studies Not Reviewed

- Analytical Methods and validation reports.
- 2-Week Dose Range Finding Study with HM10460A, a long acting G-CSF in Cynomolgus Monkeys (Study No. 7805-133)
- 2-Week Dose Range Finding Study with HM10460A, a Long Acting G-CSF, in Rats (Study No. 7805-132)

3.3 Previous Reviews Referenced

30-Day Safety Review by Mary Jane Masson Hinrichs, PhD

Primary pharmacology	**Study No.**
Determination of binding affinity between G-CSF receptor and HM10460A	EF080910
In Vitro Proliferation assay of HM10460A in Mouse Bone Marrow Cells	EF080930
In Vitro Colony Forming Assay of HM10460A	EF070910
In vivo Efficacy Study of HM10460A, Long Acting G-CSF, in Neutropenic Mouse Model	Dvo 07-031
Pharmacodynamic Study of HM10460A in Neutropenic Rats	Dvo08-004/052
Pharmacokinetics	
Pharmacokinetic/Pharmacodynamic Evaluation of HM10460A in rats	kvo-07-097
Tissue distribution in rats	Kvo-07-134
PK study in nephrectomized rats	Kvo-08-033
Pharmacokinetic and Pharmacodynamic Study for Single Dose of HM10460A in Cynomolgus Monkey	EF060904
Toxicology	
2-week dose range finding study with HM10460A in cynomolgus monkeys	7805-133
Single dose toxicity and toxicokinetic study with HM10460A in rats.	7805-134
Single-Dose Subcutaneous Injection Toxicity and Toxicokinetic Study with HM10460A in Rats	8211-319
Single-Dose Subcutaneous Injection Toxicity and Toxicokinetic Study with HM10460A, a long acting G-CSF, in Cynomolgus Monkeys	7805-135
A Single-Dose Subcutaneous Injection Toxicity and Toxicokinetic Study with HMC001 in Cynomolgus Monkeys	7805-129

4 Pharmacology

4.1 Primary Pharmacology

Eflapegrastim binds to G-CSF receptors on myeloid progenitor cells and neutrophils, triggering signaling pathways that control cell survival, proliferation, differentiation, and migration of neutrophil precursors and mature neutrophils.

In vitro data (Studies EF080910, EF080930 and EF070910)
Eflapegrastim binds to human recombinant G-CSF receptor and stimulated cellular proliferation and differentiation in mouse bone marrow cells, with similar affinity and potency to pegfilgrastim and filgrastim.

Table 2. HM10460A receptor binding affinity and activities of stimulating mouse bone marrow cell proliferation and differentiation

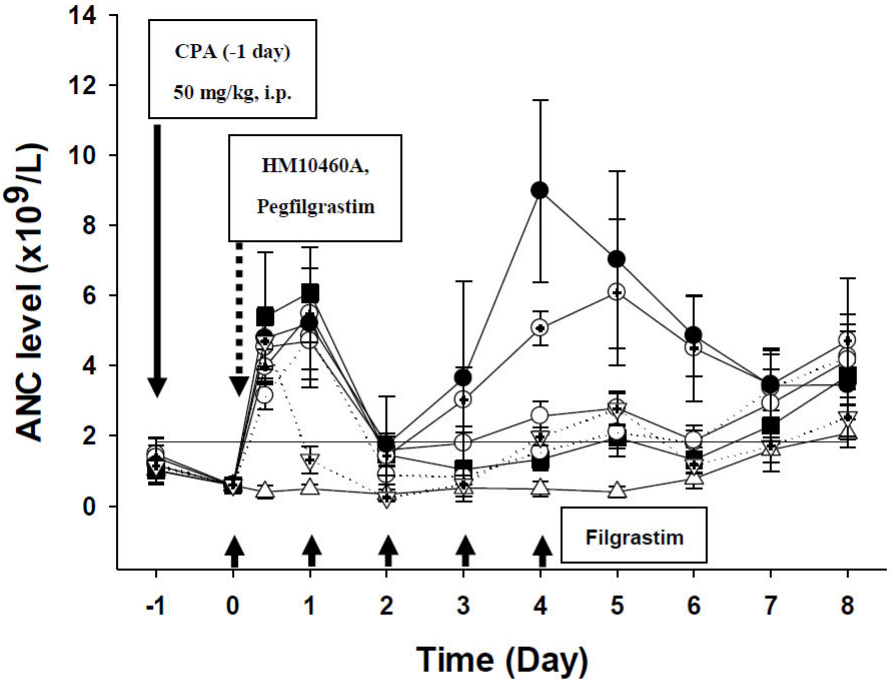
	Eflapegrastim	Pegfilgrastim	Filgrastim
G-CSF receptor binding affinity, Kd	3.6 nM	2.9 nM	0.67 nM
In vitro stimulation of mouse bone marrow cell proliferation, EC_{50}	114.4 pg/mL	60.22 pg/mL	132 pg/mL
In vitro colony formation assay, EC_{50}	1.49 ng/mL	1.92 ng/mL	0.82 ng/mL

Table excerpted from applicant submission

In vivo data
Efficacy in chemotherapy-induced neutropenic rodents

- In mice, neutropenia was induced by cyclophosphamide, doxorubicin and vincristine treatment. Administration of a single subcutaneous dose of HM10460A at 323 mcg/kg and 968.9 mcg/kg resulted in dose-related increases in neutrophil counts, with an area under effect curve of absolute neutrophil count (ANC-AUEC) of 38% and 255%, respectively, as compared to 70% with pegfilgrastim 300 mcg/kg (Study # Dvo 07-031).
- In rats, neutropenia was induced by cyclophosphamide (CPA) administered 1 day before or at the same time as the test article was administered. Administration of a single subcutaneous dose of HM10460A (32, 97, 194 or 323 mcg/kg) showed a dose-related shortening of duration of neutropenia (DN) when HMA10406A was either co-administered or given the day prior to cyclophosphamide. When co-administered, HM10460A 32 mcg/kg (0.45 nmol/kg) reduced DN from 8 to 4.2 days, while pegfilgrastim 100 mcg/kg (3.1 nmol/kg) reduced DN from 8 to 2.8 days. When HM10460A was administered one day after CPA, HM10460A 32 mcg/kg group and pegfilgrastim 100 mcg/kg groups showed similar DN (2.9 days versus 2.8 days), suggesting higher potency of HM10460A. An additional study showed similar results. (Study # Dvo08-004/052 and Dv-8028).

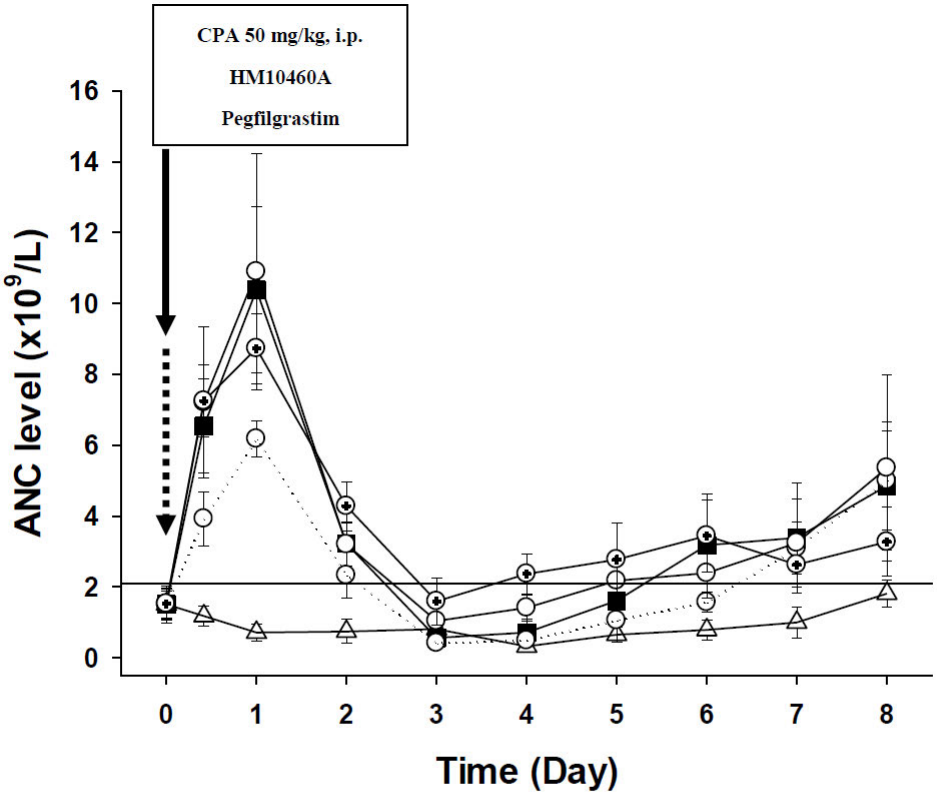
Figure 1. Duration of Neutropenia (DN) following sequential administration of cyclophosphamide and HM10460A in neutropenic rats



Effect of HM10460A (round), pegfilgrastim (square) and filgrastim (inverted triangle) on the DN study in chemotherapy induced neutropenia rat model. HM10460A and pegfilgrastim were single administrated and filgrastim was five times dosed by subcutaneous 1 day after cyclophosphamide (CPA) treated normal rats. Normal ANC level was specified as 2 x 10^9/L (—). —△— vehicle (CPA treatment), --▽-- filgrastim 20.00 μg/kg/day for 5 days, —■—pegfilgrastim 100.00 μg/kg, --⊙-- HM10460A 32.30 μg/kg (8.80 μg/kg as HM10411), —○— HM10460A 96.89 μg/kg (26.40 μg/kg as HM10411), —⊙— HM10460A 193.78 μg/kg (52.80 μg/kg as HM10411), —●— HM10460A 322.96 μg/kg (88.00 μg/kg as HM10411).

Figure excerpted from applicant submission

Figure 2. DN following co-administration of cyclophosphamide and HMA10460A in neutropenic rats

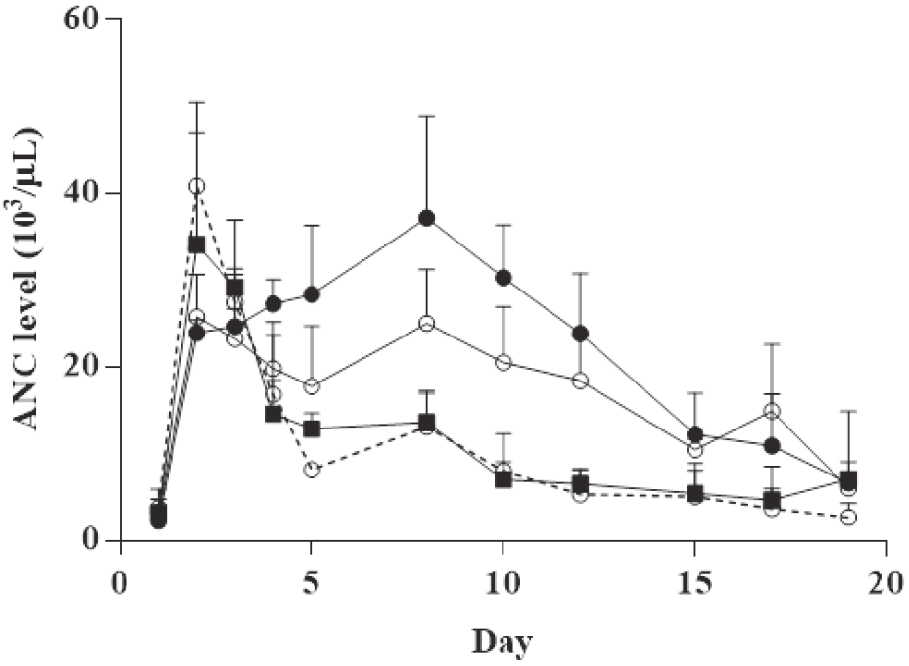


Effect of HM10460A (round) and pegfilgrastim (square) on the DN study in chemotherapy induced neutropenia rat model. HM10460A, pegfilgrastim and cyclophosphamide (CPA) were single administrated on the same time. Normal ANC level was specified as 2 x 10^9/L (—). —△— vehicle (CPA treatment), —■—pegfilgrastim 100.00 μg/kg, --⊙-- HM10460A 32.30 μg/kg (8.80 μg/kg as HM10411), —○— HM10460A 96.89 μg/kg (26.40 μg/kg as HM10411), —⊙— HM10460A 193.78 μg/kg (52.80 μg/kg as HM10411).

Figure excerpted from applicant submission

Pharmacodynamic (PD) study in healthy monkeys (Study EF060904)
Following a single subcutaneous dose of HM10460A at 0.161 (2.2umol/kg) and 0.323 mg/kg, ANC levels peaked at day 3.3 post-dosing and then slowly decreased until day 16. HM10460A plasma levels peaked at 10-hour post-dose and were still detectable at Day 7. In comparison, increase in ANC was slightly lower with pegfilgrastim at 0.1 mg/kg (2.5 umol/kg) S.C. See figure below.

Figure 3. ANC levels in monkeys



Abbreviations: ANC = absolute neutrophil count; ef-G-CSF = recombinant human granulocyte-colony stimulating factor analog (HM10411); SD = standard deviation.

Effect of subcutaneous eflapegrastim at doses of 0.161 mg/kg (—o—) and 0.323 mg/kg (—●—) and intravenous eflapegrastim at a dose of 0.161 mg/kg (···o···) (0.044, 0.088, and 0.044 mg/kg ef-G-CSF, respectively), and subcutaneous pegfilgrastim at 0.100 mg/kg (—■—) on ANC in male cynomolgus monkey (n =3/dose group). Note: Day 1 is equivalent to pre-dosing.

Figure excerpted from applicant submission

4.2 Secondary Pharmacology

- Effector function activity of HM10460A in vitro (Study ME060102)
  At a protein level, both HMC001 (de-glycosylated Fc fragment of IgG4) and HM10460A showed very low binding affinities to C1q, FcγRI, FcγRIIB, and FcγRIIIA, but a strong binding affinity to FcRnαβ2, suggesting that HM10460A is unlikely to induce CDC and ADCC, and has a long T1/2 via neonatal Fc receptor mediated mechanism. At a cellular level, HM10460A did not bind to Fcγ receptors expressed on U937 cells. HMC001 had no lytic activity on target cells through CDC or ADCC mechanisms.
- FcRn-mediated transcytosis (Study ME100716)
  The neonatal Fc receptor (FcRn) plays an important role in extending the half-life of IgG by protecting from catabolism, and in active transport across the vascular endothelium. The transcytosis of eflapegrastim via FcRn was investigated in FcRn overexpressed Madin-Darby canine kidney (MDCK) epithelial cell line. The amount of HM10460A transported through FcRn-overexpressed MCDK cells was 3-fold higher than that transported through MCDK cells.

4.3 Safety Pharmacology

CNS
A modified Irwin screen was conducted in male rats following a single subcutaneous dose of HM10460A (0.323, 1.616 and 8.07 mg/kg) at 1, 4 and 24 hours post-dosing. Increased hind limb grip strength (+22%) was noted at 8.07 mg/kg (approximately 46-fold the clinical dose of 13.2 mg for a 70 kg human). The NOAEL was 1.62 mg/kg (9-fold the clinical dose) (Study 7805-168).

Respiratory system
Effects of HM10460A on respiratory function were assessed in male rats following a single subcutaneous dose (0.323, 1.616 or 8.07 mg/kg) at 1, 4 and 6 hours post-dose. Higher tidal volume (+19.8%) from 1 through 6 hours post-dose was observed in the high dose animals. This effect was no longer present at 3 days post-dose (Study 7805-149).

Cardiovascular system
HM10460A effects on the cardiovascular system were assessed in a 4-week monkey study (Study 7805-159) where ECG was recorded at 1, 4 and 8 hours post-dosing on Days 1, 3, 7 and 24 of dosing phase. There were no remarkable findings in heart rate and wave intervals (PR, QRS, QTc) in monkeys given HM10460A at doses up to 3.23 mg/kg/week. Similarly, no treatment-related effects were observed in a 26-week monkey study at HM10460A doses up to 1.6 mg/kg/week.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption
Pharmacokinetics following a single subcutaneous dose in rats and monkeys (Studies kvo-07-097 and EF060904)
HM010460A was given to rats at 323, 969, or 3230 ug/kg via both intravenous and subcutaneous injection, and to monkeys at 161 and 323 ug/kg subcutaneously and 161 ug/kg intravenously. The dose escalation was associated with decreased clearance, and more than dose-proportional increases in AUC, potentially as a result of saturation of receptor-mediated clearance. Elimination half-life ($T_{1/2}$) was increased with increasing dose in rats, but not in monkeys which mignt be due small sample size related data variability.. The increased exposure was also associated with increased absolute neutrophil count (data not shown here). Subcutaneous bioavailability was in a range of 37% to 64% in rats and 23.3% in monkeys. See Tables 3 and 4.

Table 3. PK parameters of eflapegrastim after IV and SC administration in rats

Dosage (mg/kg)[a]		C_0 or C_{max} (ng/mL)[a]		AUC_{last} (ng·hr/mL)[a]		MRT_{last} (hr)	CL or CL/F (mL/hr/kg)	V_d or V_d/F (mL/kg)	$t_{½}$ (hr)	F (%)
Eflapegrastim	ef-G-CSF	Eflapegrastim	ef-G-CSF	Eflapegrastim	ef-G-CSF					
Intravenous										
0.323	0.088	3662.6 ± 858.7	998.0 ± 234.0	47131.7 ± 9480.7	12842.3 ± 2583.2	17.0 ± 1.5	7.0 ± 1.5	76.4 ± 16.4	7.5 ± 0.1	—
0.969	0.264	10629.2 ± 1869.6	2896.2 ± 509.4	305117.2 ± 27141.9	83138.3 ± 7395.5	36.4 ± 2.5	3.2 ± 0.3	39.9 ± 11.1	8.6 ± 1.7	—
3.23	0.880	55232.8 ± 23721.5	15049.8 ± 6463.6	1747267.8 ± 464890.3	476094.4 ± 126673.1	61.3 ± 1.8	1.9 ± 0.5	30.3 ± 6.6	11.0 ± 1.4	—
Subcutaneous										
0.323	0.088	451.8 ± 198.3	123.1 ± 54.0	17284.1 ± 6544.3	4709.5 ± 1783.2	36.5 ± 0.7	20.5 ± 7.3	231.8 ± 105.1	7.7 ± 0.8	36.7
0.969	0.264	2130.1 ± 599.2	580.4 ± 163.3	166113.1 ± 58668.1	45262.4 ± 15985.8	64.6 ± 7.8	6.3 ± 2.0	70.1 ± 33.6	7.4 ± 1.3	54.4
3.23	0.880	10239.3± 2739.4	2790.0 ± 746.4	1122993.1 ± 277816.2	305992.9 ± 75699.2	85.3 ± 4.6	3.0 ± 0.8	47.2 ± 11.1	10.9±0.4	64.3

Abbreviations: AUC_{last} = area under the curve from time 0 to the time of last measured concentration; CL (CL/F) = (apparent) plasma clearance; C_0 = back-extrapolated drug concentration following rapid intravenous injection; C_{max} = maximum plasma concentration; F = subcutaneous bioavailability; MRT_{last} = mean residence time from the time of dosing (Dosing time) to the time of the last measurable concentration; ef-G-CSF = recombinant human granulocyte-colony stimulating factor analog (HM10411); $t_{½}$ = terminal half-life; V_d (V_d/F) = (apparent) volume of distribution during the terminal phase.

(a) Dosage and AUC_{last}, and C_{max} values are presented based on total protein in eflapegrastim as well as for ef-G-CSF.

Values are mean ± SD of 3 animals per dose.

Table excerpted from applicant submission

Table 4. **Pharmacokinetic data following a single SC dose in monkeys**

Dosage (mg/kg)[a]		C_{max} or C_0 (ng/mL)[a]		AUC_{0-t} (ng·hr/mL)[a]		T_{max} (hr)	MRT_{last} (hr)	CL or CL/F (mL/hr/kg)	V_d or V_d/F (mL/kg)	$t_{½}$ (hr)	F (%)
Eflapegrastim	ef-G-CSF	Eflapegrastim	ef-G-CSF	Eflapegrastim	ef-G-CSF						
Intravenous											
0.161	0.044	4748.0 ± 1575.2	1293.7 ±429.2	83453.7 ± 18784.0	22739.5 ± 5118.4	—	12.1±0.2	2.0±0.4	19.9 ± 6.3	6.8±0.7	—
Subcutaneous											
0.161	0.044	822.8 ± 151.7	224.2 ± 41.3	19429.4 ± 3998.7	5293.9 ± 1089.6	10.0 ± 0.0	21.7±2.8	8.5±2.0	452.7 ± 71.2	37.8±7.5	23.3
0.323	0.088	1784.9 ± 508.7	486.3 ± 138.6	84263.6 ± 7226.0	22960.1 ± 1969.1	14.7 ±8.1	34.6±2.4	3.8±0.3	138.8 ±29.9	25.5±7.3	—

Abbreviations: AUC_{0-t} = area under the curve from time 0 to the time of last measured concentration; CL (CL/F) = (apparent) plasma clearance; C_{max} = maximum plasma concentration; C_0 = back-extrapolated drug concentration following rapid intravenous injection; F = subcutaneous bioavailability; MRT_{last} = mean residence time from the time of dosing (Dosing time) to the time of the last measurable concentration; ef-G-CSF = recombinant human granulocyte-colony stimulating factor analog (HM10411); $t_{½}$ = terminal half-life; V_d (V_d/F) = (apparent) volume of distribution during the terminal phase.

(a) Dosage and AUC_{0-t}, and C_{max} values are presented based on the total protein in eflapegrastim as well as for ef-G-CSF.

Values are mean ± SD of 3 animals per dose.

Table excerpted from applicant submission

Distribution

Tissue distribution in rats (Kvo-07-134)

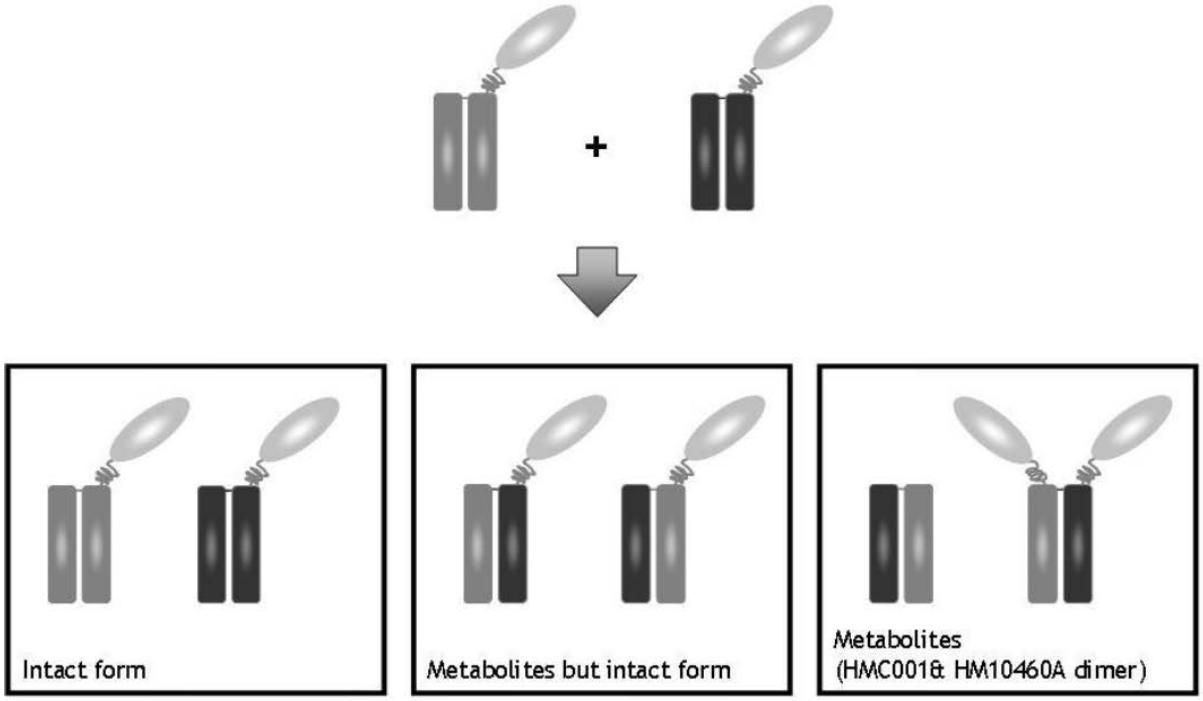
At 30 hours (Tmax) following a S.C dose of HM10460A at 323 ug/kg in rats, the highest concentration of HM10460A was observed in plasma. The tissue/plasma concentration (T/P) ratio was in order of bone marrow (8.5%) > kidney (5.6%) > heart (5.4%) > lung (5.1%) > liver (3.1%) > spleen (1.2%). At 96 hours after dosing, the concentration of HM10460A was 1.0 ± 0.5 ng/mL in the plasma, and was eliminated from most tissues.

Metabolism

Identification of chain exchange mechanism in HM10460A (ME090105, ME090128)

Following a single injection of 35.2 mg/kg HM10460A, rat blood samples were collected over a time course and analyzed for metabolites by a Western blot analysis. The results showed that about 50 kDa and 94 kDa products of new molecular entity were formed, and increased time dependently. These products are likely HM10460 dimer (94 kDa contains 72 kDa HM10460A and 22 kDa G-CSF), and HMC001 (49.8 kDa) (see Figure 4 below). Ex vivo incubation of HM10460A spiked human and rat blood, respectively resulted in similar products. Further comparison between the products from spiked rat blood incubation and in-house prepared reference HM10460A dimer and HMC001 confirmed they are the same molecules.

Figure 4. Expected products generated from HM10460A chain exchange in vivo and ex vivo



Expected products generated from HM10460A chain exchange *in vivo* and ex vivo. If the chain exchange occurs between HM10460A, three types of molecules such as intact HM10460A, HM10460A dimeric form and HMC001 can be generated.

PEG bond stability (study ME080926)

PEG bond stability was studied by examining breakdown products (3.4k-PEG-HM10411, HMC001, HM10411 and 3.4k-PEG-HMC001) in rats given a single subcutaneous dose of HM10460A 35.2 mg/kg. The results demonstrated that PEG bond of HM10460A was stable in the rat up to 45 hours post-dosing. In vitro, following incubation with human serum at 37°C for 8 days, HM10460A did not generate any breakdown products.

Investigation of the interaction between HM10460A and human IgG4 (ME090110)

To study whether chain exchange occurs between HM10460A and human IgG4, HM10460A and Biotinylated human IgG4 were co-spiked in rat whole blood or human blood and incubated at 30°C for 20 hours. The products were analyzed by Western blot using anti-human IgG Fc and anti-human GCSF antibody, as well as MALDI-TOF mass spectrometry. There were no HM10460A and human IgG4 exchange products detected.

PK study in nephrectomized rats (Kvo-08-033)

There was no difference in AUC or clearance between sham and nephrectomized rats, demonstrating that HM10460A is not renally cleared.

6 General Toxicology

6.1 Single-Dose Toxicity

Rat studies

- Study 7805-134: Single subcutaneous doses of eflapegrastim at 3.23, 9.69 or 32.3 mg/kg in rats (5/sex/dose) resulted in moribund sacrifice in two animals from mid dose group (5/sex) at Day 11 due to swollen and limited use of hind legs. Clinical pathology findings mainly consisted of increases in absolute neutrophil count (1.8- to 131-fold) and alkaline phosphatase (1.6- to 20-fold). The increased alkaline phosphatase is likely related to increases in the neutrophil count and the osteoblast activity. Other clinical pathology findings included mild to marked increased gamma glutamyltransferase (mid dose and high dose) and moderate increases in the ALT and the AST (high dose). Splenic enlargement was observed at all dose levels and correlated with histopathological findings of markedly increased hematopoiesis, decreased lymphoid follicle and acute capsule inflammation in the high dose group. No NOAEL was defined due to splenic enlargement in all treatment groups.

- Study 8211-319: To identify a NOAEL for the spleen enlargement, an additional single dose study was conducted in rats where doses of 0.01, 0.03, 0.1 and 0.3 mg/kg were used. The animals were sacrificed at 15 days post-dosing and microscopic examination of the spleen, bone marrow, kidney, liver, lymph nodes, and prostate from control and high dose groups were conducted. Treatment-related findings included increases in WBC, ANC and monocyte counts at all dose levels, slightly increased spleen weight at the high dose (1.3-fold) and increased extramedullary hematopoiesis in the spleen (dose related, minimal to moderate degrees) and the liver (males and females at high dose). The NOAEL was defined as 0.3 mg/kg dose ($AUC_{0\text{-}120h}$ males, 17.6 ug.h/mL; females, 27.6 ug.h/mL) in this study.

Monkey studies

- Study 7805-135: Monkeys (1/sex/dose) were administered a single subcutaneous dose of eflapegrastim at 0.323, 9.689 or 32.3 mg/kg and sacrificed at Day 15. In-life treatment-related findings included body weight loss (up to 8%), increases in WBC, neutrophil, and monocyte counts, decreases in RBC mass (up to -49%) and platelet counts (up to -83%), and increased ALP (moderate to marked). Necropsy findings included significantly increased spleen weight (MD and HD, 5- to 20-fold), pulmonary infiltrates of neutrophils and mononucleocytes, hemorrhage/erythrophagocytosis (males, HD; females, MD and HD), and splenic lymphoid depletion (marked) and granulopoiesis (severe) in all treatment groups. The MTD of eflapegrastim was determined to be the high dose (32.3 mg/kg) and the NOAEL was 0.323 mg/kg ($AUC_{0\text{-}336h}$ of 86.5 ug.h/mL).

6.2 Repeat-Dose Toxicity

6.2.1 Rat Studies

6.2.1.1 Four-Week Rat Study

Study title: 4-Week Subcutaneous Injection Toxicity and Toxicokinetic Study with HM10460A, a Long Acting G-CSF, in Rats with a 4-Week Recovery Period

Study no.:	7805-158
Study report location:	SDN 1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	04/14/2008
GLP compliance:	Yes, a signed GLP compliance statement is included in the report
QA statement:	Yes
Drug, lot #, and % purity:	B14030-LGK071, purity 98.1%

Key Study Findings

HM10460A was administered subcutaneously to rats at doses of 0.162, 0.969 and 3.23 mg/kg/week for four weeks. Following the second dose, all high dose animals and one mid dose animal were sacrificed in moribund conditions. Adverse clinical signs included limited use of hindlimbs, mild to moderate swelling and erythema of hindlimbs and hindpaws, and sensitive to touch. At necropsy, joint inflammation, reduced bone area, osteogenesis, and marrow necrosis were observed in the euthanized animals. Following dose reduction in the mid dose group (from 0.969 to 0.323 mg/kg/week starting at the third dose), all animals survived to terminal sacrifice; increased blood WBC and ANC, increased splenic weight (up to 2-fold), and histopathology findings of increased granulopoiesis in the bone marrow, and increased extramedullary granulopoiesis and megakaryopoiesis in the spleen, liver, kidney, tarsal joint and lymph nodes were noted. The NOAEL was defined as 0.162 mg/kg/week (Day 1 $AUC_{0\text{-}144h}$, 1.22 ug.h/mL for males, and 4.46 ug.h/mL for females, <1X MRHD) based on mortality.

TK data at Day 28 were not obtained due to development of anti-HM10460A neutralizing antibodies in most treated animals.

Methods

Doses:	0, 0.162, 0.969/0.323, 3.23 mg/kg/week
Frequency of dosing:	Weekly
Route of administration:	Subcutaneously
Dose volume:	5 mL/kg
Formulation/Vehicle:	Citric acid monohydrate (b) (4) sodium chloride (b) (4) polysorbate 80 (b) (4) mannitol (b) (4) in water
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	15/sex for control and high dose; 10/sex for low dose and mid dose groups
Age:	12-13 weeks age
Weight:	Males, 340-419 g; females, 234-258 g
Satellite groups:	Toxicokinetic animals (12/sex/dose for treatment groups and 6/sex for control) were included in the study
Unique study design:	Due to severe toxicities, mid dose was reduced from 0.969 to 0.323 mg/kg after the second dose. The detailed study design is presented below

Group[a]	No. of Animals[b] Male	Female	Dose Level as HM10411 (mg/kg/dose)	Dose Concentration as HM10411 (mg/mL)	Dose Level as HM10460A (mg/kg/dose)	Dose Concentration as HM10460A (mg/mL)
Toxicity Animals						
1 (Control)	15	15	0.0	0.0	0	0
2 (Low)	10	10	0.044	0.009	0.162	0.033
3 (Mid)[c]	10	10	0.264 (0.088)	0.053 (0.018)	0.969 (0.323)	0.194 (0.0646)
4 (High)[d]	15	15	0.880	0.176	3.23	0.646
Toxicokinetic Animals						
5 (Control)	6	6	0.0	0.0	0	0
6 (Low)	12	12	0.044	0.009	0.162	0.033
7 (Mid)[c]	12	12	0.264 (0.088)	0.053 (0.018)	0.969 (0.323)	0.194 (0.0646)
8 (High)[d]	12	12	0.880	0.176	3.23	0.646

a Groups 1 and 5 received vehicle control article/diluent only [HM10460A vehicle: [Citric acid monohydrate (b) (4) Sodium chloride (b) (4) Polysorbate 80 (b) (4) Mannitol (b) (4) prepared in sterile water for injection].

b Five toxicity animals/sex in Group 1 and three toxicity animals/sex in Group 3 were designated for the recovery sacrifice and underwent at least 4 weeks of recovery following the dosing phase.

c Due to adverse affects observed after two weeks of dosing, the dose levels of Groups 3 and 7 were lowered to the values in parenthesis for the remaining two weeks of dosing (Days 15 and 22 of the dosing phase).

d Due to observed adverse affects, all animals in Groups 4 and 8 (high dose) were sacrificed on Day 12 of the dosing phase.

Deviation from study protocol:	No deviations that affected overall interpretation of study findings or the integrity of the study occurred.

Observations and Results

Mortality

All animals in the high dose group (3.23 mg/kg), including both main study and TK study animals, and one animal in the mid dose group (0.969 mg/kg) were sacrificed on Day 12 in moribund condition.

Clinical findings in these animals included limited use of hindlimbs, swelling and erythema of hindlimbs and hindpaws, and signs of pain (sensitivity to touch and vocalization), as well as body weight loss from Days 8-12 of the dosing phase. Correlated histopathology findings of hindlimb inflammation (joints and surrounding soft tissues), decreased bone area, and marrow necrosis were observed. See histopathology section for details.

Clinical Signs
Swelling and skin erythema of the hind leg/paw and associated pain (sensitive to touch) were observed at ≥0.969 mg/kg/dose (Table 5). In the surviving animals, clinical signs continued throughout dosing. Following dose reduction in the mid dose group (reduced from 0.969 to 0.323 mg/kg/dose beginning at the third weekly dose), no additional clinical signs were observed.

Table 5. Incidence of hind leg/paw swelling on Days 10-12, Rat 4-week study

Group	Male	Female
Toxicity Animals		
1 (Control)	0/15	0/15
2 (Low)	0/10	0/10
3 (Mid)	4/10	3/10
4 (High)	13/15	1/15
Toxicokinetic Animals		
5 (Control)	0/6	0/6
6 (Low)	0/12	0/12
7 (Mid)	4/12	2/12
8 (High)	10/12	6/12

Table excerpted from applicant submission

Body Weights
Body weight gain reduction was observed in the mid dose groups (0.906/0.323 mg/kg) during Days 1-15 (male, -46%; female, -17%); following dose reduction on Day 15, body weight gain was similar or higher than control.

Food Consumption
Reduced food consumption was noted in males, but not females, given 0.969 mg/kg HM10460A, and was associated with significantly reduced body weight gain during the first 15 days of the dosing phase.

Ophthalmoscopy
No remarkable findings

ECG
Not performed.

Hematology
All high-dose animals were prematurely sacrificed at Day 12, without collection of blood samples.

Increased white blood cell and absolute neutrophil counts was observed in surviving animals, and correlated with increased myeloid:erythroid (M/E) ratio in the bone marrow smear and increased serum ALP activity. In addition, slightly reduced platelet count (up

to -25% in male groups) and red blood cell count (up to -6% in mid dose females) were also observed in treatment groups. All changes were reversible. See Table 6.

Table 6. WBC and Neutrophil counts at end of treatment, 4-week rat study

Dose, mg/kg		0	0.162	0.969/0.323
WBC, E3/uL	M	9.35	15.6	16.11
	F	8.25	9.99	11.29
Neutrophil, E3/uL	M	1.54	7.73	8.02
	F	1.43	2.5	4.34

Clinical Chemistry
Minimally decreases in blood glucose (-7.8%), total protein (-4.3%) and globulin (-8%) were observed in male treatment groups. No similar findings were seen in recovery animals after 4 weeks of recovery period.

Urinalysis
No remarkable findings

Gross Pathology
Splenic enlargement and tarsal joint enlargement were observed in the high dose animals sacrificed at Day 12. See details in histopathology section.

Organ Weights
Increased spleen weights were observed in males (LD, 1.29x; MD, 1.54x) and females (LD, 1.12x; MD, 1.74x).

Histopathology
Adequate Battery Yes. A complete battery of tissues was examined in the control, high dose and mid dose groups. The liver, spleen, lymph nodes (mandibular and mesenteric), sternum with bone marrow, and prostate were identified as target organs, processed, and examined microscopically in each low-dose (Group 2) animal.

Peer Review
Not performed

Histological Findings
Increased extramedullary granulopoiesis and/or megakaryopoiesis (spleen, liver, kidney and mesenteric lymph node) and increased granulopoiesis in the bone marrow were observed in all treatment groups. At the high dose, additional findings were observed including decreased bone area (thinning of cortical and trabecular bone, fibrosis, decreased osteoblast and increased osteoclasts) and osteogenesis (presence of trabeculae of woven or new bone) in the femur and tarsal joint bones, joint capsule inflammation in the tarsal and stifle joints, as well as marrow necrosis in the bone of tarsal joints. The joint capsule findings were characterized by a mixed inflammatory cell infiltrate (lymphocytes, macrophages, and neutrophils), edema, fibroplasia, and

synoviocyte enlargement, and occasionally deposition of fibrin in the joint space and extension of the inflammation to surrounding soft tissues. See Table 7.

Table 7. Histopathology finding incidences (severity), 4-week rat study

		Male				Female			
	Dose, mg/kg	0	0.162	0.969/0.323	3.23	0	0.162	0.969/0.323	3.23
	number examined	10	10	7	15	10	10	7	15
Liver	Granulopoiesis		2(1.0)	4(1.0)	15(2.0)			2(1.0)	15(2.0)
	Megakaryopoiesis				15(1.0)				15(1.0)
Spleen	Increased Granulopoiesis		4(1.0)	4(1.3)	15 (3.0)		2(1.0)	4(1.3)	15 (3.0)
	Increased Megakaryopoiesis		4(1.0)	5(1.3)	15(2.0)		2 (1.0)	5(1.0)	15(2.0)
	Decreased Lymphoid Follicles		1(1.0)	1(2.0)	15(2.0)				15 (2.0)
	Inflammation, Capsule				4(1.0)			1(1.0)	4(1.0)
Kidney	Granulopoiesis, Peripelvic		NA		3(1.0)		NA		1(1.0)
LN, Mandibular	Granulopoiesis		1(1.0)	3(1.0)	10(1.0)			6(1.0)	14(1.0)
Prostate	Inflammation	1(1.0)	3(1.0)	4(1.0)	11(1.4)				
Bone, Femur	Decreased Bone Area				15(1.7)				15(2.0)
	Osteogenesis				5(1.0)				13(1.4)
Marrow, femur	Increased Granulopoiesis		9(1.4)	7(1.6)	15(3.0)		3(1.0)	4(1.0)	15(3.0)
Joint, Stifle	Inflammation, Capsule				1(1.0)				1(1.0)
Joint, Tarsal	Inflammation, Capsule							1(2.0)	11(2.5)
	Decreased Bone Area				15(2.2)			1(1.0)	14(1.7)
	Osteogenesis		1(2)		11(2.3)			1(3)	9(1.7)
	Increased Granulopoiesis		6(1.1)	7(2.0)	15(2.5)		8(1.0)	7(1.6)	15(2.9)
	Necrosis, Marrow				10(1.0)				11(1.0)
Subcut. Injection Site	Granulopoiesis				3(1.3)				1(1.0)

( ) mean severity of affected animals in a scale of 5 where 1=minimal, 2=slight, 3=moderate, 4 =marked, 5=severe; Blanks indicate zero incidence

Toxicokinetics

Dose-related increase in exposure was observed on Day 1 (see Table 8).
TK analysis was not performed on Day 22 due to immunogenicity observed at 0.162 and 0.969/0.323 mg/kg and associated impact on the exposure. The TK group at 3.23 mg/kg was terminated on Day 12.

Table 8. Toxicokinetic parameters on Day 1, 4-week rat study

Dose Group	HM10460A Dose Level (mg/kg/dose)	Sex	C_{max} (pg/mL)	T_{max} (hr)	AUC_{0-144} (pg•hr/mL)	$AUC_{0-\infty}$ (pg•hr/mL)	$t_{1/2}$ (hr)
6	0.162	M	28084	10.0	1223576	1230712	19.4
		F	179809	24.0	4456298	4450846	11.8
7	0.969	M	2441156	48.0	127079481	127135991	9.21
		F	3251221	48.0	237244687	237272156	6.95
8	3.23	M	13293691	48.0	1201653216	1459934317	44.2
		F	14963455	48.0	1388486547	1660407713	44.8

Table excerpted from applicant submission

Dosing Solution Analysis

Dosing solution analysis demonstrated acceptability of the formulations (90.3 and 102% of target concentration) used for dosing on Day 1 and Day 15.

Antibody and Neutralizing Antibody Analysis

Blood samples were collected at pre-study (Day -3), end of treatment (week 4) and end of recovery (week 8). Antibodies against HM10406A were detected in most treated animals, the majority of which were neutralizing antibodies (Table 9).

Table 9. Incidence of antibodies (Tab) and neutralizing antibodies (Nab), 4-week rat study

		TAb			NAb		
Group	Sex	Predose	Week 4 (Dosing Phase)	Week 4 (Recovery Phase)	Predose	Week 4 (Dosing Phase)	Week 4 (Recovery Phase)
1 Control)	Males	0/15	0/15	0/5	Not Tested	Not Tested	Not Tested
	Females	0/15	0/15	0/5	Not Tested	Not Tested	Not Tested
2 (Low)	Males	0/10	8/10	NA	0/8	5/8	NA
	Females	0/10	9/10	NA	0/9	7/9	NA
3 (Mid)	Males	0/10	8/10	3/3	0/9	7/8	2/3
	Females	0/10	8/10	3/3	0/9	6/8	2/3
4 (High)[a]	Males	0/15	Not Tested	Not Tested	Not Tested	Not Tested	Not Tested
	Females	0/15	Not Tested	Not Tested	Not Tested	Not Tested	Not Tested

NA = Not applicable.
a Due to observed adverse affects, all animals in Groups 4 and 8 (high dose) were sacrificed on Day 12 of the dosing phase.

Table excerpted from applicant submission

6.2.2 Monkey Studies

6.2.2.1 Monkey 4-Week Study

Study title: 4-Week Subcutaneous Injection Toxicity and Toxicokinetic Study with HM10460A, a Long Acting G-CSF, in Cynomolgus Monkeys with a 4-Week Recovery Period

Study no.:	7805-159
Study report location:	SDN 1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	03/31/2008
GLP compliance:	Yes, a signed GLP compliance statement is included in the report
QA statement:	Yes
Drug, lot #, and % purity:	B14030-LGK071, purity 98.1%

Key Study Findings

Mortality due to hemorrhage in the lung or brain was observed in high dose monkeys (3.23 mg/kg/week); increased neutrophil counts (2-4x) and increased spleen weight (1.5-2.5x) in all treatment groups; dose-related increased granulopoiesis in the bone marrow and extramedullary granulopoiesis in the spleen, liver, kidney and lymph nodes. The NOAEL was 0.969 mg/kg/week (Day 1 $AUC_{0\text{-}144h}$ of 484 ug.h/mL for males and 715 ug.h/mL for females, 31X and 46X MRHD, respectively) based on acceptable macroscopic and histopatholgical findings at this dose level.

Repeat dosing resulted in reduced systemic exposures in all treatment groups, likely due to formation of anti-drug antibodies. At Day 22, $AUC_{0\text{-}144h}$ values at NOAEL (0.969 mg/kg) were 92 ug.h/mL for males and 136 ug.h/mL for females.

Methods

Doses:	0, 0.162, 0.969/0.323, 3.23 mg/kg/week
Frequency of dosing:	weekly
Route of administration:	Subcutaneously
Dose volume:	5 mL/kg
Formulation/Vehicle:	Citric acid monohydrate (b) (4) sodium chloride (b) (4) polysorbate 80 (b) (4) mannitol (b) (4) in water
Species/Strain:	Cynomolgus monkeys
Number/Sex/Group:	5/sex for control and high dose; 3/sex for low dose and mid dose groups
Age:	2-5 years age
Weight:	Males, 2.4-4.0 kg; females, 2.2- 3 kg
Satellite groups:	None
Unique study design:	Recovery animals for the control (2/sex) and high dose (1/sex) were included. The detailed study design is presented below

Group	No. of Animals[b] Male	Female	Dose Level as HM10411 (mg/kg/dose)	Dose Concentration as HM10411 (mg/mL)	Dose Level as HM10460A (mg/kg/dose)	Dose Concentration as HM10460A (mg/mL)
1 (Control)[a]	5	5	0	0	0	0
2 (Low)	3	3	0.088	0.018	0.323	0.064
3 (Mid)	3	3	0.264	0.053	0.969	0.194
4 (High)	5	5	0.880	0.176	3.230	0.646

a Group 1 received vehicle control article/diluent only [HM10460A vehicle: ([Citric acid monohydrate (b) (4) Sodium chloride (b) (4) Polysorbate 80 (b) (4) Mannitol (b) (4) prepared in sterile water for injection)].

b Two animals/sex in Group 1 and one animal/sex in Group 4 were designated for recovery sacrifice and underwent at least 4 weeks of recovery following the dosing phase.

Deviation from study protocol:	No deviations affecting overall interpretation of study findings or the integrity of the study occurred.

Observations and Results

Mortality

Three animals at 3.23 mg/kg (2 males and 1 female) were sacrificed in moribund conditions between Days 9-15 of the dosing phase. Acute pulmonary hemorrhage, and cerebral and cerebellar hemorrhage were likely the cause of deaths, although the latter was confounded with possible head trauma in one animal.

Clinical Signs

Prior to their moribund sacrifice, the three animals exhibited hunched posture, dehydration, hypoactivity, vomitus (clear, yellow), few feces, broken skin, blue skin, red skin, and scab. Treatment-related clinical signs in surviving animals were limited to nonformed feces, which were also observed during recovery phase although at a lower frequency.

Body Weights

No remarkable findings

Food Consumption

There were no clear treatment-related changes in food consumption, except in the three premature deaths where significantly decreased or absence of food consumption prior to deaths were noted.

Dermal Irritation

No remarkable findings

Ophthalmoscopy

No remarkable findings

ECG

ECG, using leads I, II, aVF, CV5RL, and CV6LL, exhibited no remarkable findings in heart rate, wave intervals (PR, QRS, QTc) when recorded at 1, 4, and 8 hours post-dosing on Days 1, 3, 7 and 24 of dosing phase.

Blood Pressure

No treatment-related findings

Hematology

Most notable findings were increased WBC counts primarily due to increased absolute neutrophil count in all treatment groups, without a clear dose-dependence. A few individuals at each dose level were relatively unaffected. Average increases were 2-3 fold for WBCs and 2-4 fold for neutrophils. Minor decrease in red blood cell count (up to -31%) along with increased reticulocytes (up to 2.7x) in mid dose and/or high dose groups, as well as increased platelet counts (1.3-1.5x) in high dose groups, were also observed. Increased neutrophil counts generally correlated with minimally to mildly increased myeloid:erythroid (M/E) ratios in the bone marrow smear. With limited animal numbers, the recovery data appear to support reversibility of the hematology findings.

Table 10. Hematology findings, 4-week monkey study

		Treatment Day 29				Recovery Day 29	
Dose, mg/kg		0	0.162	0.969	3.23	0	3.23
WBC, E3/uL	M	9.38	21.14	24.33	17.69	10.52	17.34
	F	9.32	21.98	17.23	27.82	10.21	9.46
Neutrophil, E3/uL	M	4.3	13.12	15.37	9.21	1.84	2.63
	F	4.33	16.12	10.16	18.01	4.53	2.24
RBC, E6/uL	M	5.21	5.14	4.78	4.07	5.44	5.45
	F	5.31	4.49	4.68	3.65	5.49	4.71
Reticulocyte, E3/uL	M	93.5	88	103.9	234.8	83.3	41.6
	F	105.3	71.5	215.6	288.9	41.2	31.3
Platelet, E3/uL	M	510	487	514	743	486	311
	F	474	492	546	595	354	335

Note: recovery data were obtained from 2/sex and 1/sex for control and high dose groups, respectively

Clinical Chemistry

Mildly increased globulin, decreased albumin, as well as decreased albumin-to-globulin ratio were observed in treatment groups. No differences were noted in recovery animals.

Table 11. Clinical chemistry at end of treatment, 4-week monkey study

Dose, mg/kg		0	0.162	0.969	3.23
Globulin, g/dL	M	3	3.3	3.4	3.4
	F	3.1	3.1	3.1	3.8
Albumin, g/dL	M	4.6	4.2	4.5	4.1
	F	4.5	4.2	4.5	4.3
Albumin/Globulin ratio,	M	1.5	1.4	1.3	1.2
	F	1.4	1.4	1.5	1.1

Urinalysis

No remarkable findings

Gross Pathology

Among the three moribund sacrifices, multiple foci of dark red discoloration in the cerebrum and cerebellum of the two males and in all lung lobes in the females were noted.

At the end of treatment phase, macroscopic findings were observed in one male at 3.23 mg/kg/dose, including enlarged mandibular, mesenteric and mediastinal lymph nodes, and a large spleen. Recovery animals (1/sex at 3.23 mg/kg/dose) showed no similar findings.

Organ Weights

Increased spleen weights were noted in most treatment groups (1.5-2.5x) except females at the low dose (0.162 mg/kg/dose). Females also displayed reduced organ weights in the thymus (up to -50%), uteruses (up to 70%), and ovaries (-30%), which were correlated with histopathology findings of atrophic changes. Recovery animals showed no remarkable findings.

Table 12. organ weight findings, 4-week monkey study

Dose, mg/kg		0	0.162	0.969	3.23
Spleen wt., g	M	2.469	6.079	4.384	5.456
	F	3.581	3.626	5.203	5.927
Thymus wt., g	M	1.77	2.574	2.671	1.921
	F	2.547	2.167	1.87	1.267
Uterus wt., g	F	3.55	2.243	2.999	1.122
Ovary wt., g	F	0.214	0.244	0.249	0.145

Histopathology

Adequate Battery: Yes. A complete battery of tissues was examined for all animals.

Peer Review: Not performed

Histological Findings

All three moribund animals (two males and one female in the3.23 mg/kg group) exhibited increased intravascular neutrophils in multiple organs (no details provided) in addition to foci of hemorrhage in the lung (the female) or brain (two males). Other findings in these animals were also observed in terminal sacrificed animals.

Increased granulopoiesis in the bone marrow, and extramedullary granulopoiesis in multiple other organs (spleen, liver, kidney, lymph node, testis, uterus, heart and choroid plexus) were observed in both moribund and terminal sacrificed animals. In scheduled sacrifice animals, maturing segmented cells predominated, while bands and myeloblasts were the most common cell types in moribund sacrifice animals. Increased cellularity due to increases in all cell lines was most striking in the femoral marrow, while increase in granulopoiesis associated with decreased erythropoiesis was noted in sternum marrow; the difference is likely due to background histology as femoral marrow is mostly adipose tissue and sternum has relatively little marrow fat, which limited the potential for increased hematopoietic cellularity. In moribund sacrificed animals, the cortical bone of the sternum was multifocally absent (osteolysis secondary to marrow expansion), and granulopoietic cells and segmented neutrophils extended through cortical gaps into the periosteum. Splenic extramedullary granulopoiesis was associated with increased intrasinusoidal neutrophils and sometimes lymphoid depletion.

Tissue atrophies in the thymus, uteruses (smaller cross section diameter and few and less torturous endometrial glands) and ovaries (corpora albicans but no corpora lutea were observed) were observed in high dose animals.

Recovery animals exhibited increased granulopoiesis and increased erythropoiesis in bone marrow, and extramedullary granulopoiesis in the liver and mandibular lymph node, but not other histopathological findings.

Table 13. Histopathology incidence, 4-week monkey study

		end of 4-week treatment							
		Male				Female			
	Dose, mg/kg	0	0.162	0.969	3.23	0	0.162	0.969	3.23
	number examined	3	3	3	2	3	3	3	3
Marrow, femur	Increased granulopoiesis		3(1.7)	3(3.0)	2(4.0)		3(1.7)	2(2.0)	3(3.0)
	Increased erythropoiesis		3(2.0)	3(1.3)	2(1.5)		1(1.0)	1(1.0)	3(1.0)
Marrow, sternum	Increased granulopoiesis		3(2.3)	3(2.0)	2(3.5)		3(1.0)	3(2.0)	3(1.5)
	Increased erythropoiesis			1(1.0)	1(1.0)				1(1.0)
	Decreased erythropoiesis		2(1.0)	1(1.0)	1(1.0)				
Liver	Granulopoiesis		1	3	2			1	3
Kidney	Granulopoiesis			1	1			2	3
Spleen	Increased granulopoiesis		3	2	1		2	1	2
	Lymphoid depletion			1					
Lymph node, mandibular	Granulopoiesis		2	2	1				3
Thymus	Involution								2
Ovary	Atrophy								2
Uterus	Granulopoiesis								2
	Atrophy							1	3
Subcut Injection Site	Mononuclear infiltrate	1		2			1	1	3
		end of 4-week recovery							
	Dose, mg/kg	0	0.162	0.969	3.23	0	0.162	0.969	3.23
	number examined	2			1	2			1
Marrow, femur	Increased granulopoiesis				1				1
	Increased erythropoiesis				1				1
Marrow, sternum	Increased granulopoiesis								1
	Increased erythropoiesis								1

Severity was provided for selected findings. ( ) mean severity of affected animals in a scale of 5 where 1=minimal, 2=slight, 3=moderate, 4 =marked, 5=severe; Blanks indicate zero incidence

Toxicokinetics

Systemic exposure data were obtained following Days 1 and Day 22 dosing. Plasma drug concentration was dose proportional on Day 1, but more than dose proportional on Day 22. Repeat dosing resulted in significantly reduced exposure due to unclear causes. There was no clear gender-related difference in toxicokinetic parameters.

Table 14. Toxicokinetics parameters, 4-week monkey study

		Day 1				Day 22			
		N	Cmax, ug/mL	$AUC_{0\text{-}144h}$, ug.h/mL	$T_{1/2}$, h	N*	Cmax, ug/mL	$AUC_{0\text{-}144h}$, ug.h/mL	$T_{1/2}$, h
0.323	M	3	2.68	113.3	6.6	2	0.8	20.71	NA
	F	3	1.76	63.7	15.2	2	0.45	13.97	16.3
0.969	M	3	8.47	483.9	8.8	2	2.99	91.75	28.2
	F	3	12.35	714.6	8.7	1	4.27	135.88	18.6
3.23	M	5	34.53	3077.7	31.5	1	27.58	1340.9	8.3
	F	5	39.06	3446.1	32.6	2	25.4	1021	12.9

*subjects with positive immunogenicity were excluded from TK analysis

Dosing Solution Analysis

Dosing solution analysis demonstrated acceptability of the formulations (90.9 and 104% of target concentration) used for dosing on Day 1 and Day 15.

Antibody and Neutralizing Antibody Analysis

Blood samples were collected at Days -7, 1 and 22. Anti-drug antibodies, mostly neutralizing, were identified on Day 22 in some individual animals from all treatment groups.

Table 15. Incidence of antibodies (Tab) and neutralizing antibodies (Nab), 4-week monkey study

Dose Group	Dose level (HM10460A)	Dose level (HM10411)	No. of Animals assigned per Group (M/F)	Incidence of TAb +ve animals (M/F)	Incidence of NAb +ve animals (M/F)
1	0.000	0.0	5/5	0/0	0/0
2	0.323	0.088	3/3	1/1	0/0
3	0.969	0.264	3/3	1/2	1/2
4	3.230	0.880	5/5	2[a]/3	2[a]/2

M/F = Male/Female, +ve = Positive samples.

a A male animal in the high dose group had positive sample results for total antibody and neutralizing antibody during the dosing and recovery phases.

Table excerpted from applicant submission

6.2.2.2 Monkey 26-week study

Study title: 26-Week Subcutaneous Injection Chronic Toxicity and Toxicokinetic Study with HM10460A, a Long Acting G-CSF, in Cynomolgus Monkeys with 4-Week Recovery Phase

Study no.:	8203-497
Study report location:	SDN 1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	04/09/2009
GLP compliance:	Yes, a signed GLP compliance statement is included in the report
QA statement:	Yes
Drug, lot #, and % purity:	B14099-LID032, purity 96.2%

Key Study Findings

Monkeys given test article at 0.02, 0.2, 0.8, or 1.6 mg/kg/week subcutaneously for 26 weeks exhibited transient increases (3-11 fold on Day 12) and subsequent reductions in neutrophils counts for the 0.2 and 1.6 mg/kg/week groups (Week 26, 40-50% below control; end of recovery, 70-85% below control), with correlated decreases in bone marrow M:E ratios. In three animals at 0.8 mg/kg (2 males and 1 female) increased neutrophil counts persisted to the end of 26 weeks of treatment. At necropsy, histopathology findings were limited to these three animals and included increased spleen weights, increased granulopoietic cells, decreased erythropoietic cells in the bone marrow, and extramedullary granulopoiesis in the spleen and kidneys.

Anti-HMA10460A antibodies, mostly neutralizing antibodies, were detected in the majority of treated animals and were likely the cause of the absence of expected pharmacological effects (reduction of neutrophils) in most treated animals at the end of dosing.

The NOAEL is 1.6 mg/kg/week (Day 1 $AUC_{0\text{-}144h}$, 1258 ug.h/mL, 82X MRHD).

Methods

Doses:	Cohort 1: 0, 0.2, 0.8, 1.6 mg/kg; Cohort 2: 0, 0.02 mg/kg
Frequency of dosing:	Weekly
Route of administration:	subcutaneously
Dose volume:	5 mL/kg
Formulation/Vehicle:	Citric acid monohydrate (b) (4) sodium chloride (b) (4) polysorbate 80 (b) (4) mannitol (b) (4) in water
Species/Strain:	Cynomolgus monkeys
Number/Sex/Group:	6/sex for control and high dose; 4/sex for low dose and mid dose groups
Age:	2-5 years age
Weight:	Males, 2.2-5.0 kg; females, 2.0- 3.1 kg
Satellite groups:	none
Unique study design:	Recovery animals (2/sex) for the control and high dose were included in cohort 1. Cohort 2 (groups 5 and 6) was added to the study two month after initiation of Cohort 1. The detailed study design is presented below

Group	No. of Animals[b] Male	Female	Dose Level as HM10411 (mg/kg/dose)	Dose Concentration as HM10411 (mg/mL)	Dose Level as HM10460A (mg/kg/dose)	Dose Concentration as HM10460A (mg/mL)
Cohort 1[c]						
1 (Control)[a]	6	6	0	0	0	0
2 (Low)	4	4	0.055	0.011	0.2	0.04
3 (Mid)	4	4	0.218	0.044	0.8	0.16
4 (High)	6	6	0.436	0.087	1.6	0.32
Cohort 2[c]						
5 (Control)[a]	3	3	0	0	0	0
6 (Low)	3	3	0.0055	0.0011	0.02	0.004

a Group 1 received vehicle control article/diluent only.
b Two animals/sex in Groups 1 and 4 were designated for recovery sacrifice and underwent at least 4 weeks of recovery following the dosing phase.
c Animals were grouped into two cohorts. Cohort 1 consisted of Groups 1, 2, 3, and 4. Cohort 2 consisted of Groups 5 and 6. Day 1 of the dosing phase was designated as the first day of dosing for each cohort.

Deviation from study protocol:	No deviations affecting the overall interpretation of study findings or the integrity of the study occurred.

Observations and Results

Mortality

All animals survived to terminal sacrifice.

Clinical Signs

No treatment-related findings.

Body Weights

No treatment-related findings

Food Consumption

Increased incidence of food consumption reduction was observed in males at 0.8 and 1.6 mg/kg groups only.

Dermal Irritation

No dermal irritation findings were considered treatment-related.

Ophthalmoscopy

No remarkable findings

ECG

ECG with leads I, II, aVF, CV5RL, and CV6LL showed no remarkable findings in heart rate, wave intervals (PR, QRS, QTc) in Weeks 1, 13 and 26 of dosing phase.

Hematology

Significantly increases in neutrophils and other WBC cells (lymphocytes, monocytes, eosinophils, basophils and large unstained cells), and slightly decreases in RBC mass and platelets were observed mostly on Days 12 and 26, and also on Day 57 for eosinophils. The most prominent change was increased white blood cell count due primarily to increased absolute neutrophil count, which occurred in all treatment groups and peaked at Day 12 with a 3 to 11 fold increase. The increased neutrophil counts persisted to the end of treatment only for three mid dose animals (2 males and 1 female). Reversal of neutrophil effect was observed as early as Day 26 in a few individuals, and in more animals over time. Mean neutrophil counts in all treated groups returned to pre-study levels by Day 54 , with neutrophils counts below 1000/mcL in several animals at one or more intervals during the last half of the dosing phase (Table 16). Additionally, all recovery animals had decreased absolute neutrophil counts after 4 weeks of recovery period (720-1680/mcL vs predose 3540-7000/mcL).

Table 16. Neutrophil counts -cohort 1, 26-week monkey study

Test Article		Control	HM10411/HM10460A		
Group		1	2	3	4
HM10411 (mg/kg/dose)		0	0.055	0.218	0.436
HM10460A (mg/kg/dose)		0	0.2	0.8	1.6

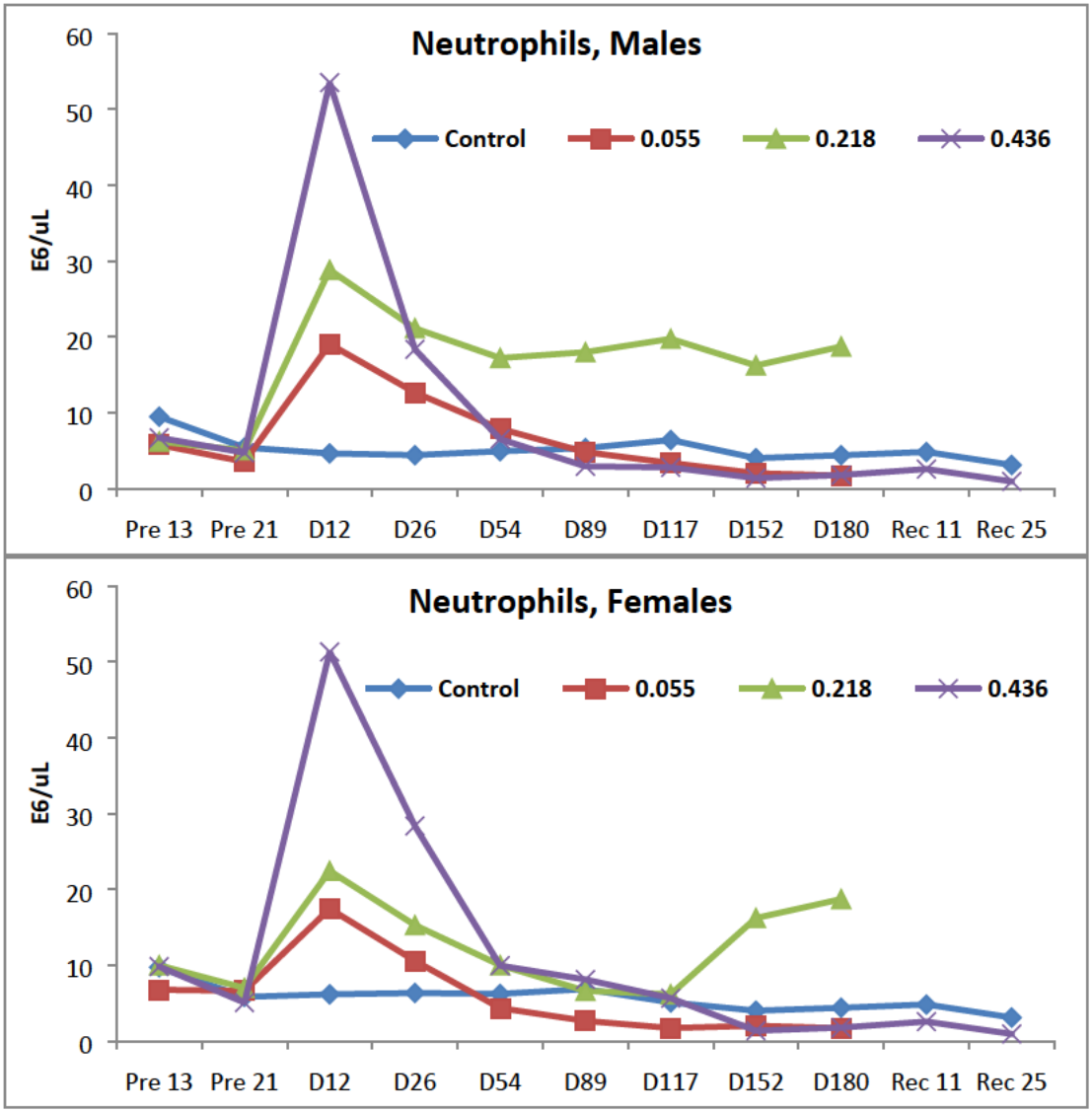
Group/ Sex		NEUT E3/uL PRED 13	PRED 21	DSNG 12	DSNG 26	DSNG 54	DSNG 89	DSNG 117
1M	Mean	9.47	5.41	4.63	4.41	4.92	5.33	6.40
	SD	1.282	2.753	0.817	1.651	2.276	1.435	4.906
	N	6	6	6	6	6	6	6
2M	Mean	5.77	3.54	19.04*	12.66*	7.88	4.80	3.37
	SD	3.279	1.493	1.615	2.419	8.280	4.164	2.371
	N	4	4	4	4	4	4	4
3M	Mean	6.19	5.03	28.82*	21.12*	17.21	17.98	19.74
	SD	2.673	1.876	9.509	11.957	17.526	17.950	19.399
	N	4	4	4	4	4	4	4
4M	Mean	6.70	4.73	53.52*	18.35*	6.52	2.93	2.79
	SD	3.714	1.514	20.550	17.104	7.571	2.036	1.260
	N	6	6	6	6	6	6	6
1F	Mean	9.73	5.83	6.17	6.34	6.23	6.86	5.10
	SD	3.369	1.775	2.613	3.237	1.180	4.515	3.506
	N	6	6	6	6	6	6	6
2F	Mean	6.77	6.62	17.45	10.58	4.35	2.66	1.72
	SD	3.132	4.532	7.028	7.260	3.037	1.268	1.083
	N	4	4	4	4	4	4	4
3F	Mean	9.99	7.00	22.44*	15.29	10.01	6.62	6.17
	SD	3.598	2.844	8.043	10.863	10.967	10.289	9.473
	N	4	4	4	4	4	4	4
4F	Mean	9.83	5.08	51.31*	28.36*	9.95	8.12	5.66
	SD	4.950	2.360	9.594	15.787	7.585	5.616	5.354
	N	6	6	6	6	6	6	6

Group/ Sex		NEUT E3/uL DSNG 152	DSNG 180	RECO 11	RECO 25
1M	Mean	4.01	4.39	4.83	3.11
	SD	1.437	1.665	0.651	0.134
	N	6	6	2	2
2M	Mean	2.02	1.70*	.	.
	SD	2.015	1.403	.	.
	N	4	4	0	0
3M	Mean	16.22	18.72	.	.
	SD	16.969	17.810	.	.
	N	4	4	0	0
4M	Mean	1.37*	1.78*	2.57	0.94
	SD	0.883	1.114	1.952	0.311
	N	6	6	2	2
1F	Mean	5.79	5.10	11.15	8.88
	SD	2.698	2.104	6.385	5.657
	N	6	6	2	2
2F	Mean	1.29*	2.59	.	.
	SD	0.509	1.577	.	.
	N	4	4	0	0
3F	Mean	6.69	6.51	.	.
	SD	10.160	7.588	.	.
	N	4	4	0	0
4F	Mean	2.41	2.77	1.36	1.33
	SD	1.769	1.106	0.276	0.495
	N	6	6	2	2

Neutrophil counts - Cohort 2

Group/ Sex		NEUT E3/uL PRED 9	PRED 14	DSNG 12	DSNG 26	DSNG 54	DSNG 89	DSNG 117	DSNG 152	DSNG 180
5M	Mean	4.97	3.79	6.20	5.34	5.31	5.22	4.36	3.80	4.41
	SD	0.720	1.314	2.774	1.481	1.480	2.431	1.969	0.962	0.881
	N	3	3	3	3	3	3	3	3	3
6M	Mean	5.67	5.83	19.63	16.56	16.62	13.88	7.29	3.35	4.73
	SD	2.647	3.461	7.451	4.867	11.233	13.838	6.806	3.011	3.442
	N	3	3	3	3	3	3	3	3	3
5F	Mean	7.23	5.03	5.77	7.19	4.54	7.04	5.30	3.94	6.06
	SD	3.435	1.896	1.164	1.396	1.965	1.399	1.632	0.337	0.526
	N	3	3	3	3	3	3	3	3	3
6F	Mean	6.74	4.96	16.27	13.86	3.15	3.26	2.62	1.38	2.98
	SD	2.141	1.510	2.881	3.954	1.330	3.117	1.933	0.710	1.330
	N	3	3	3	3	3	3	3	3	3

Tables excerpted from applicant submission



Clinical Chemistry

No remarkable findings

Urinalysis

No remarkable findings

Gross Pathology

Enlarged spleen in one high mid dose male (0.8 mg/kg/dose) and increased incidence of thickened tissue at injection sites in treated male groups were observed.

Organ Weights

Increased spleen weight (1.5-6.1x) in three animals at 0.8 mg/kg/dose that correlated with increased WBC and neutrophil counts was noted

Histopathology

Adequate Battery: Yes. A complete battery of tissues was examined for all animals.

Peer Review: Not performed

Histological Findings

Histopathological findings were associated with medullary and extramedullary granulopoiesis including increased granulopoiesis and decreased erythropoiesis in the bone marrow, and extramedullary granulosis in the spleen (neutrophil in the sinusoid) and the kidney (neutrophil aggregate within the interstitium) in the three animals at 0.8 mg/kg where increases in WBC and neutrophils persisted throughout the 26 weeks of treatment and where the anti-drug antibodies titers were low or undetectable. No remarkable findings were observed in the high dose groups after 4 weeks of recovery period.

Table 17. Incidence of histopathological findings -cohort 1, 26-week monkey study

Controls from group(s): 1	Animal sex:	-- Animals Affected -- Males --				-- Females --			
	Dosage group:	Ctls	2	3	4	Ctls	2	3	4
Tissues With Diagnoses	No. in group:	4	4	4	4	4	4	4	4
Bone, Femur	Number examined:	4	4	4	4	4	4	4	4
	Unremarkable:	4	4	4	4	4	4	4	4
Marrow, Femur	Number examined:	4	4	4	4	4	4	4	4
	Unremarkable:	4	4	2	4	4	4	3	4
Increased granulopoietic cells		0	0	2	0	0	0	1	0
Decreased erythropoietic cells		0	0	2	0	0	0	1	0
Bone, Sternum	Number examined:	4	4	4	4	4	4	4	4
	Unremarkable:	4	4	4	4	4	4	4	4
Marrow, Sternum	Number examined:	4	4	4	4	4	4	4	4
	Unremarkable:	4	4	2	4	4	4	3	4
Increased granulopoietic cells		0	0	2	0	0	0	1	0
Decreased erythropoietic cells		0	0	2	0	0	0	1	0
Spleen	Number examined:	4	4	4	4	4	4	4	4
	Unremarkable:	3	3	1	4	2	4	3	4
Eosinophilic material, Germinal center		1	1	0	0	0	0	1	0
Extramedullary granulopoiesis		0	0	2	0	0	0	0	0
Fibrosis, Capsule		0	0	1	0	2	0	0	0
Kidney	Number examined:	4	4	4	4	4	4	4	4
	Unremarkable:	1	2	2	1	4	3	3	3
Infiltrate, Lymphocytes/Macrophages		3	2	2	3	0	1	1	1
Extramedullary granulopoiesis		0	0	1	0	0	0	0	0

Table excerpted from applicant submission

Dosage groups 2, 3 and 4 received 0.2, 0.8 and 1.6mg/kg/week eflapegrastim, respectively.

Decreases in myeloid:erythroid (M/E) ratios were observed at ≥0.2 mg/kg/dose, with the exception of three animals at 0.8 mg/kg where the increased neutrophil counts persisted

to the end of treatment (animal Nos. I00544, 00545, 00563). Lower M/E ratio were also observed in in all four high dose recovery animals.

Table 18. Myeloid:Erythroid ratio, 26-week monkey study

Dose, mg/kg/week		0	0.2	0.8	1.6
Dosing Day 184	M	1.02	0.88	1.44	0.83
	F	1.12	1.05	1.08	0.83
Recovery Day 29	M	1.34			0.94
	F	1.24			0.83

Toxicokinetics

Systemic exposure (Cmax and AUC) was increased more than dose proportionally on day 1 of treatment. There were no clear gender-related differences. Due to development of anti-drug antibodies in most animals, toxicokinetic data obtained in weeks 9, 13 and 26 were limited to animals with no detectable anti-drug antibodies (ADA). Although TK was analyzed in fewer animals starting from week 9, it appears that exposure levels were reduced over time. See Table 19.

Table 19. Toxicokinetics parameters, 26-week monkey study

				Day 1		
Dose, mg/kg/w		N	Cmax, mcg/mL	$AUC_{0\text{-}144h}$, mcg.h/mL	$T_{1/2}$, h	Tmax
0.02	M	3	0.02	0.32	NA	5
	F	3	0.03	0.41	NA	6.7
0.2	M	4	1.07	32.5	9.56	24
	F	4	1.01	31.8	8.34	24
0.8	M	4	7.24	373.8	7.62	24
	F	4	7.25	393.8	7.1	24
1.6	M	6	17.05	1307.5	8.49	28
	F	6	18	1209.7	7.12	24
				Week 9		
0.02	M	1	0.007	0.22	NC	5
0.8	M	2	2.1	49.3	33.9	10
				Week 13		
0.8	M	2	3.9	107.3	37	17
				Week 26		
0.8	M	2	2.08	55.2	67.7	17

Animals with ADAs were excluded from TK analysis

Dosing Solution Analysis

Formulations prepared for cohort 1 were acceptable with mean concentrations ranging from 94.3 to 106% of target concentrations, with the exception of the 0.04 mg/mL that was in a range of 86.0-88.7% of target concentration, used for 0.2 mg/kg group during Weeks 16 and 20 of the dosing phase.

Antibody and Neutralizing Antibody Analysis

Blood samples for immunogenicity analysis were collected at weeks 4, 13, 26 and end of recovery, Week 30. Antibodies against test article were detected in the majority of treated animals, of which a large portion was neutralizing antibodies. Two males of the three animals in the 0.8 mg/kg/dose group that had persisting increases in neutrophils throughout dosing, developed no antibodies, whereas the female had a low total antibody titer (436 ng/mL) at week 4 and neutralizing antibodies beginning at week 6. The presence of neutralizing antibodies appears to be a plausible explanation for the lack of pharmacological effects in most treated animals as well as neutrophil reductions later in the dosing phase of this study.

It is noted that more animals had neutralizing antibodies at week 13 than at week 26 in Groups 3 and 4, the cause of which is unclear.

Table 20. Incidence of TAb and NAb findings, 26-week monkey study

Dose Group	Dose Level (HM10460A)	Dose Level (HM10411)	No. of Animals Assigned per Group (M/F)	Incidence of TAb +ve Animals (M/F)[b] Week				Incidence of NAb +ve Animals (M/F)[b] Week			
				4	13	26	30[a]	4	13	26	30[a]
1	0	0	6/6	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
2	0.2	0.055	4/4	2/4	4/4	4/4	-	0/2	3/4	1/3	-
3	0.8	0.218	4/4	2/4	2/4	2/4	-	0/0	2/4	2/3	-
4	1.6	0.436	6/6	5/5	6/6	6/6	2/2	1/0	5/6	1/6	1/2
5	0	0	3/3	0/1	1/1	2/1	-	0/1	0/0	1/0	-
6	0.02	0.0055	3/3	1/3	3/3	3/3	-	0/0	2/3	3/3	-

M/F = Male/Female, +ve = Positive samples. - = Not applicable.

a Week 30 represents Week 4 of the recovery phase. Only two animals/sex in Groups 1 and 4 underwent a 4 week recovery phase.

b All animals were screened for the presence of total antibodies (TAb) to HM10460A. Samples screening positive were analyzed for confirming the presence of TAb. Samples confirmed for TAb were screened for neutralizing antibodies (NAb) and subsequent confirmation for NAb.

Table excerpted from applicant submission

7 Genetic Toxicology

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

Study title:

Study no.:	7805-162
Study report location:	SDN1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	2/15/2008
GLP compliance:	Yes, a signed GLP compliance statement was included in the report
QA statement:	Yes
Drug, lot #, and % purity:	HM10460A, lot B14030-LHA281, purity 98.0%

Key Study Findings

HM10460A was not mutagenic in the Ames test.

Methods

Strains:	TA98, TA100, TA1535, TA1537 and WP2uvrA
Concentrations in definitive study:	88, 293, 880, 2200, 4400 mcg/plate with and without S9 mix.
Basis of concentration selection:	Concentration limit of 5000 mcg/plate
Negative control:	(b) (4) mannitol, (b) (4) NaCl, (b) (4) Citric acid monohydrate, (b) (4) polysorbate 80 in sterile water
Positive control:	

Tester Strain	S9 Mix	Positive Control	Dose (µg/plate)
TA98	+	benzo[a]pyrene	2.5
TA98	–	2-nitrofluorene	1.0
TA100	+	2-aminoanthracene	2.5
TA100	–	sodium azide	2.0
TA1535	+	2-aminoanthracene	2.5
TA1535	–	sodium azide	2.0
TA1537	+	2-aminoanthracene	2.5
TA1537	–	ICR-191	2.0
WP2*uvr*A	+	2-aminoanthracene	25.0
WP2*uvr*A	–	4-nitroquinoline-N-oxide	1.0

Formulation/Vehicle:	Same as negative control
Incubation & sampling time:	52±4 hours at 37°C

Study Validity

The study is valid based on the following:

- Results of both positive and negative control were as expected
- Triplicate cultures for each test condition were used
- Adequate doses were tested
- Results from dose ranging finding study and confirmatory mutagenicity assay were consistent

Results
There were no cytotoxicity or precipitation observed in any test conditions.
HM10460A did not induce increased revertants in any of the tester strains either in the presence or absence of S9 mix.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells

Study no.:	7805-163
Study report location:	SDN1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	2/15/2008
GLP compliance:	Yes, a signed GLP compliance statement was included in the report
QA statement:	Yes
Drug, lot #, and % purity:	HM10460A, lot B14030-LGK071 (purity not reported) and B14030-LHA281 (purity 98.0%)

Key Study Findings
HM10460A was negative for inducing chromosomal aberrations in CHO cells with and without metabolic activation.

Methods

Cell line:	Chinese Hamster Ovary cells
Concentrations in definitive study:	Without S9 mix, 110, 220, 440, 880, 1760, 2640, 3520, and 4400 µg/Lm; with S9 mix, 880, 1760, 2640, 3520, and 4400 µg/mL; chromosomal aberration was analyzed for concentrations of 2640, 3520 and 4400 mcg/mL with and without metabolic activation.
Basis of concentration selection:	Concentration limit of 0.5 mg/mL
Negative control:	(b) (4) mannitol, (b) (4) NaCl, (b) (4) Citric acid monohydrate, (b) (4) polysorbate 80 in sterile water
Positive control:	-S9, mitomycin 0.75 mcg/mL; +S9, cyclophosphamide, 7.5 mcg/mL
Formulation/Vehicle:	Same as negative control
Incubation & sampling time:	Without S9, 20 hour; with S9, 3 h incubation + 17 hours additional incubation

Study Validity

The study is valid based on the following:

- Results of negative and positive controls were as expected
- Duplicated cultures were employed for all test conditions
- Adequate doses were tested
- Cells scored for aberrations were adequate (100 per slide, 200 for each test condition)
- Results of initial chromosomal aberrations assay and confirmatory chromosomal aberration assay were consistent.

Results

No cytotoxicity was observed in any test conditions.
HM10460A at concentrations of 2640, 3520, and 4400 mcg/mL did not induce increased cells with chromosomal aberration, polyploidy, or endoreduplication, with and without metabolic activation.

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

Study title: *In Vivo* Rat Bone Marrow Micronucleus Assay

Study no:	7805-164
Study report location:	SDN1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	04/03/2008
GLP compliance:	Yes, a signed GLP compliance statement was included in the report
QA statement:	Yes
Drug, lot #, and % purity:	HM10460A, batches B14030-LHA281 (purity 98.0%) and B14030-LHC281 (purity 98.5%)

Key Study Findings

HM10460A subcutaneously administered to rats at doses up to 1445 mg/kg did not induce increases in micronucleated polychromatic erythrocytes in the bone marrow.

Methods

Doses in definitive study:	361.3, 722.7 and 1445.2 mg/kg
Frequency of dosing:	Single dose
Route of administration:	Subcutaneous injection
Dose volume:	10 mL/kg
Formulation/Vehicle:	(b) (4) mannitol, (b) (4) NaCl, (b) (4) Citric acid monohydrate, (b) (4) polysorbate 80 in sterile water
Species/Strain:	CD® (SD) IGS BR rats
Number/Sex/Group:	10 male/dose
Satellite groups:	none
Basis of dose selection:	Dose limit
Negative control:	Same as formulation/vehicle
Positive control:	Cyclophosphamide, 60 mg/kg PO

Study design

Target Dose Level [a] (mg/kg)	Stock Concentration as HM10460A (mg/mL)	Dosing Volume (mL/kg)	Route of Administration	Animals/Harvest Timepoint 24 Hour Male	Animals/Harvest Timepoint 48 Hour Male
Positive Control, 60	6	10	Oral Gavage	5	-
Vehicle Control, 0	0	10	Subcutaneous Injection	5	5
361.3/98.5	36.13	10	Subcutaneous Injection	5	-
722.7/196.9	72.27	10	Subcutaneous Injection	5	-
1445.2/393.8	144.523	10	Subcutaneous Injection	5	5

Vehicle Control = HM10460A vehicle, Positive Control = Cyclophosphamide
[a] The first dose level is as HM10460A, and the second dose level is as HM10411.

Study Validity

This study is considered valid based on the following.

- Dosing formulation was confirmed to be within 93% of target concentration.
- The high dose of 1445.2 mg/kg was adequate based on a dose ranging study.
- Animal number used for each group was adequate.

Results

HM10406A at subcutaneous doses up to 1445.2 mg/kg did not induce signs of clinical toxicity. HM10460A did not increase micronucleated PCEs at any test article dose examined (361.3, 722.7 and 1445.2 mg/kg). Additionally, there was no cytotoxicity to the bone marrow (no decreases in the PCE:NCE ratios) at any dose of the test article.

8 Carcinogenicity

Carcinogenicity studies were not conducted

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: Subcutaneous Injection Study of Fertility and Early Embryonic Development to Implantation with HM10460A in Rats

Study no.:	8302914
Study report location:	SDN1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	03/15/2016
GLP compliance:	Yes, a signed GLP compliance statement was included in the report
QA statement:	Yes
Drug, lot #, and % purity:	HM10460A, Batch 906615014, purity 98.5%

Key Study Findings

Subcutaneous administration of HM10460A at 0.183, 0.414 or 0.825 mg/kg/week did not affect fertility parameters (mating and fertility, and preimplantation loss). NOAEL for fertility was defined as 0.825 mg/kg/week. No toxicokinetic data were obtained in this study.

Methods

Doses:	0.183, 0.414, 0.825 mg/kg/dose
Frequency of dosing:	Once per week
Dose volume:	5 mL/kg
Route of administration:	Subcutaneous injection
Formulation/Vehicle:	(b) (4) mannitol, (b) (4) NaCl, (b) (4) Citric acid monohydrate, (b) (4) polysorbate 80 in sterile water
Species/Strain:	Sprague-Dawley rats
Number/Sex/Group:	22/sex/dose
Satellite groups:	none
Study design:	Males were dosed for at least 10 weeks - weekly for four weeks prior to mating, on the day of mating phase started, and weekly throughout the mating and post-pairing phase. Females were dosed twice during the premating phase, on the first day of mating, weekly throughout the mating phase (until confirmation) and on GD0 and 7.
Deviation from study protocol:	No deviations that affect overall interpretation of study findings or compromise the integrity of the study occurred.

Observations and Results

Mortality
Moribund sacrifices occurred in mid dose (4 males) and high dose groups (1 male, 2 females) due to swollen hind limbs, with or without limited use. The sacrifice days were in a range of premating day 21 to post-paring day 26.

Clinical Signs
Swollen hind limbs, with or without limited use were observed in the mid and high dose animals. Other than unscheduled sacrifice animals, all other affected animals recovered within a few days.

Body Weight
Slight decreases in body weight gains and lower body weights (up to 4% lower than control) were noted in mid and high dose males during the first two weeks of treatment. By the end of the study, the overall body weight gain and body weight were comparable across all groups.

Food Consumption
A transient and slight decrease in food consumption was noted in mid and high dose males in the premating phase.

Hematology
Dose related increases in WBC count and neutrophil count were observed in males and females, at postpairing Day 15 and gestation Day 8, respectively. See Table 21

Table 21. WBC and neutrophil counts, rat FED study

Dose, mg/kg		0	0.183	0.414	0.825
WBC,	M	11.8	19.5	33.8	41.1
E3/uL	F	10.3	16.5	27.9	36.7
Neutrophil,	M	2	8.1	22.3	28.8
E3/uL	F	1.7	8	18.7	25.7

Toxicokinetics
No toxicokinetic profile could be obtained as that HM10460A was only detected in a limited number of samples.

Dosing Solution Analysis
Most sample concentrations were within ±10% of target concentrations except Day 1 samples for 0.0366 mg/mL (LD formulation) and 0.828 mg/mL (mid dose formulation) that were 80.5% and 84.3% of target concentrations, respectively.

Necropsy
Splenic enlargement was noted in mid and high dose animals.

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)

Estrous cyclicity, male and female mating, fecundity, or fertility indices were not affected by treatment (Table 22).

Table 22. Fertility Parameters, rat FED study

dose/mg/kg/week		0	0.183	0.414	0.825
Mating index, %	male	95	95	86	95
	female	95	95	100	100
Fertility index %	male	95	91	81	86
	female	95	91	95	90
N of female pregnant at Cesarean section		21	20	18	18
mean corpora lutea		16.2	16.1	16.2	15.8
implantation sites		15.7	15.9	15.9	14.9
preimplantation loss		0.5	0.2	0.3	0.8
preimplantation loss, %		3	1.1	1.9	6

9.2 Embryonic Fetal Development

9.2.1 Rat Study

Study title: Subcutaneous Injection Study for Effects on Embryo-fetal Development and Toxicokinetic with HM10460A in Rats

Study no.:	8222-311
Study report location:	SDN1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	06/23/2010
GLP compliance:	Yes, a signed GLP compliance statement was included in the report
QA statement:	Yes
Drug, lot #, and % purity:	HM10460A, batch No. PGC0903, purity 98.8%

Key Study Findings

Subcutaneous administration of HM10460A to pregnant rats at nominal doses of 0.183, 0.414 and 0.825 mg/kg/2 days resulted in increased incidences of splenic enlargement in the dams (mid dose and high dose), but no remarkable treatment-related effects on pregnancy parameters or fetal development were noted. The test article in the fetal plasma was detectable (up to 7% of dam plasma concentration). NOAEL for fetal development was the high dose of 0.825 mg/kg/2 days ($AUC_{0\text{-}48h}$ GD6, 112 ug.h/mL; GD16, 22 ug.h/mL).

Methods

Doses:	0.183, 0.414, 0.825 mg/kg/dose (actual doses were 0.131-0.148, 0.296-0.340, 0.632-0.690 mg/kg/dose)
Frequency of dosing:	Every 2 days from gestation days 6 to 16
Dose volume:	5 mL/kg (split into two injections, each 2.5 mL/kg)
Route of administration:	Subcutaneous
Formulation/Vehicle:	Citric acid monohydrate (b) (4) sodium chloride (b) (4) polysorbate 80 (b) (4) mannitol (b) (4) prepared in sterile water for injection.
Species/Strain:	Sprague Dawley rats
Number/Sex/Group:	25 mated females/dose
Satellite groups:	9 mated females/dose (3 for control) were used in TK study
Study design:	Mated females were dosed by subcutaneous injection on GD6, 8, 10, 12, 14 and 16. On GD 21, animals were sacrificed, and laparohysterectomies were performed.

GROUP DESIGNATION AND DOSE LEVELS

Group[a]	No. of Animals Mated Female	Dose Level as HM10411[b] (mg/kg/dose)	Dose Concentration as HM10411[c] (mg/mL)	Dose Level as HM10460A[b] (mg/kg/dose)	Dose Concentration as HM10460A[c] (mg/mL)
Main Study Animals					
1 (Control)	25	0	0	0	0
2 (Low)	25	0.050	0.010	0.183	0.0366
3 (Mid)	25	0.113	0.0226	0.414	0.0828
4 (High)	25	0.225	0.045	0.825	0.165
Toxicokinetic Animals					
5 (Control)	3	0	0	0	0
6 (Low)	9	0.050	0.010	0.183	0.0366
7 (Mid)	9	0.113	0.0226	0.414	0.0828
8 (High)	9	0.225	0.045	0.825	0.165

a Groups 1 and 5 will receive vehicle control article only.
b The animals will be dosed on Gestation Days 6, 8, 10, 12, 14, and 16.
c Dose volume is 5 mL/kg.

Deviation from study protocol:	No deviations affecting the overall interpretation or compromising the integrity of the study occurred.

Observations and Results

Mortality

No unscheduled deaths occurred

Clinical Signs

No remarkable findings

Body Weight

No treatment related findings

Food Consumption

Reduced food consumption (-8.4%) was noted in high dose dams on GD12 to 14 only, which did not affect maternal body weight. Overall food consumption of this group throughout the dosing phase (GD6-GD18) was similar to control.

Toxicokinetics

Note: actual doses for all treatment groups were lower than nominal doses (see dosing solution analysis).

TK data were collected on GD6 and GD16 post-dose. Systemic exposure (Cmax and AUC) to HM10460A was increased more than dose proportionally. The exposure on GD16 was significantly lower than on GD6, which could be due to formation of anti-drug antibodies.

Table 23. Toxicokinetic parameter, rat EFD study

Group	Dose Level (mg/kg/dose) Nominal	Dose Level (mg/kg/dose) Actual	C_{max} (ng/mL)	DN C_{max} [(ng/mL)/ (mg/kg/dose)]	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	AUC_{0-24} (ng•hr/mL)	AUC_{0-48} (ng•hr/mL)	DN AUC_{0-48} [(ng•hr/mL)/ (mg/kg/dose)]
Gestation Day 6									
6	0.183	0.131	306	2338	24.0	7893	3929	7894	60260
7	0.414	0.296	984	3323	48.0	33450	10354	33450	113008
8	0.825	0.632	3703	5860	48.0	112115	30835	112115	177398
Gestation Day 16									
6	0.183	0.148	29.7	201	8.00	343	343	405	2740
7	0.414	0.340	124	366	12.0	1537	1266	1537	4520
8	0.825	0.696	958	1377	12.0	22150	15314	22150	31824

Table excerpted from applicant submission

Some fetal plasma samples collected at 48 hours postdose, from all treated groups, had detectable HM10460A. The mean concentrations at the mid dose and high dose groups were approximately 7% and 5%, respectively, of mean dam plasma concentrations.

Dosing Solution Analysis

Concentrations of HM10460A in dosing solution samples varied from 64.4 to 84.3% of the respective theoretical value; these results did not meet acceptance criteria of within 10% of the theoretical value. Based on the percent of target obtained on GD 6 and with backup samples on GD 16, female pregnant rats were dosed at the dose levels presented in the following table.

Table 24. Dosing solution analysis results, rat EFD study

		HM10460A in HM10460A Vehicle							
		0 mg/mL		0.0366 mg/mL[b]		0.0828 mg/mL[c]		0.165 mg/mL[d]	
Interval	Replicate	Actual	% of Target	Actual	% of Target	Actual	% of Target	Actual	% of Target
Gestation Day 6	1	DNS	-	0.0259	70.9	0.0563	67.9	0.131	79.4
	2	DNS	-	0.0266	72.7	0.0620	74.9	0.122	73.7
	Mean	-	-	-	71.8[a]	-	71.4[a]	-	76.6[a]
Gestation Day 16	1	DNS	-	0.0230	62.7	0.0536	64.7	0.112	67.7
	2	DNS	-	0.0247	67.4	0.0532	64.2	0.115	69.8
	Mean	-	-	-	65.1[a]	-	64.4[a]	-	68.8[a]
Gestation Day 16	1	-	-	0.0285	77.8	0.0690	83.3	0.138	83.9
	2	-	-	0.0306	73.6	0.0670	80.9	0.140	84.8
Back ups	Mean	-	-	-	80.7[a]	-	82.1[a]	-	84.3[a]

DNS = Detection Not Significant.
- = Not Applicable.
API (HM10411) (mg/mL) = [(mg/mL HM10460A) [redacted (b) (4)]
a Does not meet specifications; greater than 10% from theoretical.
b 0.0366 mg/mL HM10460A is equivalent to [redacted (b) (4)]
c 0.0828 mg/mL HM10460A is equivalent to
d 0.165 mg/mL HM10460A is equivalent to [redacted (b) (4)]

Table excerpted from applicant submission

Based on these results, the pregnant rats were dosed in the range of 0.131 to 0.696 mg/kg/dose HM10460A.

Table 25. Actual dose levels, rat EFD study

Group	Dose Level as HM10411 (mg/kg/dose)	Dose Level as HM10460A (mg/kg/dose)
	Gestation Day 6	
1 and 5 (Control)	0	0
2 and 6 (Low)	0.036	0.131
3 and 7 (Mid)	0.081	0.296
4 and 8 (High)	0.172	0.632
	Gestation Day 16	
1 and 5 (Control)	0	0
2 and 6 (Low)	0.040	0.148
3 and 7 (Mid)	0.093	0.340
4 and 8 (High)	0.190	0.696

Table excerpted from applicant submission

Immunogenicity

Antibodies against HM10460A were detected in all treatment groups at GD 20, with relatively lower frequency in the high dose group as compared to the low dose and the

mid dose groups. Among the antibody positive cases, approximately 10 to 30% were neutralizing antibodies. See Table 26.

Table 26. Incidences of antibody and neutralizing antibody, rat EFD study

group	LD	MD	HD
n examined	25	24	26
Ab +	20	20	10
titer of Ab+ animals	687	228.7	323.2
neutralizing Ab	8	7	1

LD, low dose; MD, mid dose; HD, high dose

Note: the highest antibody titer (>1000) was detected in one animal from each treatment group. For these animals, titer value of 1000 was included in the mean titer calculation.

Necropsy
Maternal spleen enlargement was observed in a dose-related manner (MD, 1; HD, 7). There were no remarkable changes in gravid uterine weight, corrected body weight or net change in body weight from GD4 noted when compared with control.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)
There were no treatment-related findings in the number of lutea corpora, implantation sites, pre- and post-implantation loss, and fetal viability.

Offspring (Malformations, Variations, etc.)
Fetal weight and sex ratio were unremarkable. There were no treatment-related external, visceral and skeletal variations and/or malformations.

9.2.2 Rabbit Study

Study title: Subcutaneous Injection Study for Effects on Embryo-fetal Development and Toxicokinetic with HM10460A in Rabbits

Study no.:	8222-307
Study report location:	SDN1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	07/23/2010
GLP compliance:	Yes, a signed GLP compliance statement was included in the report
QA statement:	Yes
Drug, lot #, and % purity:	HM10460A, batch No. PGC0903, purity 98.8%

Key Study Findings

Subcutaneous administration of HM10460A (nominal doses of 0.183, 0.367 and 0.734 mg/kg/2 days) to pregnant rabbits during organogenesis (GD7-GD19) resulted in increased post-implantation loss, mostly due to early resorptions, and associated reduction in the litter size and the number live fetuses. Five does had no viable fetuses. Additionally, decreased fetal weight (growth) was observed in the mid dose and the high dose groups. Fetal NOAEL was the low dose of 0.183 mg/kg ($AUC_{0\text{-}48h:}$ GD7, 28 ug.h/mL; GD19, 66.7 ng.h/mL). Maternal findings were limited decreases in neutrophil counts in all treatment groups. Maternal NOAEL was 0.734 mg/kg ($AUC_{0\text{-}48h}$ GD7, 152 ug.h/mL; GD19, 0.064 ng.h/mL).

Methods

Doses:	0.183, 0.367, 0.734 mg/kg/dose (actual doses of 0.131-0.162, 0.252-0.314, 0.526-0.650 mg/kg/dose)
Frequency of dosing:	Every 2 days from gestation days 7 to 19
Dose volume:	5 mL/kg (each dose was administered as two injections of 2.5 mL/kg)
Route of administration:	Subcutaneous
Formulation/Vehicle:	Citric acid monohydrate (b) (4) sodium chloride (b) (4) polysorbate 80 (b) (4) mannitol (b) (4) prepared in sterile water for injection
Species/Strain:	Hra: (NZW)SPF rabbits
Number/Sex/Group:	20 mated females/dose
Satellite groups:	3 mated females/dose were used in TK study
Study design:	Mated females were dosed by subcutaneous injection on Gestation Days 7, 9, 11, 13, 15, 17 and 19. C-section was performed on GD 19.

GROUP DESIGNATION AND DOSE LEVELS

Group[a]	No. of Animals Mated Female	Dose Level as HM10411[b] (mg/kg/dose)	Dose Concentration as HM10411[c] (mg/mL)	Dose Level as HM10460A[b] (mg/kg/dose)	Dose Concentration as HM10460A[c] (mg/mL)
Main Study Animals					
1 (Control)	20	0	0	0	0
2 (Low)	20	0.050	0.010	0.183	0.0366
3 (Mid)	20	0.100	0.020	0.367	0.0734
4 (High)	20	0.200	0.040	0.734	0.1468
Toxicokinetic Animals					
5 (Control)	3	0	0	0	0
6 (Low)	3	0.050	0.010	0.183	0.0366
7 (Mid)	3	0.100	0.020	0.367	0.0734
8 (High)	3	0.200	0.040	0.734	0.1468

a Groups 1 and 5 will receive vehicle control article only.
b The animals will be dosed on Gestation Days 7, 9, 11, 13, 15, 17, and 19.
c Dose volume is 5 mL/kg.

Deviation from study protocol: No deviations affecting the overall interpretation or compromising the integrity of the study occurred.

Observations and Results

Mortality

One doe from the mid dose group was sacrificed on GD18 due to moribund condition. Prior to the sacrifice, soft feces, reduced food consumption and body weight gain decrease of 13% from GD11-18 was noted in this animal. However, no remarkable findings were observed at necropsy. With the low incidence and absence of dose-relationship, it is unclear if mortality is treatment-related.

One doe from low dose group was removed from study on GD18 due to mistimed pregnancy, as evidenced by 9 live kits found in the cage. There were no evidence indicating treatment effect on the early delivery based on clinical signs, body weight, food consumption or clinical pathology examinations.

One doe from high dose group was also noted having delivered early; however, this doe and litter were processed as scheduled since the delivery occurred on the morning of the scheduled cesarean section.

Clinical Signs

No remarkable findings

Body Weight

Decreased maternal body weight gain was noted in the high dose group during the dosing phase (-53.4%, GD7-21) without a reduction in food consumption. After dosing cessation, the body weight gain in high dose group rebounded (GD21-GD29). By the end of study, mean maternal body weight in the high dose group was 4.8% lower than control. See Figure 5.

Figure 5. Mean maternal body weights during gestation, rabbit EFD study

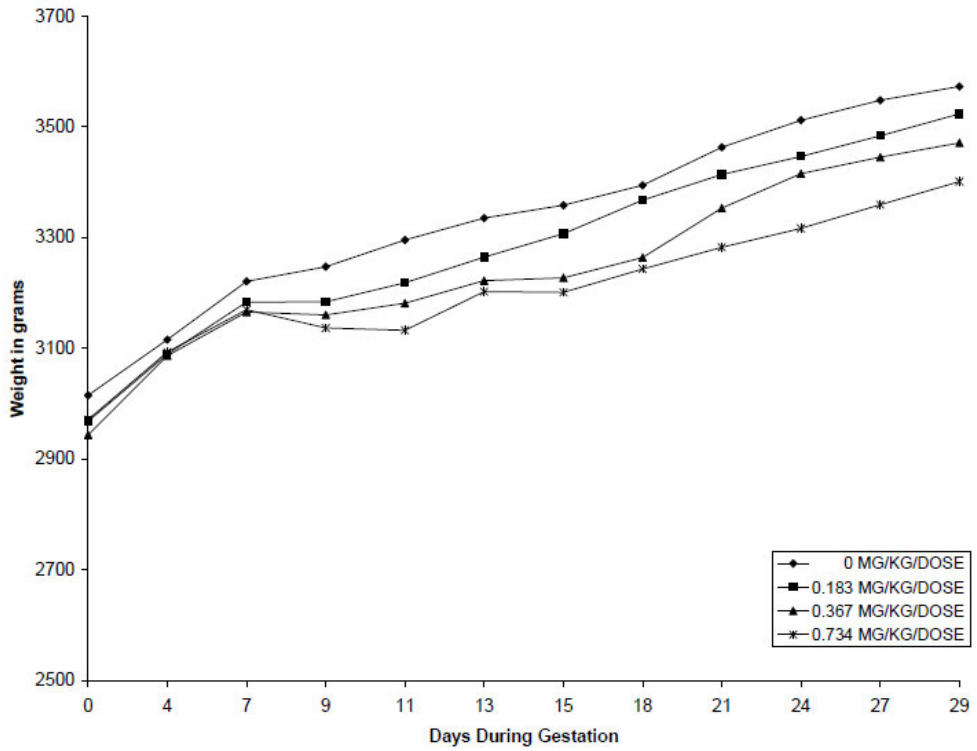


Figure excerpted from applicant submission

Food Consumption

No remarkable changes during treatment were noted. After dosing cessation, increased food consumption was observed in the high dose group.

Hematology

Hematology changes included decreases in red blood cells (up to -76.2%), white blood cells (up to -25.2%), and neutrophils (up to -76.5%); increases in reticulocyte count (up to +76.2%) and platelets (up to +32.9%). Most of these changes were observed at the mid dose and high dose, except neutrophil findings that were also observed in low dose animals.

Table 27. Hematology findings on GD29, rabbit EFD study

dose, mg/kg/2 days	0	0.183	0.367	0.734
RBC, E6/uL	5.98	5.68	5.67	5.41
Reticulocyte, E3/uL	83.1	91.4	83.7	146.1
platelet. E3/uL	377	399	501	481
WBC, E3/uL	6.02	5.2	4.5	4.93
Neutrophil, E3/uL	1.32	0.71	0.36	0.31

Toxicokinetics

Note: actual doses for all treatment groups were lower than nominal doses (see dosing solution analysis).

TK data were collected on GD7 and GD19 post-dose. Systemic exposures (Cmax and AUC) to HM10460A increased more than dose proportionally after the first dose (GD7), but less than dose proportionally after multiple doses (GD19). The exposure on GD19 was significantly lower than on GD7, which could be due to formation of anti-drug antibodies.

Table 28. Toxicokinetic parameter, rabbit EFD study

Group	Nominal Dose Level (mg/kg/dose)	Actual Dose Level (mg/kg/dose)		C_{max} (ng/mL)	DN C_{max} [(ng/mL)/ (mg/kg/dose)]	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	AUC_{0-24} (ng•hr/mL)	AUC_{0-48} (ng•hr/mL)	DN AUC_{0-48} [(ng•hr/mL)/ (mg/kg/dose)]
					Gestation Day 7					
6	0.183	0.162	Mean	979	6040	24.0	28104	12654	28104	156415
			SD	NA	NA	NA	NA	NA	NA	NA
			N	2	2	2	2	2	2	2
7	0.367	0.314	Mean	2197	6996	32.0	87923	37203	87923	280011
			SD	78	247	13.9	4486	3559	4486	14288
			N	3	3	3	3	3	3	3
8	0.734	0.650	Mean	4385	6746	36.0	152066	52226	152066	233948
			SD	NA	NA	NA	NA	NA	NA	NA
			N	2	2	2	2	2	2	2
					Gestation Day 19					
6	0.183	0.131	Mean	1.52	11.6	36.0	66.7	30.7	66.7	509
			SD	NA	NA	NA	NA	NA	NA	NA
			N	2	2	2	2	2	2	2
7	0.367	0.252	Mean	1.07	4.25	36.0	43.4	19.9	43.4	172
			SD	0.34	1.34	20.8	15.7	7.7	15.7	62
			N	3	3	3	3	3	3	3
8	0.734	0.526	Mean	1.97	3.74	14.0	63.9	30.1	63.9	122
			SD	NA	NA	NA	NA	NA	NA	NA
			N	2	2	2	2	2	2	2

NA Not applicable.

Table excerpted from applicant submission

Fetal plasma samples collected on GD 21 were below the lower limit of quantitation (625 pg/mL) in all treated groups.

Dosing Solution Analysis

The drug concentrations in the dosing solution samples were in a range of 56.4-88.6% of the respective theoretical values (Table 29); Based on the percent of target obtained with backup samples, female rabbits were dosed at 0.131-0.650 mg/kg/dose (Table 30).

Table 29. Dosing solution analysis results, rabbit EFD study

		HM10460A in HM10460A Vehicle							
		0 mg/mL		0.0366 mg/mL[a]		0.0734 mg/mL[b]		0.1468 mg/mL[c]	
Interval	Replicate	Actual	% of Target	Actual	% of Target	Actual	% of Target	Actual	% of Target
Gestation	1	DNS	-	0.0262	71.5	0.0525	71.5	0.110	74.9
Day 7	2	DNS	-	0.0259	70.8	0.0525	71.6	0.110	74.9
	Mean	-	-	-	71.1[d]	-	71.6[d]	-	74.9[d]
Gestation	1	-	-	0.0325	88.7	0.0651	88.7	0.129	87.9
Day 7	2	-	-	0.0322	88.1	0.0604	82.3	0.131	89.4
Back ups	Mean	-	-	-	88.4[d]	-	85.5[d]	-	88.6[d]
Gestation	1	DNS	-	0.0210	57.5	0.0401	54.6	0.0855	58.3
Day 19	2	DNS	-	0.0223	60.9	0.0426	58.1	0.0864	58.9
	Mean	-	-	-	59.2[d]	-	56.4[d]	-	58.6[d]
Gestation	1	-	-	0.0251	68.5	0.0498	67.9	0.105	71.7
Day 19	2	-	-	0.0274	74.9	0.0510	69.4	0.105	71.6
Back ups	Mean	-	-	-	71.7[d]	-	68.7[d]	-	71.6[d]

DNS = Detection not significant.
- = Not applicable.
API (HM10411) (mg/mL) = [(mg/mL HM10460A) (b) (4)
a 0.0366 mg/mL HM10460A is equivalent to (b) (4)
b 0.0734 mg/mL HM10460A is equivalent to
c 0.1468 mg/mL HM10460A is equivalent to
d Does not meet specifications; greater than 10.0% from theoretical.

Table excerpted from applicant submission

Table 30. Actual dose levels, rabbit EFD study

Group	Dose Level as HM10411 (mg/kg/dose)	Dose Level as HM10460A (mg/kg/dose)
	Gestation Day 7	
1 and 5 (Control)	0	0
2 and 6 (Low)	0.044	0.162
3 and 7 (Mid)	0.086	0.314
4 and 8 (High)	0.177	0.650
	Gestation Day 19	
1 and 5 (Control)	0	0
2 and 6 (Low)	0.036	0.131
3 and 7 (Mid)	0.069	0.252
4 and 8 (High)	0.143	0.526

Table excerpted from applicant submission

Immunogenicity

Blood samples were collected prior to dosing on GD7 and GD29. Anti-drug antibodies (ADA) were detected in all treated rabbits at GD29, but not on GD7, as expected. High titer detection limit was 62500. The mean titer values, calculated by this reviewer, are presented in Table 31. With a significant number of animals (LD, 10; MD, 13; HD, 14) with titer >62500, the mean titer values are likely underestimated.

Table 31. ADA incidence and mean titer, rabbit EFD study

group	LD	MD	HD
n examined	19	19	20
Ab +	19	19	20
mean titer	35973	44605	45500

LD, low dose; MD, mid dose; HD, high dose

Analysis for neutralizing activity of anti-drug antibodies was not performed.

Necropsy

Gravid uterine weight was decreased in the high dose group (-66.1%) due to an increase in the number of does with no viable fetuses (5 does had litters with no viable fetuses).

Table 32. Uterine and net body weight (g), rabbit EFD study

DOSE LEVEL		GROUP 1 0 MG/KG/DOSE	GROUP 2 0.183 MG/KG/DOSE	GROUP 3 0.367 MG/KG/DOSE	GROUP 4 0.734 MG/KG/DOSE
GRAVID UTERUS	MEAN	486.75	441.98	439.90	164.80**
	S.D.	122.49	110.59	94.09	137.67
	N	13	17	16	15
CORRECTED WEIGHT	MEAN	3086.17	3081.43	3031.35	3236.46
	S.D.	316.75	282.04	182.07	243.22
	N	13	17	16	15
NET CHANGE FROM DAY 4	MEAN	-29.75	-8.10	-62.02	136.73*
	S.D.	221.33	159.06	151.31	190.71
	N	13	17	16	15

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.

CORRECTED WEIGHT = TERMINAL BODY WEIGHT MINUS GRAVID UTERINE WEIGHT
NET WEIGHT CHANGE FROM DAY 4 = CORRECTED WEIGHT MINUS DAY 4 BODY WEIGHT

Table excerpted from applicant submission

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)
Corpora lutea, implantation sites and preimplantation loss in all treatment groups were comparable to controls. The mid dose and high dose groups showed increases in post-implantation loss (10-73.7% vs 3.8% control) and decreases in number of live fetuses in a dose related manner. When litters with no viable fetuses were excluded from calculations, a dose-dependent increase in post-implantation loss was still noted in the mid dose and high dose groups. The post-implantation loss was largely presented as early resorption. See Tables 33 and 34.

Table 33. Corpora lutea and implantation data, Rabbit EFD study

DOSE LEVEL		GROUP 1 0 MG/KG/DOSE	GROUP 2 0.183 MG/KG/DOSE	GROUP 3 0.367 MG/KG/DOSE	GROUP 4 0.734 MG/KG/DOSE
Females Mated	N	20	20	20	20
Pregnant	N	13	18	17	16
	%	65	90	85	80
Aborted	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Died	N	0	0	1	0
	%	0.0	0.0	5.0	0.0
Delivered Early	N	0	0	0	1
	%	0.0	0.0	0.0	5.0
Pregnant at C-section	N	13	17	16	16
Dams with Viable Fetuses	N	13	17	16	11
	%	100	100	100	69
Dams with no Viable Fetuses	N	0	0	0	5
	%	0.0	0.0	0.0	31
Corpora Lutea	MEAN	10.2	9.2	9.8	9.1
	S.D.	2.4	1.8	1.2	1.6
	N	12	16	16	16
	TOTAL	123	147	157	146
Implantation Sites	MEAN	9.2	8.2	9.1	8.9
	S.D.	2.9	2.6	1.3	1.7
	N	13	17	16	16
	TOTAL	120	139	146	142
Preimplantation Loss	MEAN%	8.1	13.9	7.0	2.8
	S.D.	18.1	19.6	8.4	6.8

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.

Table excerpted from applicant submission

Table 34. Post-implantation data, Rabbit EFD study

Pregnant at C-section	N	13	17	16	16
Resorptions: Total	MEAN	0.3	0.2	1.0	6.4
	S.D.	0.6	0.4	1.8	2.7
	N	13	17	16	16
	TOTAL	4	3	16	103
	MEAN%	3.5	3.1	10.0	73.7**
	S.D.	8.2	8.4	16.1	29.0
Early	MEAN	0.2	0.2	0.8	5.9
	S.D.	0.4	0.4	1.8	2.9
	N	13	17	16	16
	TOTAL	2	3	12	95
	MEAN%	1.7	3.1	7.4	67.3**
	S.D.	4.4	8.4	16.3	30.5
Late	MEAN	0.2	0.0	0.2	0.5
	S.D.	0.4	0.0	0.6	0.8
	N	13	17	16	16
	TOTAL	2	0	4	8
	MEAN%	1.7	0.0	2.6	6.4
	S.D.	4.4	0.0	5.9	10.5
Dead Fetuses	TOTAL	0	0	0	0
Postimplantation Loss	MEAN%	3.8	2.8	10.0	73.7**
	S.D.	8.4	8.6	16.1	29.0

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.

Table excerpted from applicant submission

Offspring (Malformations, Variations, etc.)

Covariate-adjusted (litter size adjusted) mean fetal weights were decreased 10.4% and 32.2% in the mid dose and high dose groups, respectively. Table 35.

There was no treatment-related fetal malformations or variations.

Table 35. Mean fetal weight, rabbit EFD study

DOSE LEVEL		GROUP 1 0 MG/KG/DOSE	GROUP 2 0.183 MG/KG/DOSE	GROUP 3 0.367 MG/KG/DOSE	GROUP 4 0.734 MG/KG/DOSE
FETAL WEIGHTS UNITS: GRAMS					
of all Viable Fetuses	MEAN	39.51	39.68	35.99	31.15
	S.D.	6.40	4.64	4.19	4.55
	N	13	17	16	11
Covariate Adjusted	MEAN	40.89	40.23	36.65	27.71**
of Male Fetuses	MEAN	40.53	40.21	36.49	32.42
	S.D.	6.53	5.10	4.86	3.63
	N	13	16	15	10
Covariate Adjusted	MEAN	42.02	40.84	37.20*	28.42**
of Female Fetuses	MEAN	38.70	39.14	35.93	30.82
	S.D.	7.09	5.03	5.57	5.75
	N	13	17	16	7
Covariate Adjusted	MEAN	39.79	39.32	36.23	27.64**

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.

Table excerpted from applicant submission

9.3 Prenatal and Postnatal Development

Study title: HM10460A: Pre/Post-natal Development and Maternal Function Study by Subcutaneous Administration in Sprague-Dawley Rats

Study no.:	D117129
Study report location:	SDN1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	10/25/2017
GLP compliance:	Yes, a signed GLP compliance statement was included in the report
QA statement:	Yes
Drug, lot #, and % purity:	HM10460A, batch 906615014, 98.5%

Key Study Findings

Administration of HM10460A to female rats during pregnancy (GD6 to GD20) and lactation (LDs 4 to 18) at doses of 0.183, 0.414 and 0.825 mg/kg/week did not affect maternal function or pre/postnatal development of F1 animals. NOAEL was 0.825 mg/kg/week (no toxicokinetic data were obtained) for both maternal function and pre- and post-natal development.

Methods

Doses:	0.183, 0.414, 0.825 mg/kg
Frequency of dosing:	Weekly (GD6, 13, 20 and LD4, 11, 18)
Dose volume:	5 mL/kg (split into 2 injections, each at 2.5 mL/kg)
Route of administration:	Subcutaneous
Formulation/Vehicle:	Citric acid monohydrate (b) (4) sodium chloride (b) (4) polysorbate 80 (b) (4) mannitol (b) (4) prepared in sterile water for injection
Species/Strain:	Sprague-Dawley rats
Number/Sex/Group:	25 females/group
Satellite groups:	None
Study design:	

Group	Animal ID			Dose Volume[a] (mL/kg)	Dose[b] (mg/kg)
	F0 Females	F1 Animals Male	F1 Animals Female		
VC	1-25	1M001-1M025	1F001-1F025	5	0
T1	26-50	2M026-1M050	2F026-2F050	5	0.183
T2	51-75	3M051-3M075	3F051-3F075	5	0.414
T3	76-100	4M076-4M100	4F076-4F100	5	0.825

[a] The vehicle control and formulated test item were subcutaneously administered as two injections of 2.5 mL/kg.
[b] The vehicle control and formulated test item were subcutaneously administered to GD 6, 13, 20 and LD 4, 11, 18.

Deviation from study protocol:	No deviations affecting results interpretation or study integrity occurred

Observations and Results

F_0 Dams

Survival:	All animals survived to terminal sacrifice.
Clinical signs:	Swollen hindlimbs and limping were observed in one mid dose female.
Body weight:	No treatment-related findings
Food consumption:	No treatment-related findings
Uterine content:	No remarkable findings
Necropsy observation:	Enlarged spleen was observed in treated females (LD, 1; MD, 1; HD, 4).
Hematology:	Increases in total white blood cells (2.1x), absolute neutrophil (7.0x) and basophil counts (2.7x) were observed in the mid dose group at postnatal Day 21. The findings in the high dose animals were less severe or absent.
Toxicokinetics:	Not obtained
Dosing Solution Analysis	Samples of dosing formulation were 92.9-96.3% of target concentration.

F_1 Generation

Survival:	No treatment-related findings in viability index, weaning index or delivery index
Clinical signs:	No remarkable findings
Body weight during lactation:	No treatment-related findings
Body weight after weaning:	No treatment-related findings
Physical development:	No treatment-related findings were noted. Pinna detachment, fur development, incisor eruption and eye-lid opening were not affected by treatment.
Neurological assessment:	No treatment-related findings in behavioral function or learning and memory were observed.
Reproduction:	No treatment-related findings in sexual maturation (vaginal opening, preputial separation), precoital time, fertility or Caesarean section examination were observed
Other:	Hematology and necropsy at LD21 revealed no remarkable findings.

F_2 Generation: Not assessed

10 Special Toxicology Studies

Toxicology studies on human IgG4 FC fragment (HMC001)

Single-Dose HMC001 study in rats

In this dose ranging study, rats (main study, 5/sex/dose; TK study, 9/sex/dose) were given a single dose of HMC001 (human IgG4 FC fragment) at 0, 3, 9 or 30 mg/kg subcutaneously and sacrificed on Day 15. Assessment of toxicity based on a standard battery of observations without macroscopic examinations revealed no remarkable findings. Exposure to HMC001 was generally dose proportional without sex related differences. NOEL was 30 mg/kg (Cmax of 90.8 ug/mL; $AUC_{0\text{-}inf}$ of 5272.0 ug.h/mL).

Single-Dose HMC001 study in monkeys

In this dose ranging study, monkeys (macaca fascicularis, 2/sex/dose) were given a single dose of HMC001 at 0, 3, 9, or 30 mg/kg subcutaneously and sacrificed on Day 15. Toxicity evaluation on a standard battery of observations and examinations including ECG and histopathology revealed no remarkable findings. The NOEL was 30 mg/kg (toxicokinetic data were not obtained).

4-Week HMC001 study in rats

Study title: 4-Week Subcutaneous Injection Toxicity and Toxicokinetic Study with HMC001 in Rats with a 4-Week Recovery Period

Study no.:	7805-130
Study report location:	SDN 1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	01/8/2008
GLP compliance:	Yes, a signed GLP compliance statement is included in the report
QA statement:	Yes
Drug, lot #, and % purity:	B11030-PGL011, purity 99.3%

Key Study Findings

Rats given HMC001 for four weeks at 0.3, 1.5 or 6 mg/kg twice weekly by subcutaneous injection showed no remarkable toxicity findings. NOAEL was 6 mg/kg ($AUC_{0\text{-}72h}$: Day 1, 938 ug.h/mL; Week 4, 1563 ug.h/mL).

Methods

Doses:	0, 0.3, 1.5, 6 mg/kg
Frequency of dosing:	Twice weekly
Route of administration:	Subcutaneously
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.002M sodium phosphate monobasic dihydrate, 0.02M sodium phosphate dibasic anhydrate; 0.1 M sodium chloride in distilled water
Species/Strain:	Sprague-Dawley rats
Number/Sex/Group:	15/sex for control and high dose; 10/sex for low dose and mid dose groups
Age:	7 weeks age
Weight:	Males, 190-234 g; females, 161-202 g
Satellite groups:	Toxicokinetic animals (6/sex/dose) were included in the study
Unique study design:	

Group[a]	No. of Animals[b] Male	Female	Dose Level[c] (mg/kg/dose)	Dose Concentration (mg/mL/dose)
Toxicity Animals				
1 (Control)	15	15	0	0
2 (Low)	10	10	0.3	0.06
3 (Mid)	10	10	1.5	0.3
4 (High)	15	15	6	1.2
Toxicokinetic Animals				
5 (Control)	6	6	0	0
6 (Low)	6	6	0.3	0.06
7 (Mid)	6	6	1.5	0.3
8 (High)	6	6	6	1.2

a Group 1 and 5 received control article only.
b Toxicity animals designated for recovery sacrifice (the first five/sex in Groups 1 and 4) were sacrificed after a 4 week-recovery phase following dose administration for at least 4 weeks.
c Groups 1-8 were dosed twice weekly for 4 weeks.

Deviation from study protocol:	No deviations affecting the overall interpretation of study findings or the integrity of the study occurred.

Observations and Results

Mortality

None

Clinical Signs

No remarkable findings

Body Weights

No treatment-related findings

Food Consumption

During treatment phase, food consumption reduction was noted in all male treatment groups, more prominently in the mid dose group (up to -17%), with no related body weight changes.

Ophthalmoscopy
No remarkable findings

ECG
Not performed

Clinical Pathology
No remarkable findings

Urinalysis
Not performed

Gross Pathology
No treatment-related findings

Organ Weights
No remarkable findings

Histopathology
Adequate Battery Yes. A complete battery of tissues was examined for the control and high dose groups. Macroscopic lesions and last injection sites were also examined for animals in the low dose and mid dose groups.

Peer Review
Not performed

Histological Findings
No treatment-related findings were noted.
Findings of testicular degeneration and accumulation of cellular debris within ductal lumen and decreased sperm in the epididymis in one high dose rat at the end of treatment, and unilateral testicular atrophy (seminiferous tubules lined by only vacuolated Sertoli cells and slight degeneration of germinal epithelium) in one high dose rat at end of recovery phase were considered incidental due to low incidence and/or unilateral nature.

Toxicokinetics
Systemic exposures (Cmax and AUC) were dose proportional. Over the 4 weeks of treatment, exposure accumulation was observed in the high dose group only (1.3-2 fold). There were no gender-related differences.

Table 36. TK parameters for HMC001 in rat serum

Group	Dose Level (mg/kg/dose)	Sex	C_{max} (ng/mL)	DN C_{max} [(ng/mL)/ (mg/kg/dose)]	T_{max} (hr)	AUC_{0-t} (ng·hr/mL)	DN AUC_{0-t} [(ng·hr/mL)/ (mg/kg/dose)]	AUC_{0-72} (ng·hr/mL)
					Day 1			
6	0.3	M	1140	3801	24.0	46644	155479	46644
		F	925	3082	24.0	40347	134490	40347
7	1.5	M	5565	3710	24.0	247450	164967	247450
		F	4775	3184	24.0	226713	151142	226713
8	6	M	18345	3058	24.0	877531	146255	877531
		F	22611	3769	24.0	998844	166474	998844
					Week 4			
6	0.3	M	1137	3791	24.0	118238	394126	58738
		F	904	3014	24.0	62801	209335	45521
7	1.5	M	4212	2808	24.0	238362	158908	181569
		F	6430	4286	24.0	298004	198670	213144
8	6	M	39184	6531	24.0	2399210	399868	1760430
		F	34092	5682	24.0	1746834	291139	1335190

Table excerpted from applicant submission

Dosing Solution Analysis

Dosing solutions were within 6% of nominal concentrations and therefore acceptable.

Antibody and Neutralizing Antibody Analysis

Blood samples collected at predosing on Days 1, 8, 22 during treatment phase and at end of recovery phase (Day 41) were generally negative for anti-drug antibodies. Antibodies were primarily seen at Week 4 collection interval at the dilution of 10. Table 37.

Table 37. Incidence of anti-HMC001 antibodies in rat serum

Group/ Sex	Interval	Negative Titer (No. of Animals)	Positive Titer (No. of Animals)
1M	DP Day 1	15	0
	DP Week 2	15	0
	DP Week 4	15	0
	RP Week 2	5	0
1F	DP Day 1	15	0
	DP Week 2	15	0
	DP Week 4	15	0
	RP Week 2	5	0
2M	DP Day 1	10	0
	DP Week 2[a]	10	0
	DP Week 4	4	6
2F	DP Day 1	10	0
	DP Week 2	9	1
	DP Week 4	1	9
3M	DP Day 1	10	0
	DP Week 2	10	0
	DP Week 4[a]	8	2
3F	DP Day 1[a]	10	0
	DP Week 2	10	0
	DP Week 4[a]	7	3
4M	DP Day 1[a]	15	0
	DP Week 2	15	0
	DP Week 4	15	0
	RP Week 2	2	3
4F	DP Day 1	15	0
	DP Week 2	15	0
	DP Week 4	14	1
	RP Week 2	3	2

a = Unconfirmed results are not counted as positive.

Table excerpted from applicant submission

4-Week HMC001 study in monkeys

Study title: 4-Week Subcutaneous Injection Toxicity and Toxicokinetic Study with HMC001 in Cynomolgus Monkeys with a 4-Week Recovery Period

Study no.:	7805-131
Study report location:	SDN 1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	0/14/2008
GLP compliance:	Yes, a signed GLP compliance statement is included in the report
QA statement:	Yes
Drug, lot #, and % purity:	B11090-PHA251, purity 98.4%

Key Study Findings

Monkeys given HMC001 by subcutaneous injection at 0.3, 1.5 or 6 mg/kg twice weekly showed no significant toxicity findings other than reversible mammary gland ductal hyperplasia and periductal fibroplasia/edema in males. NOAEL was 6 mg/kg (no toxicokinetic date obtained).

Methods

Doses:	0, 0.3, 1.5, 6 mg/kg
Frequency of dosing:	Twice weekly
Route of administration:	Subcutaneously
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.002M sodium phosphate monobasic dihydrate, 0.02M sodium phosphate dibasic anhydrate; 0.1 M sodium chloride in distilled water
Species/Strain:	Sprague-Dawley rats
Number/Sex/Group:	5/sex for control and high dose; 3/sex for low dose and mid dose groups
Age:	2 years age
Weight:	2-5 kg
Satellite groups:	None
Unique study design:	Main study animals were sacrificed on Day 33 (3/sex/dose), and recovery animals (2/sex for control and high dose) were sacrificed on Day 29 of recovery phase.

Group[a]	No. of Animals[b] Male	Female	Dose Level (mg/kg/day)	Dose Concentration (mg/mL)
1 (Control)	5	5	0	0
2 (Low)	3	3	0.3	0.06
3 (Mid)	3	3	1.5	0.3
4 (High)	5	5	6.0	1.2

a Group 1 will receive control article only.
b Animals designated for recovery sacrifice (2 animals/sex in Groups 1 and 4) will undergo 4 weeks of recovery following dose administration.

Deviation from study protocol:	No deviations affecting the overall interpretation of study findings or the integrity of the study occurred.

Observations and Results
Mortality
None

Clinical Signs
No remarkable findings

Body Weights
No treatment-related findings

Food Consumption
Not reported

Ophthalmoscopy
No remarkable findings

ECG
ECGs (leads I, II, aVF, CV_5RL and CV_6LL) were conducted at prestudy and week 4 intervals. One high dose male (6.0 mg/kg/day) has sinus tachycardia, likely attributable to the effect of anesthesia.

Clinical Pathology
No remarkable findings

Urinalysis
Not performed

Gross Pathology
No treatment-related findings

Organ Weights
Decreased in mandibular salivary gland weight was noted in all female treated groups and in high dose males at the end of treatment but not after 4 weeks of recovery period (Table 38). There were no histopathologic correlated findings. Treatment relationship is unclear.

Table 38. Salivary gland weight (g), 4-w monkey study with HMC001

dose, mg/kg	0	0.3	1.5	6
male	2.127	2.617	2.346	1.715
female	2.369	1.555	1.795	1.615

Histopathology
Adequate Battery Yes. A complete battery of tissues was examined for the control and high dose groups. Macroscopic lesions and last injection site were also examined for animals in the low dose and mid dose groups.

Peer Review
Not performed

Histological Findings
Mammary gland ductal hyperplasia and periductal fibroplasia/edema were present in males (2 at low dose, minimal to slight severity; 3 at high dose, slight to moderate severity). This finding was likely treatment-related as all males on this study were prepubertal. At the end of the recovery phase, this finding was not present in the control or in high dose males, and therefore, had resolved.

The hyperplasia of the mammary glands occurred in all treated female groups during the dosing phase. The change was consistent with normal mammary development, characterized by proliferation (slight to moderate severity) of small acinar/lobule structures with little or no lumen secretory activity. This change was also present in the recovery phase in one female (slight severity) given 6.0 mg/kg/day and in one control female (minimal severity). Table 39.

Table 39. incidence of histopathology findings at end of treatment, 4-week monkey study with HMC001

Controls from group(s): 1	Animal sex:	-- Animals -- Males --				Affected -- Females --			
Tissues With Diagnoses	Dosage group:	Ctls	2	3	4	Ctls	2	3	4
	No. in group:	3	3	3	3	3	3	3	3
Mammary, Female	Number examined:					3	3	3	3
	Unremarkable:					3	0	2	1
Hyperplasia, Physiologic Infiltrate, Lymphocytes/Macrophages						0	2	1	2
						0	1	0	0
Mammary, Male	Number examined:	3	3	3	3				
	Unremarkable:	3	1	3	0				
Ductal hyperplasia with periductal fibroplasia/edema		0	2	0	3				

Table excerpted from applicant submission

Additionally, one high dose recovery female had remarkable changes in the heart and the kidney. In the heart, a slight inflammation in the small to medium sized arteries and minimal mononuclear infiltrate in the bicuspid were observed. The renal findings included bilateral slight inflammation of the arcuate arteries at the corticomedullar junction, slight mononuclear infiltrate involving the interstitium and slight basophilic tubules. The vascular findings are consistent with monkey spontaneous findings reported in literature[1]

Toxicokinetics
No data obtained

Dosing Solution Analysis
The concentrations of dosing solutions were within 11% of target concentrations and thus considered acceptable.

[1] Chamanza, R., et al. "Spontaneous Lesions of the Cardiovascular System in Purpose-Bred Laboratory Nonhuman Primates", Toxicologic Pathology, 34:357-363 (2006)

Antibody and Neutralizing Antibody Analysis
Blood samples were collected at predosing on Day 1, Weeks 2 and 4, and during Weeks 2 and 4 of the recovery phase. Anti-HMC001 antibodies were generally negative, although some positive were detected at week 4 at the dilution of 10.

Table 40. Incidence of anti-HMC001 positive animals

	Group 1 0 mg/kg/day		Group 2 0.3 mg/kg/day		Group 3 1.5 mg/kg/day		Group 4 6.0 mg/kg/day	
Interval	Male	Female	Male	Female	Male	Female	Male	Female
DP Day 1	0	0	0	0	1	0	0	0
DP Week 2	0	0	0	0	1	1	0	0
DP Week 4	0	0	3	2	0	1	1	1
RP Week 2	0	0	N/A	N/A	N/A	N/A	0	0
RP Week 4	0	1	N/A	N/A	N/A	N/A	0	0

Unconfirmed results are not counted as positive.
DP – Dosing Phase
RP – Recovery Phase
Table excerpted from applicant submission

Toxicology studies on impurities

2-Week rat study on HM10460A truncated form
Title: 2-weeks Subcutaneous Repeated Dose Toxicity Study of HM10460A and HM10460A Truncated Form in SD Rats

Summary
[redacted](b) (4), participates in the drug substance process in the same way as its native form. Due to a deviation of manufacturing process (batch 107618006), a large amount of truncated [redacted](b) (4) was produced. As part of the deviation investigation, a toxicity evaluation of HM10460A manufactured with truncated [redacted](b) (4) was conducted.

Male rats (5/group) were administered two doses (Days 1 and 8) of HM10460A native (batch PDFGC1807-1, >99% purity) or truncated form (batch PDFGC18072, purity >99%). A standard battery of toxicology endpoints including macroscopic, and histopathology examinations of selected organs, were conducted at Day 15. See Table 41.

Table 41. Study design, 2-w rat study with truncated HM10460A

	Treatment Group	Dose (mg/kg)	Concentration (mg/mL)	Route	Schedule	No. of animal	Individual No.
G1	Vehicle control	-	-	s.c	Q1W×2	5	1 – 5
G2	HM10460A (native)	3.96	0.79	s.c	Q1W×2	5	6 – 10
G3	HM10460A (native)	13.20	2.64	s.c	Q1W×2	5	11 – 15
G4	HM10460A (truncated)	3.96	0.79	s.c	Q1W×2	5	16 – 20
G5	HM10460A (truncated)	13.20	2.64	s.c	Q1W×2	5	21 – 25

Table excerpted from applicant submission

Similar toxicity profiles were observed with HM10460A native form and HM10460A truncated form. Treatment-related findings included swollen tarsal joint, increased absolute neutrophil counts, increased white blood cell counts, elevated alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT), and the splenic enlargement in all treatment animals given both HM10460A forms. The incidence and severity of these findings were similar to those reported in HM10460A toxicology studies.

Table 42. Treatment related findings in rats, 2-w rat study with truncated HM10460A

test article		HM10460A native		HM10460A truncated	
dose, mg/kg	0	3.96	13.2	3.96	13.2
swollen tarsal joints, n	0	1	1	0	1
WBC counts, x103/uL	6.16	146.59	340.68	77.39	362.08
nutrophil count, $x10^3$/uL	0.79	119.77	290.77	60.7	310.41
ALP, U/L	1262	5684	10798	3000	11234
GGT, U/L	0.8	6.8	12.5	2.2	15.8
Spleen weight, g	0.67	2.93	3.59	2.95	3.73
lung weight, g	1.25	1.42	1.58	1.41	1.7
enlarged spleen, n	0	5	5	5	5

In conclusion, there was no difference in toxicity between HM10460A native form and HM10460A truncated form.

11 Integrated Summary and Safety Evaluation

Spectrum Pharmaceuticals submitted this BLA for their product, Rolontis® (Eflapegrastim), a long-acting granulocyte-colony stimulating factor (G-CSF) indicated to reduce the incidence of infection, as manifested by febrile neutropenia, in patients with non-myeloid malignancies receiving myelosuppressive anticancer drugs.

Pharmacology, pharmacokinetics, general toxicology, reproductive toxicology and genotoxicity studies were conducted with eflapegrastim by the subcutaneous route of administration.

Pharmacology

In vitro, eflapegrastim binds to human G-CSF receptor ([redacted] (b) (4) and stimulates mouse bone marrow cells proliferation (EC_{50}: 114 pg/mL) and differentiation (EC_{50}: 1.5 ng/mL). In vivo, eflapegrastim increased neutrophil counts in chemotherapy-induced neutropenic mice and rats, and in normal monkeys.

Eflapegrastim and its Fc fragment bind to FcRn$\alpha\beta_2$, thereby extending eflapegrastim half-life via neonatal Fc receptor-mediated recycling, but do not bind to C1q, FcγRI, FcγRIIB, and FcγRIIIA suggesting low potential to induce Fc-mediated effector functions such as complement dependent cytotoxicity (CDC) and antibody dependent cell mediate cytotoxicity (ADCC).

There were no significant safety pharmacology findings in the CNS and respiratory system in rats, or in the cardiovascular system in monkeys.

ADME

Subcutaneous bioavailability of eflapegrastim was 37-64% in rats and 23% in monkeys. C_{max} was reached at 5-72 hours post-dose in rats and 10-48 hours post-dose in monkeys, following a single subcutaneous dose.

Tissue distribution studies in rat indicated that the highest concentration of eflapegrastim was in the plasma, followed by the bone marrow with a tissue/plasma concentration ratio of 8.5%. Eflapegrastim crossed the placental barrier in rat dams, with a fetal /dam plasma levels ratio of 5-7%.

In vitro and in vivo metabolism studies showed formation of chain exchange products including HM10460 dimer (HM14060A+ G-CSF) and HMC001 (IgG4 Fc fragment). There was no interaction of eflapegrastim and human IgG4 (via Fc fragment), and the PEG bond linking G-CSF to the Fc fragment was shown to be stable.

HM10460A is not cleared through the kidney as no difference in AUC or clearance between sham and nephrectomized rats was observed. Similar to pegfilgrastim, HM10460A is expected to be cleared primarily via neutrophil G-CSF receptor binding, in addition to metabolism.

Toxicology

The clinical route of administration, subcutaneous injection, was used in all pivotal nonclinical studies.

General toxicology studies were conducted in rats and monkeys with treatment durations of a single dose and 4 weeks in both species, and 26 weeks in monkeys.

Treatment-related findings in both rats and monkeys were consistent with the known pharmacological effects of G-CSF, mainly increases in blood neutrophil counts, granulopoiesis in the bone marrow, and increased extramedullary granulopoiesis in the spleen, liver, kidney, and lymph nodes. In the 4-week studies, high doses were associated with mortality and severe toxicities including joint inflammation, reduced bone area, marrow necrosis in rats (≥11-fold MRHD) and hemorrhage in the lung and brain in monkeys (212-fold MRHD) in the early sacrificed animals. A 26-week study in monkeys did not reveal additional toxicities other than neutrophil count reduction (-78% by Week 26, -85% by end of the recovery) after a transient increase during the first 4 weeks. The neutrophil counts reduction was likely attributable to the presence of neutralizing anti-drug antibodies. NOAELs were 0.16, 0.97, 1.6 mg/kg, representing 0.2-, 39- and 82-fold the MRHD in the 4-week rat study, 4-week monkey study and 26-week monkey study, respectively.

Eflapegrastim was not mutagenic or clastogenic in a standard battery of genotoxicity studies, including the Ames assay, a chromosomal aberration assay in Chinese hamster ovary cells, and an *in vivo* micronucleus study in rats.

Eflapegrastim did not affect fertility, embryofetal development, and pre- and post-natal development in rats at doses up to 7-fold the MRHD. Pregnant rabbits given eflapegrastim during organogenesis (GD7-GD19) exhibited increased post-implantation loss (mostly early resorption) and related reduction in litter size, increased incidence of dams with no viable fetuses, as well as decreased fetal weights at ≥6-fold MRHD).

Repeat dosing resulted in reduced systemic exposures in all animal studies (57-99.9% reduction in AUC as compared to Day 1). Considering the indicated clinical use of a single dose once per chemotherapy cycle, safety margins to the maximum recommended human dose (MRHD) of 13.2 mg were calculated based on Day 1 AUC values. The following table presents safety margins of pivotal toxicology studies. See table 43.

Table 43. Safety margins in rodents and monkeys

		NOAEL		Human safety margin, AUC
		dose	Day 1 AUC, ug.h/mL	
general toxicology	Rat single dose	0.323 mg/kg	23	1.5x
	Monkey single dose	0.323 mg/kg	87	5.6x
	Rat 4-week study	0.162 mg/kg/week	3	0.2x
	Monkey 4-week study	0.969 mg/kg/week	600	39x
	Monkey 26-week study	1.6 mg/kg/week	1258	82x
reproductive toxicology	FEED in rats	0.825 mg/kg/week	112*	7x
	EFD in rats	0.825 mg/kg/2 days	112	7x
	EFD in rabbits	0.183 mg/kg/2 days	28	1.8x
	PPND in rats	0.825 mg/kg/week	112*	7x

Day 1 AUC of animal study/Day 1 human AUC (15.4 ug.h/mL at 13.2 mg based on 70 kg human body weight) was used to calculate safety margin; *referenced to data from EFD rat study as no TK data were obtained in FEED and PPND studies

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/s/

HUIQING HAO
06/25/2020 01:36:11 PM

FEDERICA BASSO
06/25/2020 01:52:57 PM
I concur