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APPLICATION NUMBER:

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PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	206947
Supporting document/s:	001
Applicant's letter date:	August 14, 2014
CDER stamp date:	August 14, 2014
Product:	LENVIMA (Lenvatinib)
Indication:	Radioiodine-refractory differentiated thyroid cancer
Applicant:	Eisai Inc. 155 Tice Blvd. Woodcliff Lake, New Jersey 07677
Review Division:	Division of Hematology Oncology Toxicology (Division of Oncology Products 2)
Reviewers:	Emily Fox, PhD, Stephanie Aungst, PhD
Supervisor/Team Leader:	Whitney Helms, PhD
Division Director:	John Leighton, PhD (Patricia Keegan, MD)
Project Manager:	Deanne Varney

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Reference ID: 3698119

Pharmacology/Toxicology Labeling Review of Lenvatinib

The following table describes the labeling decisions made by the pharmacology/toxicology team for the initial approval of LENVIMA (lenvatinib) for the treatment of patients with progressive, radioiodine-refractory differentiated thyroid cancer.

The Applicant Proposed	FDA Recommends	Reasoning/Final Agreement
Highlights **WARNINGS AND PRECAUTIONS** (b) (4)	**Highlights** **WARNINGS AND PRECAUTIONS** Embryofetal Toxicity: Can cause fetal harm. Advise of potential risk to a fetus and use of effective contraception (5.12, 8.1, 8.3).	Label was updated to reflect CFR. The Applicant accepted these changes.
5 WARNINGS AND PRECAUTIONS **5.11 Embryo-Fetal Toxicity** (b) (4)	**5 WARNINGS AND PRECAUTIONS** **5.12 Embryofetal Toxicity** Based on its mechanism of action and data from animal reproduction studies, LENVIMA can cause fetal harm when administered to a pregnant woman. In animal reproduction studies, oral administration of lenvatinib during organogenesis at doses below the recommended human dose resulted in embryotoxicity, fetotoxicity, and teratogenicity in rats and rabbits. Advise pregnant women of the potential risk to a fetus. Advise females of reproductive potential to use effective contraception during treatment with LENVIMA and for at least 2 weeks following completion of therapy *[see Use in Specific Populations (8.1, 8.3)]*.	Label was updated in accordance with CFR and to reflect results of reproductive toxicology studies submitted to the NDA. Duration of contraception use in females was added based on the half-life of lenvatinib in humans, which is ~20-34 hours at the recommended clinical dose. Use of contraception for at least 2 weeks was recommended because it is greater than 6 times the half-life of lenvatinib and takes into account fetal harm caused by lenvatinib based on mechanism of action and data from animal reproduction studies. During labeling revisions, the Applicant wanted to change the length of recommended contraception from 2 to (b) (4) weeks (b) (4) The nonclinical team discussed this proposed change with the Maternal Health review team. (b) (4)

Reference ID: 3698119

		(b) (4) Thus, these (b) (4) do not support the proposed change in contraception duration from 2 to (b) weeks, and the review team retained 2 weeks in the label. The Applicant proposed the same change in Sections 5.12, 8.3, and 17, and the review team retained 2 weeks as the recommended duration of contraception in these sections as well.
8 USE IN SPECIFIC POPULATIONS **8.1 Pregnancy** (b) (4) *Risk Summary* (b) (4) *Animal Data* (b) (4)	**8 USE IN SPECIFIC POPULATIONS** **8.1 Pregnancy** *Risk Summary* Based on its mechanism of action and data from animal reproduction studies, LENVIMA can cause fetal harm when administered to a pregnant woman *[see Clinical Pharmacology (12.1)]*. In animal reproduction studies, oral administration of lenvatinib during organogenesis at doses below the recommended human dose resulted in embryotoxicity, fetotoxicity, and teratogenicity in rats and rabbits *[see Data]*. There are no available human data informing the drug-associated risk. Advise pregnant women of the potential risk to a fetus. The background risk of major birth defects and miscarriage for the indicated population is unknown; however, the background risk in the U.S. general population of major birth defects is 2-4% and of miscarriage is 15-20% of clinically recognized pregnancies. *Data* <u>Animal Data</u>	**8 USE IN SPECIFIC POPULATIONS** **8.1 Pregnancy** *Risk Summary* Based on its mechanism of action and data from animal reproduction studies, LENVIMA can cause fetal harm when administered to a pregnant woman *[see Clinical Pharmacology (12.1)]*. In animal reproduction studies, oral administration of lenvatinib during organogenesis at doses below the recommended human dose resulted in embryotoxicity, fetotoxicity, and teratogenicity in rats and rabbits *[see Data]*. There are no available human data informing the drug-associated risk. Advise pregnant women of the potential risk to a fetus. The background risk of major birth defects and miscarriage for the indicated population is unknown; however, the background risk in the U.S. general population of major birth defects is 2-4% and of miscarriage is 15-20% of clinically recognized pregnancies. *Data* <u>Animal Data</u>

(b) (4)	In an embryofetal development study, daily oral administration of lenvatinib mesylate at doses greater than or equal to 0.3 mg/kg [approximately 0.14 times the recommended human dose based on body surface area (BSA)] to pregnant rats during organogenesis resulted in dose-related decreases in mean fetal body weight, delayed fetal ossifications, and dose-related increases in fetal external (parietal edema and tail abnormalities), visceral (b) (4) and skeletal anomalies. (b) (4) Daily oral administration of lenvatinib mesylate to pregnant rabbits during organogenesis resulted in fetal external (short tail), visceral (retroesophageal subclavian artery), and skeletal anomalies at doses greater than or equal to 0.03 mg/kg (approximately 0.03 times the human dose of 24 mg based on body surface area). At the 0.03 mg/kg dose, increased post-implantation loss, (b) (4) was also observed. Lenvatinib was abortifacient in rabbits, resulting in late abortions in approximately one-third of the rabbits treated at a dose level of 0.5 mg/kg/day (approximately 0.5 times the recommended clinical dose of 24 mg based on BSA).	In an embryofetal development study, daily oral administration of lenvatinib mesylate at doses greater than or equal to 0.3 mg/kg [approximately 0.14 times the recommended human dose based on body surface area (BSA)] to pregnant rats during organogenesis resulted in dose-related decreases in mean fetal body weight, delayed fetal ossifications, and dose-related increases in fetal external (parietal edema and tail abnormalities), visceral, and skeletal anomalies. Greater than 80% postimplantation loss was observed at 1.0 mg/kg/day (approximately 0.5 times the recommended human dose based on BSA). Daily oral administration of lenvatinib mesylate to pregnant rabbits during organogenesis resulted in fetal external (short tail), visceral (retroesophageal subclavian artery), and skeletal anomalies at doses greater than or equal to 0.03 mg/kg (approximately 0.03 times the human dose of 24 mg based on body surface area). At the 0.03 mg/kg dose, increased post-implantation loss, including one fetal death, was also observed. Lenvatinib was abortifacient in rabbits, resulting in late abortions in approximately one-third of the rabbits treated at a dose level of 0.5 mg/kg/day (approximately 0.5 times the recommended clinical dose of 24 mg based on BSA).
Justification for 8.1:	The label was updated to reflect the Pregnancy and Lactation Labeling Rule (PLLR), "Content and Format of Labeling for Human Prescription Drug and Biological Products; Requirements for Pregnancy and Lactation Labeling," published in the CFR on December 4, 2014.	During the labeling revisions, the Applicant wanted to remove (b) (4)

	As required by PLLR the (b) (4) cross-references to Section 12.1 and *Data* were added, and a background statement regarding the risk of major birth defects and miscarriage was added. The label was updated to reflect results of reproductive toxicology studies submitted to the NDA, and dose ratios based on BSA were updated to reflect the Pharmacology/Toxicology Reviewer's calculations. The NOAEL was considered unnecessary and subsequently removed.	(b) (4) . The nonclinical team accepted this deletion based on the reasoning and the additional historical control data provided. During the labeling revisions, the Applicant wanted to remove (b) (4) The nonclinical team accepted this deletion based on the laboratory historical control data provided and because the 80% incidence of postimplantation loss observed at the 1 mg/kg dose level, representing a fraction of the expected exposure clinically, remains in the label.
(b) (4) (b) (4)	**8.2 Lactation** *Risk Summary* It is not known whether LENVIMA is present in human milk. Lenvatinib and its metabolites are excreted in rat milk at concentrations higher than in maternal plasma *[see Data]*. Because of the potential for serious adverse reactions in nursing infants from LENVIMA, advise women to discontinue breastfeeding during treatment with LENVIMA. *Data* <u>Animal Data</u> Following administration of radiolabeled lenvatinib to lactating Sprague Dawley rats, lenvatinib-related radioactivity was approximately 2 times higher (based on AUC) in milk compared to maternal plasma.	Label format and content was updated according to PLLR. A cross-reference to *Data* was added, as required by PLLR. Label was updated to include results of a nonclinical pharmacokinetic milk transfer study submitted to the NDA. The Applicant accepted these changes.

8.8 Females and Males of Reproductive Potential *Contraception* Based on its mechanism of action, LENVIMA can cause fetal harm when administered to a pregnant woman [*see Use in Specific Populations (8.1)*]. (b) (4)	**8.3 Females and Males of Reproductive Potential** *Contraception* Based on its mechanism of action, LENVIMA can cause fetal harm when administered to a pregnant woman [*see Use in Specific Populations (8.1)*]. Advise females of reproductive potential to use effective contraception during treatment with LENVIMA and for at least 2 weeks following completion of therapy. *Infertility* Females LENVIMA may result in reduced fertility in females of reproductive potential [see *Nonclinical Toxicology (13.1)*]. Males LENVIMA may result in damage to male reproductive tissues leading to reduced fertility of unknown duration [see *Nonclinical Toxicology (13.1)*].	Label was updated according to PLLR. Duration of contraception use in females was added based on the half-life of lenvatinib in humans, which is ~20-34 hours at the recommended clinical dose. Use of contraception for at least 2 weeks was recommended because it is greater than 6 times the half-life of lenvatinib and takes into account fetal harm caused by lenvatinib based on mechanism of action and data from animal reproduction studies. An *Infertility* subsection was added, in accordance with PLLR. This section reflects fertility/effects on reproductive organ data submitted to the NDA in general toxicology studies. Cross-references to *Nonclinical Toxicology (13.1)* were added, as required by PLLR. During labeling revisions, the Applicant wanted to change the length of recommended contraception from 2 to (b) (4) weeks (b) (4) For the reasons discussed above, the nonclinical team disagreed with this change and retained 2 weeks in the label.
8.4 Pediatric Use (b) (4) of LENVIMA in pediatric patients have not been established. *Juvenile Animal Data* Daily oral administration of lenvatinib mesylate (b) (4) rats for 8 weeks starting on postnatal day 21 resulted in growth retardation (decreased body weight gain and decreased food consumption),	**8.4 Pediatric Use** The safety and effectiveness of LENVIMA in pediatric patients have not been established. *Juvenile Animal Data* Daily oral administration of lenvatinib mesylate to juvenile rats for 8 weeks starting on postnatal day 21 (approximately equal to a human pediatric age of 2 years) resulted in growth retardation (decreased body weight gain, decreased food	**8.4 Pediatric Use** The safety and effectiveness of LENVIMA in pediatric patients have not been established. *Juvenile Animal Data* Daily oral administration of lenvatinib mesylate to juvenile rats for 8 weeks starting on postnatal day 21 (approximately equal to a human pediatric age of 2 years) resulted in growth retardation (decreased body weight gain, decreased food

secondary delay of physical development, (b) (4) [redacted]	consumption, and decreases in the width and/or length of the femur and tibia) and secondary delays in physical development and reproductive organ immaturity at doses greater than or equal to 2 mg/kg (approximately 1.2 to 5 times the clinical exposure by AUC at the recommended human dose). Decreased length of the femur and tibia persisted following 4 weeks of recovery. (b) (4) [redacted] In general, the toxicologic profile of lenvatinib was similar between juvenile and adult rats, though toxicities including broken teeth at all dose levels and mortality at the 10 mg/kg/day dose level (attributed to primary duodenal lesions) occurred at earlier treatment time-points in juvenile rats.	consumption, and decreases in the width and/or length of the femur and tibia) and secondary delays in physical development and reproductive organ immaturity at doses greater than or equal to 2 mg/kg (approximately 1.2 to 5 times the clinical exposure by AUC at the recommended human dose). Decreased length of the femur and tibia persisted following 4 weeks of recovery. In general, the toxicologic profile of lenvatinib was similar between juvenile and adult rats, though toxicities including broken teeth at all dose levels and mortality at the 10 mg/kg/day dose level (attributed to primary duodenal lesions) occurred at earlier treatment time-points in juvenile rats.
Justification for 8.4:	The label was initially updated to reflect the results of a juvenile toxicology study submitted to the NDA, including effects on femur/tibia growth (b) (4) [redacted]. Data deemed unnecessary or not fully supported by submitted data was removed. Exposure ratios were updated to reflect the steady-state individual adult AUC range (1610 to 6960 ng·h/mL) at the recommended human dose provided by the Clinical Pharmacology reviewer.	During the labeling revisions, the Applicant wanted to remove the sentence stating that (b) (4) [redacted] (b) (4) [redacted] however, upon further detailed review (b) (4) [redacted] in the context of the rest of the study, the nonclinical team agreed to remove this sentence (b) (4) [redacted]

12.1 Mechanism of Action Lenvatinib is a (b) (4) receptor tyrosine kinase (RTK) inhibitor that inhibits the kinase activities of vascular endothelial growth factor (VEGF) receptors VEGFR1 (FLT1), VEGFR2 (KDR), and VEGFR3 (FLT4). (b) (4) in pathogenic angiogenesis, tumor growth, and cancer progression including fibroblast growth factor (FGF) receptors FGFR1, 2, 3, and 4; the platelet derived growth factor (PDGF) receptor PDGFRα; KIT; and RET.	**12.1 Mechanism of Action** Lenvatinib is a receptor tyrosine kinase (RTK) inhibitor that inhibits the kinase activities of vascular endothelial growth factor (VEGF) receptors VEGFR1 (FLT1), VEGFR2 (KDR), and VEGFR3 (FLT4). Lenvatinib also inhibits other RTKs that have been implicated in pathogenic angiogenesis, tumor growth, and cancer progression in addition to their normal cellular functions, including fibroblast growth factor (FGF) receptors FGFR1, 2, 3, and 4; the platelet derived growth factor receptor alpha (PDGFRα), KIT, and RET.	Grammatical edits were made and the phrase "in addition to their normal cellular functions" was added to decrease the promotional potential of the sentence and to reflect the potential toxicity of the drug. The Applicant accepted these changes.
13 NONCLINICAL TOXICOLOGY **13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility** Carcinogenicity studies have not been conducted with lenvatinib. Lenvatinib mesylate was (b) (4) No specific studies with lenvatinib have been conducted in animals to evaluate the effect on fertility. However, (b) (4)	**13 NONCLINICAL TOXICOLOGY** **13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility** Carcinogenicity studies have not been conducted with lenvatinib. Lenvatinib mesylate was not mutagenic in the in vitro bacterial reverse mutation (Ames) assay. Lenvatinib was not clastogenic in the in vitro mouse lymphoma thymidine kinase assay or the in vivo rat micronucleus assay. No specific studies with lenvatinib have been conducted in animals to evaluate the effect on fertility; however, results from general toxicology studies in rats, monkeys, and dogs suggest there is a potential for lenvatinib to impair fertility. Male dogs exhibited testicular hypocellularity of the seminiferous epithelium and desquamated seminiferous epithelial cells in the epididymides at lenvatinib exposures approximately 0.02 to 0.09 times the clinical exposure by AUC at the recommended human dose. Follicular atresia of the ovaries was observed in monkeys and rats at exposures	Label was updated to clarify mutagenesis data submitted to the NDA. *tk* was replaced with its full name (thymidine kinase). Label was updated to reflect fertility/effects on reproductive organ data submitted to the NDA in general toxicology studies. Exposure ratios were updated to reflect the steady-state individual adult AUC range (1610 to 6960 ng·h/mL) at the recommended human dose provided by the Clinical Pharmacology reviewer. During the labeling revisions, the Applicant asked the FDA to provide the basis for these exposure calculations. These explanations (based on comparisons to multiple toxicology studies) were subsequently conveyed to the Applicant, who agreed with the review team's explanations and had no further comments.

	0.2 to 0.8 times and 10 to 44 times the clinical exposure by AUC at the 24 mg clinical dose, respectively. In addition, in monkeys, a decreased incidence of menstruation was reported at lenvatinib exposures lower than those in humans at the 24 mg clinical dose.	

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/s/

EMILY M FOX
02/05/2015

STEPHANIE L AUNGST
02/06/2015

WHITNEY S HELMS
02/06/2015

MEMORANDUM

Date: January 14, 2015
From: Whitney S. Helms, PhD
Pharmacology Supervisor
Division of Hematology Oncology Toxicology for Division of Oncology Products 2
To: File for NDA # 206947
Lenvima (lenvatinib)
Re: Approvability of Pharmacology and Toxicology

On August 14, 2014 Eisai submitted New Drug Application (NDA) 206947 for the use of lenvatinib for the treatment of patients with locally recurrent or metastatic progressive radioactive iodine-refractory differentiated thyroid cancer. Non-clinical studies examining the pharmacology and toxicology of lenvatinib provided to support NDA 206947 were reviewed in detail by Emily M. Fox, PhD, and Stephanie L. Aungst, PhD. The findings of these studies are summarized in the "Executive Summary" of the NDA review and reflected in the product label.

Lenvatinib is a small molecule kinase inhibitor administered orally at the recommended dose of 24 mg daily. In the clinical trials used to support the approval of lenvatinib at this dose level, there was a variability in patient exposure with a range of 210-520 ng/mL (~0.4-1 μM) and 1610 to 6960 ng*hr/mL in Cmax and AUC, respectively. Lenvatinib was ≥ 90% protein bound in all species tested, thus, a Cmax of 1 μM would result in a free drug concentration of no more than 100 nM in plasma at the recommended dose. In biochemical screening assays lenvatinib was able to inhibit the kinases in the vascular endothelial growth factor receptor family (VEGFR1, 2, and 3), the fibroblast growth factor receptor family (FGFR1-4), RET, KIT, and platelet derived growth factor receptors α and β with IC_{50}s of less than 10 nM. Lenvatinib also demonstrated inhibition of VEGF-mediated angiogenesis both in vitro and in vivo at clinically relevant concentrations and showed in vivo anti-tumor activity against several thyroid tumor models implanted into immunodeficient mice.

Metabolism of lenvatinib appeared similar between species and unchanged parent was the major product in plasma. Elimination was primarily through the fecal route in animals and humans. Lenvatinib was widely distributed in animal tissues with high levels of the drug observed in the GI tract, liver, kidney, and aorta all target organs identified clinically. High levels of lenvatinib were also noted in the adrenal gland in rats and in the eye, particularly the choroid, in monkeys.

The major target organs identified in general toxicology studies conducted in rats, monkeys, and dogs included the gastrointestinal tract, kidney, liver, pancreas, bone marrow, growth plates, teeth, secondary lymphoid organs, adrenal gland, and pituitary. Gastrointestinal toxicity, renal toxicity, and hepatotoxicity have been observed clinically and are included in the Warnings and Precautions section of the label for lenvatinib. Increased epiphyseal growth plates and broken teeth are commonly seen in rodent studies investigating the effects of VEGF pathway inhibition and are likely more relevant for a pediatric population.

In a safety pharmacology study, no significant behavioral or physiological changes were observed following a single dose of lenvatinib. The drug is, however, able to cross the blood brain barrier, at least at low levels. Lenvatinib demonstrated low potential for causing direct QTc prolongation in the in vitro hERG assay or in vivo in a single dose cardiac safety pharmacology conducted in monkeys, though QTc prolongation is observed with many VEGFR inhibitors, has been noted clinically with lenvatinib treatment, and is included in the Warnings and Precautions section of the label. Vascular lesions sometimes associated with hemorrhage were observed in multiple rat and monkey tissues, consistent with its pharmacologic activity as an inhibitor of VEGF signaling and the arterial thromboembolic and hemorrhagic events, including fatal intracranial hemorrhage, that have been observed in patients administered lenvatinib. Transient elevations in blood pressure were observed in a single dose safety pharmacology study conducted in monkeys though no clear effects on cardiovascular function were recorded at the time-points included for ECG monitoring in repeat-dose studies. Some of the vascular toxicity noted in the repeat-dose toxicology studies, including arterial fibroid necrosis in the choroid plexus in monkeys and ischemic malacia in the cerebral cortex of rats, is, however, suggestive of lenvatinib-mediated hypertension. Hypertension and reversible posterior leukoencephalopathy syndrome (RPLS) have been reported clinically.

Based on discussions with the clinical team, the intended clinical population for treatment with lenvatinib does fall under the scope of the International Conference on Harmonization (ICH) S9 Guidance for pharmaceuticals intended to treat patients with advanced cancer. Consistent with this guidance, carcinogenicity studies using lenvatinib were neither conducted nor required to support the approval of this drug in the intended patient population. Lenvatinib was not mutagenic in the bacterial reverse mutation assay or clastogenic in the in vitro mouse lymphoma thymidine kinase assay or the in vivo micronucleus test.

In embryofetal development studies conducted in both rats and rabbits, lenvatinib was embrotoxic, fetotoxic, and teratogenic. Based on body surface area (BSA), at doses greater than or equal to 0.03 (rats) and 0.14 times (rabbits) the recommended clinical dose of 24 mg, oral administration of lenvatinib to pregnant animals resulted in increased post-implantation loss as well as external, visceral, and skeletal anomalies. In rabbits, lenvatinib was abortifacient, resulting in late abortions in 7 of 20 rabbits treated at the 0.5 mg/kg/day dose level (approximately 0.5 times the recommended clinical dose based on BSA); dead fetuses were also observed in rabbits at doses of 0.03 and 0.1 mg/kg/day. A warning for embryofetal toxicity is recommended and females of reproductive potential are advised to use contraception during treatment with lenvatinib and, based on the half-life in humans of approximately 28 hours, for up to 2 weeks following cessation of treatment. Lenvatinib was detected in milk from lactating rats treated with the drug at levels approximately twice as high as those seen in plasma; breastfeeding is not recommended during treatment with lenvatinib.

Reproductive studies investigating the effects of lenvatinib on fertility or on post-natal development were not submitted and were not required for approval in this patient population. Follicular atresia of the ovaries was, however, observed in general toxicology studies conducted in both rats and monkeys. In addition, monkeys demonstrated a decreased incidence of menstruation suggesting that lenvatinib may impair female fertility, at least transiently. In dogs, administration of lenvatinib was associated with decreases in hypocellularity of the seminiferous

epithelium and desquamated seminiferous epithelial cells in the epididymides at doses resulting in exposures significantly lower than the expected clinical exposure at the recommended dose of 24 mg, suggesting the potential for at least transiently reduced male fertility following treatment with lenvatinib as well. Though not required, the Applicant did conduct a study investigating the effects of lenvatinib on juvenile animals. Treatment of juvenile animals beginning on Postnatal Day (PND) 21, equivalent to approximately 2 years of age in humans, resulted in toxicities similar to those noted in adult animal studies; however, toxicities including mortality and broken teeth were observed following shorter treatment durations at the same dose levels used in adults. Administration of lenvatinib to juvenile animals at doses ≥ 2 mg/kg (approximately 1.2 to 5 times the clinical exposure at the recommended dose of 24 mg) also resulted in delayed growth and maturation (b) (4). These findings are included in the Pediatric Use (8.4) section of the label.

Recommendations: I concur with the conclusion of Drs. Fox and Aungst that the pharmacology and toxicology data support the approval of NDA 206947 for LENVIMA. There are no outstanding nonclinical issues related to the approval of LENVIMA for the treatment of patients with locally recurrent or metastatic progressive radioactive iodine-refractory differentiated thyroid cancer.

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/s/

WHITNEY S HELMS
01/14/2015

MEMORANDUM

Lenvima (lenvatinib)

Date: January 14, 2015
To: File for NDA 206947
From: John K. Leighton, PhD, DABT
Acting Director, Division of Hematology Oncology Toxicology
Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting and labeling reviews for Lenvima conducted by Drs. Aungst and Fox, and secondary memorandum and labeling provided by Dr. Helms. I concur with Dr. Helms' conclusion that Lenvima may be approved and that no additional nonclinical studies are needed for the proposed indication.

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/s/

JOHN K LEIGHTON
01/14/2015

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	206947
Supporting document/s:	001
Applicant's letter date:	August 14, 2014
CDER stamp date:	August 14, 2014
Product:	LENVIMA (Lenvatinib)
Indication:	Radioiodine-refractory differentiated thyroid cancer
Applicant:	Eisai Inc. 155 Tice Blvd. Woodcliff Lake, New Jersey 07677
Review Division:	Division of Hematology Oncology Toxicology (Division of Oncology Products 2)
Reviewers:	Emily M. Fox, PhD, Stephanie L. Aungst, PhD
Supervisor/Team Leader:	Whitney Helms, PhD
Division Director:	John Leighton, PhD (Patricia Keegan, MD)
Project Manager:	Deanne Varney

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Reference ID: 3686430

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1 Executive Summary

1.1 Introduction

Lenvatinib is a multiple receptor tyrosine kinase inhibitor that targets vascular endothelial growth factor (VEGF) receptors VEGFR1 (FLT1), VEGFR2 (KDR), and VEGFR3 (FLT4), fibroblast growth factor (FGF) receptors FGFR1, 2, 3, and 4, the platelet derived growth factor receptor alpha (PDGFRα), KIT, and RET. Eisai has submitted New Drug Application (NDA) 206947 for lenvatinib (LENVIMA) for the treatment of patients with locally recurrent or metastatic, progressive, radioiodine-refractory differentiated thyroid cancer.

1.2 Brief Discussion of Nonclinical Findings

Against a panel of recombinant human kinases, lenvatinib inhibited VEGFR1, VEGFR2, VEGFR3, RET, FGFR1-4, KIT, PDGFRα, and PDGFRβ at concentrations that have been achieved clinically at the 24 mg dose level used in clinical trials conducted to support the approval of the drug. In vitro, lenvatinib inhibited VEGF-induced VEGFR2 phosphorylation, endothelial tube formation, and proliferation in human umbilical vein endothelial cells at clinically achievable concentrations. In vivo, lenvatinib exhibited anti-angiogenic activity in a human anaplastic thyroid cancer xenograft model and inhibited tumor growth in multiple thyroid cancer xenograft models in mice.

In single-dose nonclinical pharmacokinetic (PK) studies, lenvatinib exhibited low clearance, a slightly shorter Tmax (0.5 to 2 hrs) than that observed in humans (2 to 4 hrs), and a shorter terminal elimination half-life (~1.7-5.3 hrs) than that observed in humans (~28 hrs following single and multiple doses). Following a single oral administration of radiolabeled lenvatinib to rats and monkeys, tissue distribution was consistent with nonclinical and clinical findings of gastrointestinal (GI) and liver toxicity. Lenvatinib distribution to the brains of rats and monkeys was ~2-14% of that in plasma, suggesting that lenvatinib is able to across the blood-brain barrier, at least at low levels. There were no clinical signs of CNS toxicity in animals, and no significant effects were observed on behavior, general physical condition, or respiratory function of rats administered single oral doses of lenvatinib up to 600 mg/m^2.

Six metabolites were detected in vitro in human liver microsomes, with the demethylated form of lenvatinib (M2) being the major metabolite. All of these metabolites were also found in microsomes from the animal species tested. There were no major circulating metabolites identified in clinical studies. Consistent with this, the unchanged parent compound was the major component of rat and monkey plasma following a single oral administration of radiolabeled lenvatinib. Adequate exposure to M2 occurred in animals to account for potential metabolite-mediated toxicity, so further metabolite evaluation is not warranted at this time. In keeping with findings in the clinic, lenvatinib excretion was primarily fecal via bile in rats and monkeys.

Target organs of lenvatinib-mediated toxicity identified in repeat-dose toxicology studies with daily dosing for up to 26 and 39 weeks in rats and monkeys, respectively, included

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the kidney, duodenum, stomach, pancreas, adrenals, liver, spleen (lymphoid depletion), thymus (lymphoid depletion and atrophy), pituitary, choroid plexus (eosinophilic exudate, arterial fibroid necrosis, and hemorrhage), femur (increased epiphyseal growth plate), teeth (rats; broken/discolored), sternum (rats; increased epiphyseal cartilage), femoral and sternal marrow (rats; hypocellularity), common bile duct (rats), and gallbladder (monkeys). Many of the lenvatinib-induced findings were attributable to the primary pharmacology of VEGF inhibition. In keeping with this, vascular lesions sometimes associated with hemorrhage were observed in multiple rat and monkey tissues, and arterial thromboembolic and hemorrhagic events including fatal intracranial hemorrhage have been observed in patients administered lenvatinib.

Potential cardiovascular effects of lenvatinib were examined both in vitro and in single and repeat dose toxicology studies in vivo. Daily oral dosing of lenvatinib at the 24 mg dose used in clinical trials to support its approval in the proposed patient population resulted in a mean C_{max} of 210-520 ng/mL and a median steady-state AUC of 3840 ng·h/mL. The IC_{50} for the inhibitory effect of lenvatinib on hERG potassium current was > 10 μM, which is not clinically achievable at the recommended dose of 24 mg. Although lenvatinib did not significantly affect blood pressure or QT prolongation in repeat-dose toxicology studies in dogs and monkeys at dose levels up to 10 mg/m^2 and 360 mg/m^2, respectively, in a single dose safety pharmacology study there was a statistically significant increase in mean blood pressure in male dogs following administration of lenvatinib at 6 or 30 mg/kg compared to controls. These increases were not dose-dependent. In addition, histopathological findings in repeat dose studies, including findings in the choroid plexus of hemorrhage, potentially suggestive of reverse posterior leukoencephalopathy syndrome (RPLS) that has been reported clinically following treatment with lenvatinib, were observed. Both QT interval prolongation and hypertension have been observed clinically with lenvatinib.

In the 26-week toxicology study in rats, mortality occurred at lenvatinib exposures approximately 9 to 39 times the clinical exposure at the recommended human dose of 24 mg due to severe duodenal and renal findings. The duodenal lesions were associated with cholangitis and bacterial infection in the liver and/or common bile duct, as well as two cases of pancreatitis. Pancreatitis in rats correlated with elevated serum amylase and lipase levels in humans treated with lenvatinib; however, there was no clinically significant difference in the incidence of pancreatitis in patients treated with lenvatinib versus placebo. In the 39-week toxicology study in monkeys, mortality occurred at lenvatinib exposures approximately 1.4 to 6 times the clinical exposure at the recommended human dose due to inflammatory changes in the lower large intestine and severe duodenal changes. Treatment with lenvatinib induced clinical signs of GI toxicity in animals and extensive histological findings throughout the GI tract consistent with clinical findings of decreased weight, appetite, diarrhea, and GI perforation or fistula. Lenvatinib induced renal toxicity in rats and monkeys, including proteinuria, glomerulopathy, and glomerulonephropathy at exposures approximately 1.6 to 11 times, 0.2 to 1 times, and 9 to 39 times the clinical exposure at the recommended human dose of 24 mg, respectively. These nonclinical findings correlate with clinical findings of renal

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impairment/failure and proteinuria. Increased ALT and AST observed in rats and monkeys, indicative of hepatotoxicity, was also seen in the clinic.

Lenvatinib was not mutagenic in the in vitro Ames Assay. Lenvatinib was not clastogenic in the in vitro mouse lymphoma thymidine kinase assay, or the in vivo rat bone marrow micronucleus assay.

Fertility/embryonic development studies were not conducted with lenvatinib; however, results from repeat-dose toxicology studies suggest that lenvatinib has the potential to impair male and female fertility. Female monkeys and rats exhibited follicular atresia of the ovaries at lenvatinib exposures approximately 0.2 to 0.8 times and 9 to 39 times, respectively, the clinical exposure by AUC at the recommended human dose of 24 mg. Decreased incidence of menstruation was also observed in monkeys. Male dogs exhibited testicular hypocellularity of the seminiferous epithelium and desquamated seminiferous epithelial cells in the epididymides at lenvatinib exposures approximately 0.02 to 0.09 times the clinical exposure by AUC at the recommended human dose of 24 mg.

Lenvatinib can cross the placental barrier in pregnant rats. Following administration of 3 mg/kg ^{14}C-lenvatinib to lactating rats, lenvatinib-related radioactivity was approximately 2 times higher (based on AUC) in milk compared to maternal plasma. Thus, women should be advised to avoid breastfeeding during treatment with lenvatinib.

Embryofetal development studies demonstrated that lenvatinib is embryotoxic, fetotoxic, and teratogenic in rats and rabbits. Daily oral administration of lenvatinib to pregnant rats and rabbits during organogenesis resulted in maternal toxicity at doses approximately 0.5 times the recommended human dose based on body surface area (BSA). Daily oral administration of lenvatinib to pregnant rats at doses ≥ 0.3 mg/kg (approximately 0.14 times the recommended human dose of 24 mg based on BSA) resulted in dose-related decreases in mean fetal body weight, delayed fetal ossifications, and increases in fetal external (parietal edema and tail abnormalities), visceral (retroesophageal subclavian artery), and skeletal anomalies. Dose-related increases in post-implantation loss were observed beginning at 0.1 mg/kg.

Daily oral administration of lenvatinib to pregnant rabbits at doses ≥ 0.03 mg/kg (approximately 0.03 times the recommended human dose of 24 mg based on BSA) resulted in increased post-implantation loss and fetal external (short tail), visceral (retroesophageal subclavian artery), and skeletal anomalies. One and three dead fetuses were observed at the 0.03 and 0.1 mg/kg dose levels, respectively. Lenvatinib was abortifacient in rabbits, resulting in late abortions in approximately one-third of the rabbits treated at the 0.5 mg/kg dose level (approximately 0.5 times the recommended human dose based on BSA). A warning for embryofetal toxicity is recommended for LENVIMA. Based on a half-life of lenvatinib of ~20-34 hours, it is advised that females of reproductive potential use effective contraception during treatment with lenvatinib and for at least 2 weeks following cessation of therapy.

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Daily oral administration of lenvatinib to juvenile rats for 8 weeks starting on postnatal day 21 (approximately equal to a human pediatric age of 2 years) resulted in mortality attributed to primary duodenal lesions at lenvatinib exposures approximately 6 to 24 times the clinical exposure at the recommended human dose of 24 mg. The toxicologic profile of lenvatinib was generally similar between juvenile and adult rats, though broken teeth at all dose levels and mortality at the 10 mg/kg dose level occurred at earlier treatment time-points in juvenile rats compared to adults. Treatment with lenvatinib at doses ≥ 2 mg/kg (approximately 1.2 to 5 times the clinical exposure by AUC at the recommended human dose of 24 mg) resulted in growth retardation, decreases in the width and/or length of the femur and tibia, and secondary delays in physical development and reproductive organ immaturity. Decreased length of the femur and tibia persisted following 4 weeks of recovery. Potential evidence of delayed learning indicated by longer navigation times through a water maze was also observed in males treated with lenvatinib at the 2 mg/kg dose level. Overall, these data suggest that administration of lenvatinib to pediatric patients may result in growth retardation and delayed learning/development. Lenvatinib has not been investigated clinically in pediatric patients.

1.3 Recommendations

1.3.1 Approvability

Recommended for approval. The nonclinical studies submitted to this NDA provide sufficient information to support the use of lenvatinib for the treatment of patients with locally recurrent or metastatic, progressive, radioiodine-refractory differentiated thyroid cancer.

1.3.2 Additional Non Clinical Recommendations

None.

1.3.3 Labeling

A separate labeling review will be provided.

2 Drug Information

2.1 Drug

2.1.1 CAS Registry Number: 857890-39-2

2.1.2 Generic Name: None

2.1.3 Code Name: E7080; ER-203492-13; JM1; JM6

2.1.4 Chemical Name:
4-[3-Chloro-4-(*N*'-cyclopropylureido)phenoxy]-7-methoxyquinoline-6-carboxamide methanesulfonate

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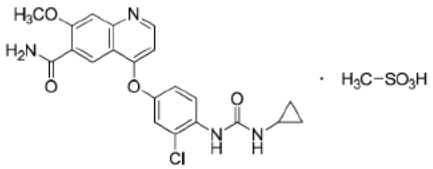
2.1.5 Molecular Formula/Molecular Weight: $C_{21}H_{19}ClN_4O_4 \bullet CH_4O_3S$/522.96 (mesylate salt)

2.1.6 Structure



2.1.7 Pharmacologic Class:
Kinase Inhibitor

2.2 Relevant IND/s:

IND (b) (4), IND (b) (4), IND 113656, IND (b) (4), and IND (b) (4).

2.3 Clinical Formulation

2.3.1 Drug Formulation

Lenvatinib mesilate is manufactured at the Kashima Plant, Eisai Co., Ltd. Ibaraki-ken, Japan.

Lenvatinib is supplied as 4 and 10 mg hypromellose (b) (4) capsules.

Table 1: Composition of Lenvatinib Capsules

Component	Lenvatinib 4 mg Capsule [a]	Lenvatinib 10 mg Capsule [a]	Function	Specification Reference
	Amount (mg)			
(b) (4)				
Lenvatinib mesilate (equivalent to free base)	(4.0)	(10.0)	Drug substance	In-house
Calcium carbonate	(b) (4)		(b) (4)	USP
Mannitol				USP
Microcrystalline cellulose				NF
Hydroxypropyl cellulose				NF
(b) (4) hydroxypropyl cellulose				NF
(b) (4)				USP
(b) (4)				
				NF
Talc				USP
(b) (4				-
Capsule				
(b) (4				-
Hypromellose capsule [c]			Capsule	JP
Total Weight			-	-

JP = Japanese Pharmacopoeia, NF = National Formulary (US), q.s. = quantum sufficit, USP = United States Pharmacopeia.

(b) (4)

(Excerpted from Applicant's submission)

2.3.2 Comments on Novel Excipients:

N/A

2.3.3 Impurities – Comments and Qualification

The Applicant examined the genotoxic potential of 14 identified impurities in valid in vitro Ames Assays using the pre-incubation method in the presence or absence of S9 mix and bacterial strains *Salmonella typhimurium* TA100, TA1535, TA98, TA1537, and

Escherichia coli WP2 *uvrA*(pKM101). The impurities listed in Table 2 were determined to have mutagenic potential under the conditions tested.

Table 2: Potentially Mutagenic Impurities as Determined by In Vitro Ames Assays

Impurity	Study #	Mutagenic effects	Mutagenicity in presence/absence of S9
(b) (4)	K12026	TA100, TA98, and TA1537	Presence of S9
	K13008	TA100	Presence and absence of S9
	USA-R-08034	WP2 *uvrA*	Absence of S9
	USA-R-08040	TA1539	Presence and absence of S9
	USA-R-08576	TA100 and TA98	Presence of S9

* = Present in drug substance and/or drug product and required qualification, which is summarized below

The CMC review team identified four impurities in the lenvatinib drug substance and one in the drug product for qualification. At the recommended clinical dose for lenvatinib of 24 mg, the resulting daily doses of (b) (4) at the proposed specifications of mg/m^2/day, respectively. These impurities were present at the same levels in the nonclinical batch used in the 13-week study; at the highest dose administered to rats at which was there no mortality in this study ((b) (4)), animals received (b) (4) above the proposed human intake of these impurities. Thus, the two non-genotoxic impurities specified in the lenvatinib drug substance, impurities (b) (4) were acceptably qualified at the proposed specification limits of (b) (4)%, respectively.

The genotoxic impurity (b) (4) will be controlled at (b) (4) ppm in lenvatinib mesilate. Based on a clinical dose of 24 mg/day, the daily intake for (b) (4) at the proposed specification of (b) (4) ppm would be (b) (4) µg/person/day. This is below the threshold of toxicological concern of 1.5 µg/day based on a lifetime risk of cancer. Thus, the proposed acceptance criteria for genotoxic impurity (b) (4) is acceptable. (b) (4) is also qualified in the 4-week rat toxicology study # S03016.

Genotoxic impurity (b) (4) is also a degradant (b) (4) At the proposed limit of (b) (4) ppm in the drug product, the daily intake for (b) (4) resulting from the clinical dose of 24 mg would be (b) (4) µg/person/day. While this amount is above the acceptable clinical intake of 10 µg/day defined in the ICH M7 Guidance for Industry, "Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk" for an individual genotoxic impurity taken for between 1 and 10 years, this guidance does not apply to the pharmaceuticals intended for the treatment of patients with advanced cancer. Given the intended patient population, the proposed drug product specification is acceptable based on ICH Q3B(R2), since (b) (4) µg/person/day is well below the qualification threshold of 120 µg/day (0.5% of 24 mg) specified in this guidance. (b) (4) is

also qualified in the 4-week rat toxicology study. In summary, the identified genotoxic impurities in the lenvatinib drug substance and drug product are controlled to acceptable levels for the treatment of patients with cancer.

The CMC review team consulted the pharmacology/toxicology review team regarding the acceptance criterion of [redacted] (b) (4) used in the manufacturing process of the lenvatinib drug substance, which is not listed in the ICH Q3C guidance. Based on the process outlined in the ICH Q3C guidance, the Applicant calculated a permitted daily exposure of [redacted] (b) (4) using a NOEL/NOAEL (20 mg/kg/day) identified in a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG422) in rats (public information in the CHRIP database and reviewed by the Hazard-Data Evaluation Committee of the National Institute of Technology and Evaluation in Japan in 2010; http://www.safe.nite.go.jp/english/sougou/view/ComprehensiveInfoDisplay_en.faces, accessed January 9, 2015). This approach was acceptable. Dose levels tested were 0, 4, 20 and 100 mg/kg/day for 42 days for males and 42-54 days for females. At the recommended clinical dose for lenvatinib of 24 mg, the resulting daily dose of [redacted] (b) (4) at the proposed specification of [redacted] (b) (4) ppm would be [redacted] (b) (4) µg/person/day. Thus, the proposed acceptance criterion for [redacted] (b) (4) is acceptable.

2.6 Proposed Clinical Population and Dosing Regimen

The proposed clinical dose of lenvatinib is 24 mg administered orally as two 10 mg capsules and one 4 mg capsule once daily.

2.7 Regulatory Background

- IND [redacted] (b) (4) for lenvatinib was submitted to the Office of Hematology and Oncology Products in March, 2005.
- IND 113,656 was opened in the Division of Oncology Products 2 in November, 2011 via an administrative split for the thyroid cancer indication.
- Orphan drug designation was granted on December 27, 2012.

3 Studies Submitted

3.1.1 Studies Reviewed

Pharmacology

Study #	Study title
M03009	E7080: Selective Inhibitory Activities in Receptor Tyrosine Kinase Assays
W-20120814	E7080: IC50 Determination study of JM1 and JM2 prepared for Eisai Co., Ltd.
W-20120816	IC50 Determination study of 6 compounds prepared for Eisai Co., Ltd.
M06002	E7080 Effects of E7080 Metabolites M1-M3 Produced by Liver Microsomes on Vascular Endothelial Growth-Factor Driven Proliferation of Human Umbilical Vein Endothelial Cell
W-20140522	X-Ray Structure Analysis of Human KDR (VEGFR2) in Complex with Either Lenvatinib and Sorafenib

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Study #	Study title
M03005	E7080: Effect on a Vascular Endothelial Growth Factor-driven Human Umbilical Vein Endothelial Cell Sandwich Tube Formation Model
M03006	E7080: Effect on Vascular Endothelial Growth Factor-driven Proliferation of Human Umbilical Vein Endothelial Cell
M03008	E7080: Effect on a Vascular Endothelial Growth Factor-driven Kinase Insert Domain Receptor Phosphorylation of Human Umbilical Vein Endothelial Cell
A10004	E7080: The Anti-tumor Effect of E7080 in a Mouse Xenograft Model of Human Thyroid Carcinoma Cell Line K1
M13003	Antitumor Activity of Lenvatinib Mesilate through the Inhibition of Antiangiogenic Activity in Human Anaplastic Thyroid Cancer Cell Line 8305C Xenografts in Mice
W-20120359	The Antitumor Effects of E7080 in Mouse Xenograft Models of Human Thyroid Carcinoma Cell Line TT and SW579
6633	In vitro Pharmacology: ExpresSProfile Study of E7080

Safety Pharmacology

Study #	Study title
DJNR1029	E7080: Effect on hERG Tail Current Recorded from Stably Transfected HEK293 Cells
B030401	Effects on General Physical Condition and Behavior by Oral Administration in Rats
B030402	E7080: Effects on Cardiovascular System and Body Temperature by Oral Administration in Conscious Dogs
B030403	E7080: Effects on Action Potential Parameters in Isolated Papillary Muscles of Guinea-Pig
S03019	E7080: Effects on Respiratory Function by Oral Administration in Rats

Pharmacokinetics

Study #	Study title
AE-4150-G	Pharmacokinetic Study of E7080: Absorption, Distribution, and Excretion of Radioactivity after a Single Oral Administration of ^{14}C-E7080 to Rats
AE-4151-G	Pharmacokinetic Study of E7080: Absorption, Distribution, and Excretion of Radioactivity after a Single Oral Administration of ^{14}C-E7080 to Monkeys
AE-6750-G	E7080: Placental Transfer and Transfer into Milk after a Single Oral Administration of ^{14}C-E7080 in Rats
B03025	E7080: Structural Analysis of E7080 Metabolites Produced by Mouse, Rat, Dog, Monkey, and Human Liver Microsomes
AE-6748-G	E7080: Metabolism in Rats and Monkeys after Single Administration of ^{14}C-E7080

General Toxicology/Toxicokinetics

Study #	Study title
S03016	E7080: A 4-Week Oral Toxicity Study in Rats
S04001	E7080: A 4-Week Oral Toxicity Study with 4-Week Recovery Period in Rats
S03077	E7080: A 4-Week Oral Toxicity Study with 4-Week Recovery Period in Dogs
SBL47-86	E7080: A 4-Week Oral Toxicity Study with 4-Week Recovery Period in Monkeys

Reference ID: 3686430

Study #	Study title
S08037	E7080: A 26-Week Oral Toxicity Study in Rats
SBL038-031	E7080: A 39-Week Oral Toxicity Study in Monkeys

Genetic toxicology

Study #	Study title
S03007	E7080: Reverse Mutation Assay in Bacteria
S03008	E7080: Mouse Lymphoma TK Assay
S05032	E7080: Micronucleus Assay in Rats Following Oral Administration
K11027	(b) (4) Reverse Mutation Assay in Bacteria
K11028	Reverse Mutation Assay in Bacteria
K12025	Reverse Mutation Assay in Bacteria
K12026	Reverse Mutation Assay in Bacteria
K13008	Reverse Mutation Assay in Bacteria
K13018	Reverse Mutation Assay in Bacteria
K13019	Reverse Mutation Assay in Bacteria
USA-R-08034	Reverse-Mutation Assay of (b) (4) in Bacteria
USA-R-08037	Reverse Mutation Test of (b) (4) using Bacteria
USA-R-08038	Reverse Mutation Test of (b) (4) using Bacteria
USA-R-08039	Reverse Mutation Test of (b) (4) using Bacteria
USA-R-08040	Reverse-Mutation Assay of (b) (4) using Bacteria
USA-R-08576	Reverse-Mutation Assay of (b) (4) Hydrate in Bacteria
USA-R-10121	(b) (4) Reverse Mutation Assay in Bacteria

Reproductive toxicology

Study #	Study title
S05152	E7080: An Oral Embryo-Fetal Development Study in Rats
S06009	E7080: An Oral Embryo-Fetal Development Study in Rabbits
J13193	E7080: A 8-Week Repeated Dose Oral Toxicity Study with 4-Week Recovery Period in Juvenile Rats

Other toxicology

Study #	Study title
SBL038-070	E7080: Phototoxicity Test in Balb/c 3T3 Cells

3.2 Studies Not Reviewed

Pharmacology

Study #	Study title
JW1012	Antitumor Activity of E7389 (Eribulin Mesylate) In Combination with E7080 (Lenvatinib Mesylate) On Growth Of Subcutaneous A375 Human Malignant Melanoma Xenografts in Athymic Mice
K06008	E7080 Anti-tumor Effect in Combination with Paclitaxel in Human Gastric Cancer MKN-74 Xenograft Model in Mice
K06053	Anti-tumor Effect in Combination with Cisplatin or Carboplatin in Human Non-small Cell Lung Cancer A549 Xenograft Model in Mice
K08004	The Anti-tumor Effect of E7080 in a Mouse Xenograft Model of Human Hepatocellular Carcinoma Cell Line PLC/PRF/5
K08038	The Anti-tumor Effect in Combination with Temozolomide in a Mouse

Reference ID: 3686430

Study #	Study title
	Xenograft Model of Human Malignant Melanoma Cell Line A375
M03007	E7080 Effect on Tumor Cells (H460 and Colo205) Proliferation
M03011	E7080 Anti-tumor Effect in Human Colorectal Cancer Colo205 Xenograft Model in Mice
M03012	E7080 Anti-tumor Effect in Human Lung Cancer H460 Xenograft Model in Mice
M13012	Antitumor Activity of Lenvatinib Mesilate and Sorafenib Tosylate in Human Papillary Thyroid Cancer Cell Line K1 Xenografts in Mice
M13013	Antitumor Activity of Lenvatinib Mesilate and Sorafenib Tosylate in Human Follicular Thyroid Cancer Cell Line RO82-W-1 Xenografts in Mice
W-20100606	Inhibitory Effect of E7050 with or without E7080 on Vascular Endothelial Growth Factor and/or Hepatocyte Growth Factor-driven Proliferation of Human Umbilical Vein Endothelial Cells
W-20100607	Experimental combination therapy of E7050 with E7080 in HGF-expressing human cancer xenograft models in mice
W-20120815	Mode of Inhibitory Action Study of JM1 and JM2 Prepared for Eisai Co., Ltd.
W-20130793	Comparison of Antitumor Effects of E7080 and Sorafenib on PLC/PRF/5 Human Hepatocellular Carcinoma Xenografts in Mice
W-20131038	Additional Statistical Analysis for the Results of Study Report Nos. K08038, JW1012, K06053, K06008, and W-20100607
W-20140526	Kinetic Interaction Analysis of Lenvatinib Mesylate and Sorafenib Tosylate Against VEGFR2

Pharmacokinetics

Study #	Study title
CFQ14045	Preparative Details of [quinoline-2-14C] E7080
CP-3896	Synthesis of [14C] E7080
JCL023451	Validation of the Analytical Method for the Determination of E7080 in Rat Plasma by High-Performance Liquid Chromatography
JCL023461	Validation of the Analytical Method for the Determination of E7080 in Dog Plasma by High-Performance Liquid Chromatography
JCL023471	Validation of the Analytical Method for the Determination of E7080 in Mouse Plasma by High-Performance Liquid Chromatography
TKV0303	Validation of the Bioanalytical Method for the Measurement of E7080 in Monkey Plasma
AE-6917-G	E7080: Absorption, Distribution and Excretion after a Single Administration of ^{14}C-E7080 Radio-labeled on Chlorbenzene Moiety in Monkeys
B03014	E7080: Pharmacokinetic Profile of E7080 after a Single Oral and Intravenous Administration to Female Nude Mice
B03015	E7080: Pharmacokinetic Profile of E7080 after a Single Oral and Intravenous Administration to Rats
B03016	E7080: Pharmacokinetic Profile of E7080 after a Single Oral and Intravenous Administration to Dogs
B04003	E7080: Pharmacokinetic Profile of E7080 after a Single Oral and Intravenous Administration to Monkeys
AE-6918-G	E7080: Metabolism in Monkeys after Single Administration of ^{14}C-CB-E7080
B10006	E7080: Determination of Metabolite of E7080 after Oral Administration of

Reference ID: 3686430

Study #	Study title
	^{14}C-E7080 in Rats and Monkeys
C10320	E7080: NMR Analysis of Three In Vivo Metabolites, Met 13, Met 16, and Met 20 Isolated from ^{14}C-E7080-Administered Monkey Urine
C11465	E7080: Structure Elucidation of an ER-203534-00 Metabolite Produced by Monkey Liver Cytosol and S9
DMPKA2013-156	Simulation of Drug-Drug Interaction Between lenvatinib and CYP3A4 Substrate Midazolam, and CYP2C8 Substrate

General Toxicology

Study #	Study title
TKB02006	E7080: Dose Escalation Oral Toxicity Study in Rats
S04094	E7080: Dose Escalation Oral Toxicity Study in Rats
TKB02022	E7080: Dose Escalation Oral Toxicity Study in Dogs
S03060	E7080: Dose Escalation Oral Toxicity Study in Monkeys
TKB02007	E7080: A 7-Day Oral Dose Range Toxicity Study in Rats (1)
TKB02008	E7080: A 7-Day Oral Dose Range Toxicity Study in Rats (2)
S05039	E7080: A 13-Week Oral Toxicity Study in Rats
TKB02027	E7080: A 7-Day Oral Dose Range Toxicity Study in Dogs
B-5108	E7080: A 4-Week Oral Toxicity Study in Dogs
SBL47-83	E7080: A 2-Week Oral Dose Range Toxicity Study in Monkeys
SBL47-94	E7080: A 13-Week Oral Toxicity Study in Monkeys

Genetic Toxicology

Study #	Study title
K12066	E7080: Reverse Mutation Assay in Bacteria (2)
K12067	E7080: Mouse Lymphoma *Tk* Assay (2)

Reproductive toxicology

Study #	Study title
S05104	E7080: An Oral Dose Range Toxicity Study in Pregnant Rats
S05062	E7090: A 4-Day Oral Dose Range Toxicity Study in Non-Pregnant Rabbits
S05063	E7080: An Oral Dose Range Toxicity Study in Pregnant Rabbits
S05119	E7090: An Oral Dose Range Toxicity Study in Pregnant Rabbits-Follow-up Study
J12412	E7080: A 2-Week Oral Repeated Dose Range Finding Study with 2-Week Recovery Period in Juvenile Rats

Other toxicology

Study #	Study title
T12002	E7080: In Vitro Exploratory Phototoxicity Test

3.3 Previous Reviews Referenced

Pharmacology/toxicology review of IND (b) (4) by Wei Chen, Ph.D.

Reference ID: 3686430

4 Pharmacology

4.1 Primary Pharmacology

M03009: E7080: Selective Inhibitory Activities in Receptor Tyrosine Kinase Assays

The Applicant examined the kinase insert domain receptor (KDR) tyrosine kinase inhibition and the enzyme selectivity profile of E7080 using an in vitro homogenous proximity tyrosine kinase assay with time-resolved fluorescence (HTRF) and ELISA. Recombinant KDR, Flt-1(fms-like tyrosine kinase 1), FGFR1 (fibroblast growth factor receptor 1), c-Met (met proto-oncogene), and PDGFRβ (platelet-derived growth factor receptor beta) kinase domains of receptors were expressed in baculovirus then purified, and EGFR (epidermal growth factor receptor) kinase domain was obtained from Sigma-Aldrich. Serial dilutions of E7080 (Lot No.: 12112750; 0.1 – 10000 nmol/L) were mixed in a 96 well plate with 10 µL of kinase domain solution, 16 µL of poly(GT) solution and 10 µL of ATP solution. After 30 minutes incubation at 30°C, the reaction was stopped. Assays were repeated three times independently.

E7080 inhibited KDR kinase activity with an IC_{50} of 4.0 nmol/L. E7080 inhibited several other kinases as well, but showed weak activity against EGFR and c-Met (see Table 3).

Table 3: IC_{50} of E7080 in receptor tyrosine kinase assays

Selective inhibitory activities of E7080 in receptor tyrosine kinase assays

Enzyme	IC50 (nmol/L)	95% C.I. (nmol/L)
KDR	4.0	1.8-8.7
Flt-1	22	11-45
FGFR1	46	18-120
EGFR	6500	5000-8500
c-Met	1900	1500-2500
PDGFRβ	39	12-130

(Excerpted from Applicant's submission)

E7080 was selective for KDR tyrosine kinase activity, and inhibited Flt-1, FGFR1 and PDGFRβ tyrosine kinase activity.

W-20120814: E7080: IC50 determination study of JM1 and JM2 prepared for Eisai Co., Ltd.

The Applicant measured IC_{50} values for JM1 (lenvatinib) and JM2 (sorafenib) against the following kinases: FGFR1, FGFR2, FGFR3, FLT1, FLT4, KDR, KIT, RET, FGFR3 [K650E], and FGFR3 [K659M]. Concentrations in µM (10, 3, 1, 0.3, 0.1, 0.03, 0.01, 0.003, 0.001, and 0.0003) of JM1 and JM2 were used in an off-chip mobility shift assay (MSA) to determine IC_{50}.

Reference ID: 3686430

Table 4: IC_{50} values for JM1, JM2, and the positive Ref. control, Staurosporine

Kinase	IC50(M) JM1	JM2	Ref.
FGFR1	6.08E-08	3.35E-07	2.47E-09
FGFR2	2.66E-08	1.46E-07	9.14E-10
FGFR3	5.16E-08	3.39E-07	3.41E-09
FGFR3[K650E]	1.13E-07	1.04E-07	1.26E-09
FGFR3[K650M]	2.51E-07	1.10E-07	9.13E-10
FLT1	4.67E-09	2.10E-08	2.54E-09
FLT4	2.32E-09	1.57E-08	4.44E-10
KDR	3.02E-09	2.06E-08	3.41E-09
KIT	8.54E-08	1.44E-07	2.20E-09
RET	6.35E-09	1.46E-08	1.80E-09

(Excerpted from Applicant's submission)

JM1 (lenvatinib) inhibited the receptor tyrosine kinases FGFR1-3 and KIT with IC_{50} values < 100 nM, and FLT1, FLT4, KDR, and RET with IC_{50} values < 10 nM.

W-20120816: IC50 determination study of 6 compounds prepared for Eisai Co., Ltd.

The Applicant measured IC_{50} values for the 6 compounds at concentrations listed in Table 5 against 66 kinases listed in Table 6.

Table 5: List of compounds tested and their respective concentrations

Name	Test concentration (μM)
JM6	10, 3, 1, 0.3, 0.1, 0.03, 0.01, 0.003, 0.001, 0.0003
JM7	10, 3, 1, 0.3, 0.1, 0.03, 0.01, 0.003, 0.001, 0.0003
JM8	10, 3, 1, 0.3, 0.1, 0.03, 0.01, 0.003, 0.001, 0.0003
JM9	10, 3, 1, 0.3, 0.1, 0.03, 0.01, 0.003, 0.001, 0.0003
JM10	10, 3, 1, 0.3, 0.1, 0.03, 0.01, 0.003, 0.001, 0.0003
JM11	50, 15, 5, 1.5, 0.5, 0.15, 0.05, 0.015, 0.005, 0.0015

(Excerpted from Applicant's submission)

Table 6: List of target kinases by compound tested

Target kinase
JM6, JM7
ABL, BRK, CSK, EGFR, EphA1, EphB2, EphB3, EphB4, FAK, FGFR4, FGR, FLT3, FRK, HER2, HER4, IGF1R, INSR, JAK2, LCK, MET, MUSK, PDGFRa, PDGFRb, SRC, SYK, TIE2, KIT[D816V], KIT[T670I], KIT[V560G], KIT[V654A], PDGFRa[T674I], PDGFRa[V561D], RET[M918T], AKT1, AurA, BRAF, CDK2/CycE1, CDK4, CDK6, CHK1, COT, GSK3b, HGK, IKKb, IRAK4, MLK1, NEK2, p70S6K, PIM1, PKACa, PLK1, PRKX, RAF1, SGK3, WEE1, BRAF[V600E]
JM8, JM9, JM10, JM11
ABL, BRK, CSK, EGFR, EphA1, EphB2, EphB3, EphB4, FAK, FGFR1, FGFR2, FGFR3, FGFR4, FGR, FLT1, FLT3, FLT4, FRK, HER2, HER4, IGF1R, INSR, JAK2, KDR, KIT, LCK, MET, MUSK, PDGFRa, PDGFRb, RET, SRC, SYK, TIE2, FGFR3[K650E], FGFR3[K650M], KIT[D816V], KIT[T670I], KIT[V560G], KIT[V654A], PDGFRa[T674I], PDGFRa[V561D], RET[M918T], AKT1, AurA, BRAF, CDK2/CycE1, CDK4, CDK6, CHK1, COT, GSK3b, HGK, IKKb, IRAK4, MLK1, NEK2, p70S6K, PIM1, PKACa, PLK1, PRKX, RAF1, SGK3, WEE1, BRAF[V600E]

(Excerpted from Applicant's submission)

Kinase activity was measured by ELISA and off-chip mobility shift assays (MSA). JM6 (lenvatinib) inhibited 5 kinases with an IC_{50} <100 nmol/L.

Table 7: IC_{50} values for JM6 (lenvatinib)

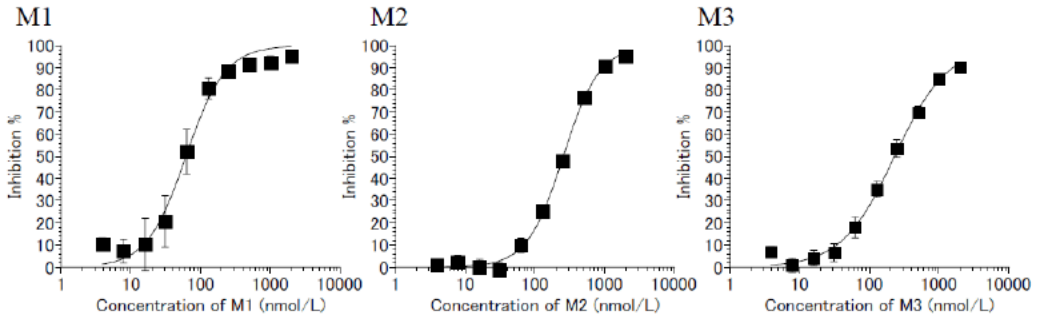
Kinase	IC_{50} nmol/L
KIT (V560G)	0.7
RET (M918T)	12
PDGFRα (V561D)	25
PDGFRα	29
FGFR4	43

M06002: E7080: Effects of E7080 Metabolites M1-M3 produced by Liver Microsomes on Vascular Endothelial Growth Factor-driven Proliferation of Human Umbilical Vein Endothelial Cell

Summary

The Applicant examined the effects of three E7080 (lenvatinib) metabolites, M1-M3, produced by liver microsomes on VEGF-driven proliferation of HUVECs. Briefly, HUVECs were plated onto a microtiter plate and incubated for 3 days with serial dilutions of M1, M2, and M3 at final concentrations of 3.9 – 2000 nmol/L, and VEGF at final concentration of 20 ng/ml, after which viable cell number was determined. M1, M2, and M3 showed anti-proliferative activity against VEGF-driven proliferation of HUVECs in a dose dependent manner with IC_{50} values of 57, 250, and 230 nmol/L, respectively.

Figure 1: Effects of E7080 metabolites M1, M2, and M3 on VEGF-driven proliferation of HUVECs



Data points represent mean ± SE from 3 separate experiments performed in quadruplicate.
(Excerpted from Applicant's submission)

Conclusion: The M1, M2, and M3 metabolites demonstrated the ability to inhibit VEGF-mediated proliferation at concentrations approximately 10 to 60-fold higher than the parent compound.

Study #	Title
M03005	E7080: Effect on a Vascular Endothelial Growth Factor-driven Human Umbilical Vein Endothelial Cell Sandwich Tube Formation Model
M03006	E7080: Effect on Vascular Endothelial Growth Factor-driven Proliferation of Human Umbilical Vein Endothelial Cell
M03008	E7080: Effect on a Vascular Endothelial Growth Factor-driven Kinase Insert Domain Receptor Phosphorylation of Human Umbilical Vein Endothelial Cell

The Applicant evaluated the effects of E7080 on VEGF-driven activation of kinase insert domain receptor (KDR, also known as VEGFR2), and proliferation and angiogenesis using a Human Umbilical Vein Endothelial Cell (HUVEC) model system in studies M03005, M03006, and M03008.

E7080 inhibits tyrosine kinase activity of KDR, a receptor for VEGF. In study M03008, the Applicant examined the effect of E7080 on VEGF-driven KDR activation by using a cell-based autophosphorylation assay. Briefly, HUVECs plated at 4.0 x 10^5 cells/well were incubated overnight, and then incubated for 1 hour after addition of serial dilutions of E7080 (Lot No.: 12112750; 0.16, 0.31, 0.63, 1.3, 2.5, 5, and 10 nmol/L). A final concentration of 20 ng/mL recombinant VEGF was then added to wells and incubated for 5 minutes. Cell lysate proteins separated via SDS-PAGE were immunoblotted with an antibody against KDR or phosphorylated-KDR and band intensity measured using 1D Image Analysis Software. Percent inhibition of phosphorylation was determined by the formula below taken from the Applicant's submission:

$$\text{Ratio in each lane} = \frac{\text{chemiluminescence intensity of phosphorylated-KDR}}{\text{chemiluminescence intensity of KDR}}$$

$$\text{Inhibition (\%)} = \frac{\text{ratio value of control} - \text{ratio value of treatment}}{\text{ratio value of control} - \text{ratio value of blank}} \times 100$$

E7080 inhibited VEGF-driven KDR phosphorylation in HUVECs in a dose-dependent manner with an IC_{50} of 0.25 nmol/L (95% confidence interval: 0.21 to 0.29; Figure 2 and Figure 3). E7080 can block VEGF-driven activation of cellular KDR tyrosine kinase.

Figure 2: Effect of E7080 on VEGF-driven KDR phosphorylation in HUVEC

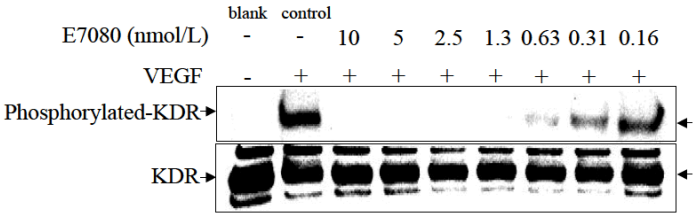


Figure 1 Effect of E7080 on VEGF-driven KDR phosphorylation of HUVEC.

(Excerpted from Applicant's submission)

Figure 3: Inhibition curve of band intensity of E7080 inhibition of KDR phosphorylation

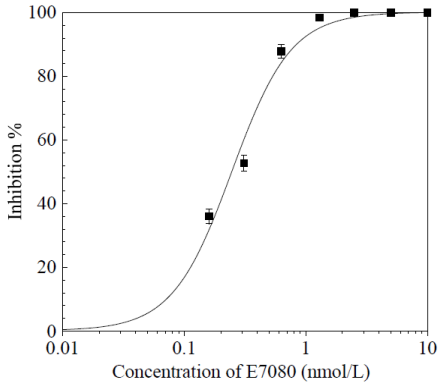


Figure 2 Inhibition curve of band intensity treated with E7080.

Each point represents the mean ± S.E.M. from three separate experiments.

(Excerpted from Applicant's submission)

In study M03005, the Applicant evaluated the effect of E7080 on VEGF-driven tube formation in a HUVEC sandwich tube formation (sTF) model. HUVECs were plated on type I collagen gel, incubated overnight, overlaid with Type I collagen gel, and then gelatin. Serial dilutions of E7080 (Lot No.: 12112750; 0.1, 0.3, 1, 3, 10, 30, and 100 nmol/L) and 20 ng/mL recombinant human VEGF (final concentration) were added to culture wells, incubated for 4 days, and then stained with MTT and imaged with light microscopy. Tube length was measured using image analysis (Angiogenesis Image Analyzer, ver1.04, KURABO, Osaka, Japan). Three experiments were performed in quadruplicate. Percent inhibition of tube formation was determined by the formula below taken from the Applicant's submission:

$$\text{Inhibition (\%)} = \frac{\text{Average value of tube length of control wells*} - \text{tube length of treated wells}}{\text{Average value of tube length of control wells*} - \text{Average value of tube length of blank wells*}} \times 100$$

*Average of quadruplicate

E7080 showed a dose-dependent inhibition of VEGF-driven tube formation with IC_{50} of 2.1 nmol/L (95% C.I.: 2.0 to 2.3; Figure 4). E7080 exhibits anti-angiogenic activity against in vitro tube formation.

Figure 4: Effect of E7080 on a VEGF-driven HUVEC sTF model

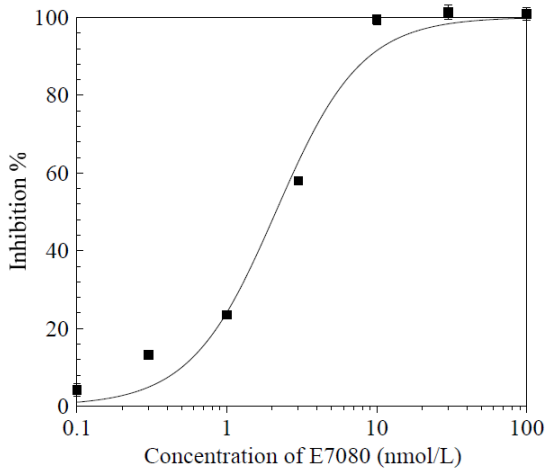


Figure 1 Effect of E7080 on a VEGF-driven HUVEC sandwich TF model
Each point represents the mean ± S.E.M. from three separate experiments, each performed in quadruplicate.

(Excerpted from Applicant's submission)

In study M03006, the Applicant examined the effect of E7080 on VEGF-driven proliferation of HUVECs. HUVECs were plated at 1,500 cells/well into a microtiter plate, incubated overnight, and then serial dilutions of E7080 (Lot No.: 12112750; 0.39, 0.78, 1.6, 3.1, 6.3, 13, 25, 50, 100, 200, and 400 nmol/L) and 20 ng/mL VEGF (final concentration) were added to culture wells and incubated for 3 days. WST-1 reagent was added in each well and viable cell number determined by measurement of absorbance with a microplate reader. Three experiments were performed in quadruplicate. Percent inhibition of cell growth was determined by the formula below taken from the Applicant's submission:

$$\text{Inhibition (\%)} = \frac{\text{Average value of OD of control wells* − OD of treated wells}}{\text{Average value of OD of control wells* − Average value of OD of blank wells*}} \times 100$$

*Average of quadruplicate

E7080 had dose-dependent anti-proliferative activity with an IC_{50} value of 3.4 nmol/L (95% confidence interval: 1.4 to 8.4; Figure 5) against VEGF-driven proliferation of HUVEC.

Figure 5: Effect of E7080 on VEGF-driven proliferation in HUVEC

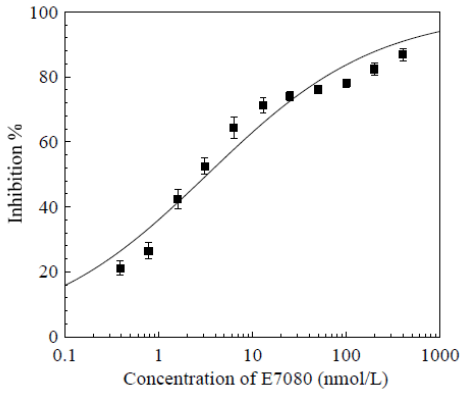


Figure 1 Effect of E7080 on VEGF-driven proliferation of HUVEC.
Each point represents the mean ± S.E.M. from three separated experiments, each performed in quadruplicate.

(Excerpted from Applicant's submission)

Conclusions: E7080 blocks VEGF-driven activation of cellular KDR tyrosine kinase, and exhibits anti-angiogenic and anti-proliferative activity against endothelial cells.

W-20140522: X-Ray Structure Analysis of Human KDR (VEGFR2) in Complex with Either Lenvatinib and Sorafenib

The Applicant had X-ray structural analysis performed on human KDR (VEGFR2) in complex with lenvatinib or sorafenib to determine the detailed binding mode of both inhibitors. The results for sorafenib correlated with previous reports (McTigue, Murray, et al. 2012). The amino acid residues located near lenvatinib and sorafenib in this structure were identified as the protein's ATP-binding site including a gate keeper residue and a non-conserved allosteric neighboring region. Lenvatinib and sorafenib could bind to the ATP-binding site at their common core from the urea group to quinoline ring (lenvatinib) and pyridine (sorafenib), and bind the neighboring region via the cyclopropane ring (lenvatinib) and 4-chloro-3-(trifluoromethyl) phenyl ring (sorafenib). Both compounds could exert their kinase inhibitory activity through binding to the ATP-binding site and their kinase selectivity through binding the neighboring region.

A10004: E7080: The Anti-tumor Effect of E7080 in a Mouse Xenograft Model of Human Thyroid Carcinoma Cell Line K1

Methods

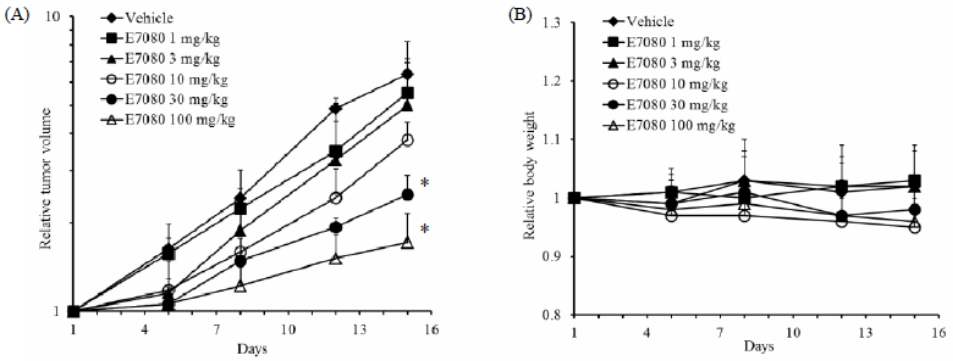
Drug:	E7080
Doses:	0, 1, 3, 10, 30, and 100 mg/kg
Frequency of dosing:	Once daily for 14 days
Route of administration:	Oral gavage
Dose volume:	0.2 mL/10 g body weight
Formulation/Vehicle:	Sterile distilled water

Species/Strain:	Mouse / CAnN.Cg-Foxn1^{nu}/CrlCrlj
Number/Sex/Group:	6 females per group
Age:	6 – 7 weeks
Weight:	~18 – 23 g
Study design:	Human thyroid carcinoma cell line, K1, suspended at a density of 5 × 10^7 cells/mL in PBS and Matrigel™ subcutaneously inoculated into the right flank region

The Applicant evaluated the anti-tumor effect of E7080 on tumor growth of human papillary thyroid carcinoma cell line, K1, in a mouse xenograft model. E7080 inhibits tyrosine kinase activity of kinase insert domain receptor (KDR), a receptor for vascular endothelial growth factor (VEGF). The K1 cell line was subcutaneously inoculated in to female athymic mice. When tumor volume (TV) reached 201 mm^3, mice were dosed with vehicle or E7080 orally once a day from Day 1 – 14 (6 mice/group); tumor volume and body weight were measured on Days 1, 5, 8, 12, and 15.

Daily administration of E7080 for 14 days to mice implanted with the K1 human thyroid carcinoma cell line resulted in delayed tumor-growth in a dose-dependent manner without statistically significant effects on body weight (Figure 6).

Figure 6: The effect of E7080 on tumor-growth (A) and body weight (B) in human thyroid carcinoma cell line, K1 xenograft model in mice



Data expressed as mean ± SD (n = 6), *p < 0.05 versus vehicle, Dunnett's type multiple comparison test.
(Excerpted from Applicant's submission)

M13003: Antitumor Activity of Lenvatinib Mesilate through the Inhibition of Antiangiogenic Activity in Human Anaplastic Thyroid Cancer Cell Line 8305C Xenografts in Mice

Methods

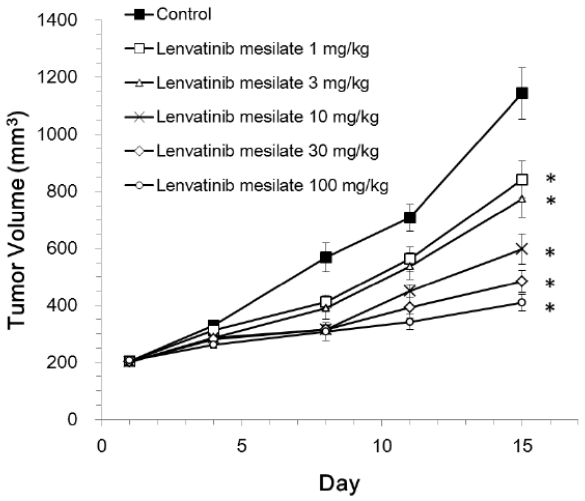
Drug:	Lenvatinib mesilate
Doses:	0, 1, 3, 10, 30, and 100 mg/kg
Frequency of dosing:	Once daily for 14 days
Route of administration:	Oral gavage
Dose volume:	0.2 mL/10 g body weight
Formulation/Vehicle:	Sterile distilled water

Species/Strain:	Mouse / CAnN.Cg-Foxn1^{nu}/CrlCrlj
Number/Sex/Group:	6 females/dose
Age:	7 weeks
Weight:	~18 – 25 g
Study design:	Human anaplastic thyroid cancer cell line 8305C, subcutaneously inoculated into the right flank region

The Applicant evaluated the effect of lenvatinib mesilate on both tumor growth and tumor angiogenesis in human anaplastic cancer cell line 8305C xenografts in athymic mice. The cell line was inoculated into the right flank region of female athymic mice. When mean tumor volume (TV) reached ~194 mm^3, mice were treated with lenvatinib or vehicle orally once a day from Day 1 – 14 (6 mice/group); TV and body weight were measured on Days 1, 4, 8, 11, and 15. After TV and body weight measurements on Day 15, tissue fragments from isolated tumor specimens were used for immunohistochemical staining of endothelial marker, CD31, for microvessel density (MVD) analysis. MVD was conducted by Aperio Microvessel Analysis algorithm using Aperio ImageScope™.

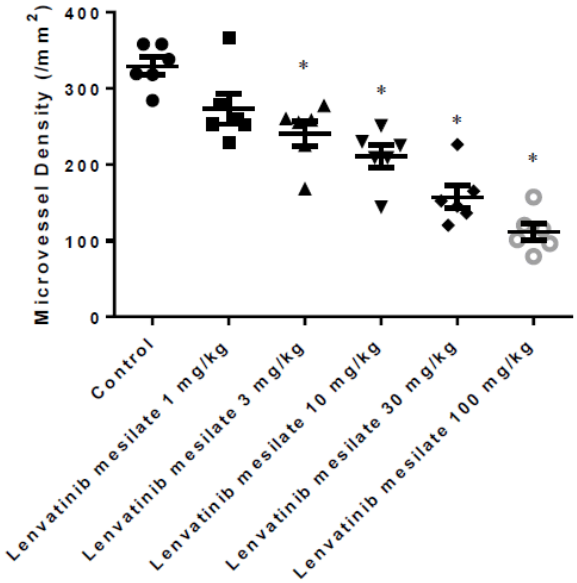
Lenvatinib mesilate had a significant dose-dependent reduction in TV compared to vehicle control on Day 15 (Figure 7). Lenvatinib mesilate in the dose range of 3 to 100 mg/kg significantly and dose-dependently decreased MVD in the 8305C tumors on Day 15 (Figure 8). There was no significant effect on body weight.

Figure 7: Anti-tumor activity of lenvatinib mesilate on human anaplastic thyroid cancer cell line 8305C xenografts in athymic mice



Data expressed as mean ± SEM (n=6), * $p < 0.05$ versus control group, Dunnett's multiple comparison test. (Excerpted from Applicant's submission)

Figure 8: Anti-angiogenic activity of lenvatinib mesilate on human anaplastic thyroid cancer cell line 80305C xenografts in athymic mice



Data expressed as mean ± SEM (n= 6), *p < 0.05 versus control group, Dunnett's multiple comparison test. (Excerpted from Applicant's submission)

Once-daily oral administration of lenvatinib mesilate for 14 days significantly and dose-dependently inhibited tumor growth and angiogenesis in human anaplastic thyroid cancer cell line 8305C xenografts in athymic mice at the doses of 3, 10, 30, and 100 mg/kg.

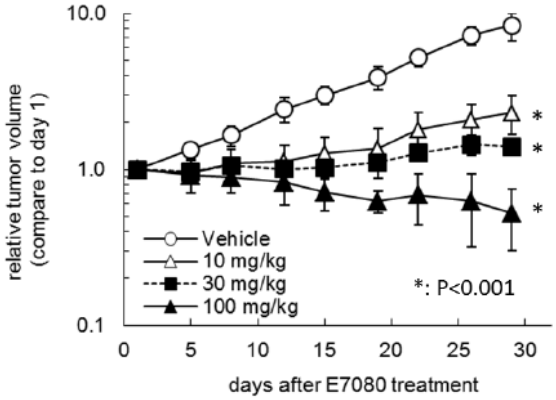
W-20120359: The Antitumor Effects of E7080 in Mouse Xenograft Models of Human Thyroid Carcinoma Cell Line TT and SW579
Methods

Drug:	E7080
Doses:	10, 30, and 100 mg/kg for TT 3, 10, 30, and 100 mg/kg for SW579
Frequency of dosing:	Once daily for 28 days for TT Once daily for 14 days for SW579
Route of administration:	Oral gavage
Dose volume:	0.2 mL/10 g body weight
Formulation/Vehicle:	Sterile distilled water
Species/Strain:	Mouse / CAnN.Cg-Foxn1^{nu}/CrlCrlj
Number/Sex/Group:	4 - 5 females/dose
Age:	9 - 10 weeks
Weight:	20 - 26 g
Study design:	A 0.1-mL aliquot of cell suspension at a density of 10×10^7 cells/mL was transplanted subcutaneously into the right flank region.

The Applicant evaluated the anti-tumor activity of E7080 in two thyroid carcinoma xenograft models in vivo. TT is a medullary thyroid carcinoma cell line, and SW579 is a thyroid-derived squamous cell carcinoma. E7080 is an inhibitor of both kinase insert domain receptor (KDR, also known as vascular endothelial growth factor receptor 2) and ret proto-oncogene (RET). When tumor volume (TV) reached 131 mm^3 for TT and 154 mm^3 for SW579, E7080 was administered orally for 28 or 14 days, respectively. TV and body weight were measured nine times (Days 1, 5, 8, 12, 15, 19, 22, 26, and 29) for TT xenograft mice, and measured seven times (Days 1, 3, 5, 8, 10, 12, and 15) for SW570 xenograft mice.

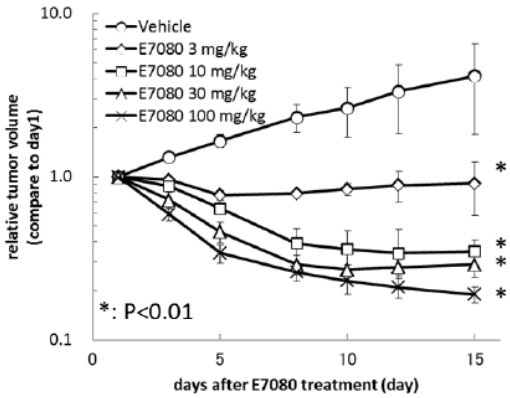
E7080 showed a significant anti-tumor effect against subcutaneous TT xenografts at doses ranging from 10 to 100 mg/kg (Figure 9), and a significant anti-tumor effect against subcutaneous SW579 xenografts at doses ranging from 3 to 100 mg/kg (Figure 10).

Figure 9: Anti-tumor activity of E7080 in subcutaneous TT xenograft model



Data expressed as mean ± SD (n = 4-5), *p < 0.001 versus vehicle, Dunnett's multiple comparison test.
(Excerpted from Applicant's submission)

Figure 10: Anti-tumor activity of E7080 in subcutaneous SW579 xenograft model



Data expressed as mean ± SD (n = 4-5), *p < 0.01 versus vehicle, Dunnett's multiple comparison test.
(Excerpted from Applicant's submission)

4.2 Secondary Pharmacology

6633: In vitro Pharmacology: ExpresSProfile Study of E7080

The Applicant evaluated the off-target binding of E7080 to various physiologically important cellular targets in vitro. E7080 (Lot No. 12112750) was tested at concentrations of 10^{-6} and 10^{-5} M against a panel of 50 receptors (ExpresSProfile) in duplicate. E7080 binding was calculated as % inhibition of the binding of a radioactively labeled ligand specific for each target; the specific ligand binding to the receptors was defined as the difference between the total binding and the nonspecific binding determined in the presence of an excess of unlabeled ligand. No significant binding (>50% inhibition) to any receptor of the ExpresSProfile was observed at the tested concentrations except for 5-HT_{1B} receptor and human NE transporter at 10^{-5} M. Note that there was a 38% inhibition for 5-HT_{1A} receptors.

Table 8: Off-target binding of E7080

Receptor	E7080 concentration (M)	% inhibition
5-HT_{1B}	1×10^{-5}	58
NE transporter	1×10^{-5}	50
5-HT_{1A}	1×10^{-5}	38

While there was ≥ 50% inhibition of the 5-HT_{1B} receptor and human NE transporter, and 38% inhibition of 5-HT_{1A}, the 1×10^{-5} M concentration of E7080 is not physiologically relevant. The C_{max} from human pharmacokinetic data ranges from 0.04×10^{-5} M to 0.1×10^{-5} M. No significant inhibition was observed when the lenvatinib concentration was lowered to 1×10^{-6} M.

4.3 Safety Pharmacology

Study title: Effects on General Physical Condition and Behavior by Oral Administration in Rats

Study no.:	B030401
Study report location:	EDR 4.2.1.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 10, 2003
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	E7080 / 13033151 / 97.7% (anhydrous basis), 79.6% (as is)

Methods

Doses:	10, 30, and 100 mg/kg
Frequency of dosing:	Single dose
Route of administration:	Oral gavage
Dose volume:	2 mL/kg
Formulation/Vehicle:	75% PEG400
Species/Strain:	Male Sprague-Dawley Rats
Number/Sex/Group:	6 males per group
Age:	6 weeks
Weight:	214 – 254 g

The Applicant assessed the effects of E7080 on the general physical condition and behavior in male Sprague-Dawley rats after single oral administration using Irwin's method. A previous 7-day oral dose-range toxicity study in rats showed toxicological findings at doses of ≥ 100 mg/kg, thus the high dose was set at 100 mg/kg. The Irwin battery of multiple observations occurred prior to administration of E7080 and at 1, 2, 4, and 8 hours post-dosing.

Concentrations of suspensions were analyzed sampled from the top, middle, and bottom of the suspension using HPLC; all concentrations fell within the acceptable range ± 10%.

All doses up to 100 mg/kg showed no effects on general physical condition and behavior in rats.

Table 9: Effects of E7080 on general physical conditions and behavior in adult male rats

Table 1 Effects of E7080 on general physical condition and behavior in rats

Test substance	Dose (mg/kg p.o.)	Signs	Before	Time after administration (hour)			
				1	2	4	8
Vehicle [1)]	-	No observable effect	6/6 [2)]	6/6	6/6	6/6	6/6
E7080	10	No observable effect	6/6	6/6	6/6	6/6	6/6
	30	No observable effect	6/6	6/6	6/6	6/6	6/6
	100	No observable effect	6/6	6/6	6/6	6/6	6/6

1) 75% PEG400

2) Number of animals showing the sign / Number of animals tested.

(Excerpted from Applicant's submission)

Study title: E7080: Effects on Respiratory Function by Oral Administration in Rats

Study no.:	S03019
Study report location:	EDR 4.2.1.3
Conducting laboratory and location:	Kawashima Research Drug Safety Research Laboratories, Eisai Co., Ltd.

Reference ID: 3686430

	1 Takehaya, Kawashima-cho, Hashima-gun, Gifu, JAPAN
Date of study initiation:	May 28, 2003
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	E7080 / 13033151 / 97.7% (anhydrous basis), 79.6% (as is)

Methods

Doses:	10, 30, and 100 mg/kg
Frequency of dosing:	Single dose
Route of administration:	Oral gavage
Dose volume:	2 mL/kg
Formulation/Vehicle:	75% PEG400, water
Species/Strain:	Sprague-Dawley rats – males
Number/Sex/Group:	6 males / group
Age:	8 weeks
Weight:	228 – 276 g
Study design:	Whole body unrestrained plethysmograph (BioSystem XA, Buxco Electronics, Inc., CT, USA). Respiratory rate (breaths/min) and tidal volume (mL/breath) measured pre-dose, 1, 2, 4, and 8 hours post-dose, and minute volume (mL/min) was calculated by multiplying tidal volume by respiratory rate.

Dose selection was based on a 7-day oral toxicity study in rats (at doses of 100, 300, and 1000 mg/kg) where decreased platelet count, increased ALT and AST, and pathologic changes occurred at ≥ 100 mg/kg; therefore, 10, 30, and 100 mg/kg were selected.

Key Study Findings:

- Oral administration of E7080 as a single dose at dose levels of 10, 30, or 100 mg/kg had no statistically significant effects on measured respiratory function parameters in male Sprague-Dawley rats.

Study title: E7080: Effect on hERG Tail Current Recorded from Stably Transfected HEK293 Cells

Study no.:	DJNR1029
Study report location:	EDR 4.2.1.3
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	June 16, 2003
GLP compliance:	Yes

QA statement: Yes
Drug, lot #, and % purity: E7080 / 13033151 / 97.7% (anhydrous basis), 79.6% (as is)

Objective: Evaluate the functional effects of E7080 on human ether-a-go-go-related gene (hERG)-encoded tail current recorded from human embryonic kidney 293 (HEK293) cells stably transfected with hERG complimentary DNA.

Methods

Concentrations: 0.3, 1, 3, 10, and 30 μM n = 4 cells/concentration
Vehicle control: DMSO (final concentration 0.1%) in bath solution (in mM: NaCl 137; KCl 4; $CaCl_2$ 1.8; $MgCl_2$ 1.0; D-glucose 10; HEPES 10; pH 7.4 with 1M NaOH) n = 4 cells
Positive control: 100 nM E-4031 n = 2 cells

Key Study Findings:

- E7080 significantly inhibited hERG tail current at concentrations ≥ 10 μM
- IC_{50} = 11.89 μM
- E7080 stock solution stability was confirmed up to 24 hours following formulation and determined to be within ± 10% of nominal concentration.
- E7080 is not considered to pose a significant risk based on an IC_{50} above 10 μM.

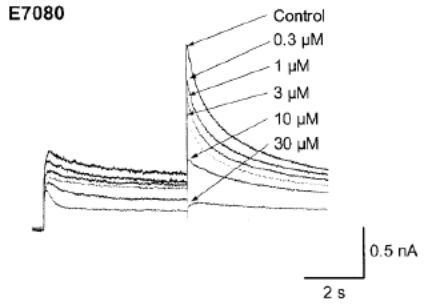
E7080 inhibited hERG tail current in a concentration-dependent manner with statistically significant inhibition of tail current observed at concentrations of 10 and 30 μM ($p < 0.01$, compared to vehicle; one-way ANOVA followed by Dunnett's t-test). When E7080 and vehicle treated groups were compared, 0.3, 1, and 3 μM E7080 had no statistically significant inhibitory effect on hERG tail current. E-4031 (100 nM), the positive control, inhibited hERG tail current by 90.8%.

Table 10: Concentration-response relationship of E7080 on hERG tail current

Concentration of E7080 (μM)	Tail Current (% Control)	Vehicle Corrected Tail Current (% Control)
0.3	86.3 ± 4.7	108.7 ± 5.9
1	76.7 ± 5.9	96.6 ± 7.4
3	67.0 ± 4.9	84.3 ± 6.2
10	45.7 ± 3.3**	57.5 ± 4.1
30	14.1 ± 1.0**	17.7 ± 1.3

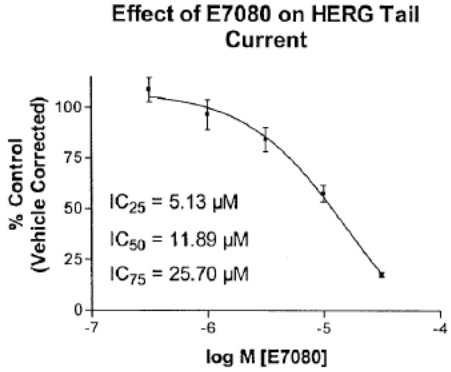
**p<0.01 compared to vehicle, one-way ANOVA, Dunnett's t-test. Data are mean ± SE. (Excerpted from Applicant's submission)

Figure 11: Effect of E7080 on hERG tail currents



(Excerpted from Applicant's submission)

Figure 12: Effect of E7080 on hERG tail current



(Excerpted from Applicant's submission)

Study title: E7080: Effects on Action Potential Parameters in Isolated Papillary Muscles of Guinea-Pig

Study no.:	B030403
Study report location:	EDR 4.2.1.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 12, 2003
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	E7080 / 13033151 / 97.7% (anhydrous basis), 79.6% (as is)

The Applicant evaluated the effects of E7080 on the following action potential parameters of the papillary muscle of guinea pigs: resting membrane potential (RMP), action potential amplitude (APA), maximal upstroke velocity of depolarization (V max), and action potential duration at 50% (APD_{50}) and 90% (APD_{90}) repolarization. Papillary muscle was isolated from the right ventricle of the heart and fixed in Tyrode's solution (in mM: 125 NaCl, 4 KCl, 25 $NaHCO_3$, 1.8 NaH_2PO_4, 0.5 $MgCl_2$, 2.7 $CaCl_2$, 5.5 glucose), and aerated with a 95 – 5% O_2 – CO_2 mix. E7080 diluted in DMSO was

perfused on to the papillary muscle of guinea pigs at 1 and 10 µmol/L (6 / group) and parameters were recorded via glass microelectrode filled with 3 M KCl (resistance: 10 - 30 MΩ), signals were amplified and recorded using pCLAMP software (Axon Instruments).

E7080 had no significant effects on action potential parameters (RMP, APA, APD_{50}, APD_{90}, and V max) in the isolated papillary muscles from male guinea pigs.

Table 11: Effects of E7080 on action potential parameters in the isolated papillary muscles of the male guinea pig

Table 1 Effects of E7080 on action potential parameters in the isolated papillary muscles of the guinea-pig

Test substance	Concentration (µmol/L)	Percentage of value just before application of the test substance				
		RMP	APA	APD_{50}	APD_{90}	V max
Vehicle [1)]	---	100.6 ± 0.5	100.1 ± 0.2	98.8 ± 0.8	99.0 ± 0.7	99.2 ± 2.0
E7080	1	100.4 ± 0.2	100.4 ± 0.3	99.3 ± 0.8	99.5 ± 0.6	101.8 ± 1.4
	10	100.0 ± 0.3	100.0 ± 0.0	99.6 ± 1.0	101.5 ± 0.8	100.7 ± 0.7

Each value represents the mean ± S.E. of 6 preparations

RMP: resting membrane potential, APA: action potential amplitude, APD_{50} and APD_{90}: action potential durations at 50% and 90% repolarization, respectively, V max: maximal upstroke velocity.

1) Dimethyl sulfoxide (Tyrode's solution containing 0.1% dimethyl sulfoxide).

Data represented as percent ratio of before value / 30 minutes incubation value (before treatment value / after treatment value x 100). (Excerpted from Applicant's submission)

Study title: E7080: Effects on Cardiovascular System and Body Temperature by Oral Administration in Conscious Dogs

Study no.:	B030402
Study report location:	EDR 4.2.1.3
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	May 27, 2003
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	E7080 / 13033151 / 97.7% (anhydrous basis), 79.6% (as is)

Methods

Doses: 6 and 30 mg/kg
Frequency of dosing: Single dose
Route of administration: Oral gavage
Dose volume: 0.5 oz gelatin capsule (lot # 1231) filled up 10-fold trituration with lactose
Formulation/Vehicle: Lactose
Species/Strain: Dog Beagle
Number/Sex/Group: 3 / group of each sex
Age: 7 – 8 months
Weight: 6.8 – 8.8 kg

Groups	Doses mg/kg p.o.	Number of animals* Males	Females
Lactose	270	3	3
E7080	6	3	3
E7080	30	3	3

*The same 3 males and females received all dose levels with a 6 day washout period between doses.

The Applicant evaluated the effects of E7080 on heart rate (HR), blood pressure (BP), electrocardiograms (PR interval, QRS duration, and QT interval), and body temperature (BT) after single oral administration in male and female Beagle dogs using a telemetry system. Parameters were measured pre-dose, 1, 2, 4, and 8 hours after administration.

HR in male dogs decreased 23% 4 hours post dose at 6 mg/kg compared to vehicle treated dogs. BP in male dogs increased 29% and 23% 2 hours post dose at 6 and 30 mg/kg, respectively, compared to vehicle treated dogs. QRS duration in male dogs decreased 13% and 11% at 1 and 4 hours post dose at the 30 mg/kg dose level compared to vehicle treated dogs. Though QT prolongation has been observed in clinical trials, there were no clear effects on QTc observed in this study.

Table 12: ECG parameters in male and female dogs dosed with E7080

ECG Parameter	Dose (mg/kg)	Time post-dose (hours)	Significant % change vs. vehicle control animals	% change vs. within group baseline (not significant)
Heart Rate	6	4	↓23%	↓13%
Blood Pressure	6	2	↑29%	↑25%
	30	2	↑23%	↑13%

All significant parameter changes occurred in males only. QTc was not calculated because E7080 showed no consistent effect on heart rate. Changes were only significant when compared to the vehicle control groups at the same time point. Comparison of predose baseline did not give significance, so the Applicant stated that E7080 had no effects on ECG parameters.

Table 13: E7080 human equivalent doses based on dog doses in cardiovascular and body temperature evaluation

Single dose in dogs	HED	Indicated use dose
6 mg/kg	~70 mg	24 mg daily
30 mg/kg	~350 mg	

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Table 14: Summary of Pharmacokinetic Parameters Following a Single Oral or Intravenous Administration of Lenvatinib to Animals

Species/Strain/Gender	Dosing Route	Doses[a] (mg/kg)	$AUC_{(0\text{-}inf)}$ (µg·h/mL)	CL_p (mL/h/kg)	V_{ss} (mL/kg)	$t_{½}$ (h)	C_{max} (µg/mL)	t_{max} (h)	F (%)
Mouse / BALB/c AnNCrj-nu/nu / Female	IV	3	8.686	345.4	714.3	2.05	7.0542[b]	NA	NA
	PO	3	5.596	NA	NA	2.09	1.9651	0.5	64.4
	PO	10	27.720	NA	NA	1.74	10.5100	0.5	NC
	PO	30	118.198	NA	NA	1.85	31.2565	1	NC
Rat/SD/Male	IV	3	30.107	100.2	391.5	3.65	14.0567[b]	NA	NA
	PO	3	20.697	NA	NA	3.61	6.1671	0.5	68.7
	PO	10	78.321	NA	NA	5.27	16.6450	0.5	NC
	PO	30	145.580	NA	NA	4.95	23.2015	1	NC
Dog/Beagle/Male	IV	3	8.417	368.3	1610.0	5.27	2.2889[b]	NA	NA
	PO	3	5.481	NA	NA	4.76	1.2717	2	70.4
Monkey/Cynomolgus/ Male	IV	3	12.900	237.7	793.7	4.28	4.6427[b]	NA	NA
	PO	3	10.272	NA	NA	4.07	2.5013	2	78.4

Doses and plasma concentrations for lenvatinib were expressed as those of the mesilate salt, and related parameters were calculated. In mice, each parameter except t_{max} was calculated with the average concentration of 3 animals, and in other species, each value except t_{max} represents the mean of 4 animals. The t_{max} represents the mode value, except for mice. F was calculated using the formula: $AUC_{(0\text{-}inf)}$ in oral dosing / $AUC_{(0\text{-}inf)}$ in intravenous dosing × 100.

$AUC_{(0\text{-}inf)}$ = area under the concentration-time curve from zero time extrapolated to infinite time, CL_p = total plasma clearance, C_{max} = maximum observed concentration, F = absolute bioavailability, IV = intravenous, NA = not applicable, NC = not calculated, PO = oral, SD = Sprague Dawley, $t_{1/2}$ = terminal elimination phase half-life, t_{max} = time at which the highest drug concentration occurs, V_{ss} = volume of distribution at steady state.

a: Lenvatinib mesilate was administered as solution in all administration groups.

b: Concentration at 5 minutes for intravenous dosing.

Source: Study Nos. B03014 (mouse), B03015 (rat), B03016 (dog), and B04003 (monkey).

(Excerpted from Applicant's submission)

AE-4150-G: Pharmacokinetic Study of E7080: Absorption, Distribution, and Excretion of Radioactivity after a Single Oral Administration of ^{14}C-E7080 to Rats

Methods

Drug:	^{14}C-E7080
Lot, specific activity (SA), radiochemical purity (RP)	CFQ14045, 4.053 MBq/mg, ≥ 96% CP-3028, 3.951 MBq/mg
Dose, frequency, ROA, dose volume:	3 mg/kg (11.853-12.159 MBq/kg), once, oral (administered into stomach using oral tube and syringe), 10 mL/kg
Formulation/Vehicle:	3% 0.1 mol/L hydrogen chloride in distilled water
Species/Strain:	~7 wk old fasted male Sprague Dawley rats
Number/Group:	3/group
Sample collection:	Blood: 5, 15, 30 min and 1, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hr post-dose Urine: 0-6, 6-12, 12-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hr post-dose Feces: 0-24, 24-48, 48-72, 72-96, 96-120, 120-144, and

144-168 hr post-dose
Bile: 0-2, 2-4, 4-6, 6-8, 8-12, 12-24, 24-48 hr post-dose

The Applicant examined the absorption, distribution, and excretion of lenvatinib following a single oral administration of 3 mg/kg ^{14}C-E7080 to fasted male rats. Radioactivity was measured by a liquid scintillation counter (LSC). Biliary excretion was investigated in bile-duct cannulated rats up to 48 hrs after ^{14}C-E7080 administration.

Table 15: Pharmacokinetic Parameters following a single oral dose of ^{14}C-E7080 to Rats

t_{max} (median)				30 min [15-60 min]
C_{max} (µg eq./mL)				3.1007 ± 0.3890
apparent $t_{1/2}$	1st phase	(0.5, 1 or 2-12 hr)	(hr)	4.50 ± 0.22
	2nd phase	(12-72 hr)	(hr)	13.6 ± 0.2
	3rd phase	(72-168 hr)	(day)	1.90 ± 0.13
AUC		(0-168 hr)		24.98 ± 2.54
(µg eq.·hr/mL)		(0-∞)		25.34 ± 2.60

Data are expressed as the mean values ± S.E.M. of three animals.
Figures in brackets are represented the range of individual t_{max}.

(Excerpted from Applicant's submission)

Following a single oral administration of ^{14}C-E7080 to intact rats, fecal and urinary excretion of radioactivity was 87.2% and 12.2% of the radioactivity dose, respectively. 99.4% of radioactivity was recovered in the excreta by 7 days post-dosing. Forty-eight hrs after administration of a single oral dose of ^{14}C-E7080 to bile-duct cannulated rats, excretion into the bile, urine, and feces was 41.6%, 18.1%, and 27.2% of the dose, respectively. Radioactivity remaining in the GI contents and carcass 48 hrs post-dose was 4.7% and 5.6% of the radioactivity dose, respectively. These data suggest that the predominant route of lenvatinib excretion in rats is fecal via bile, and at least 65% of the administered dose was absorbed.

Table 16: Excretion of Radioactivity following a single oral dose of ^{14}C-E7080 to Intact Rats

	Excretion of radioactivity (% of dose)		
Time (hr)	Urine	Feces	Total
0 - 6	5.0 ± 0.4	–	–
12	7.9 ± 0.4	–	–
24	10.5 ± 0.6	82.3 ± 1.2	92.8 ± 0.8
48	11.4 ± 0.8	86.2 ± 0.7	97.7 ± 0.2
72	11.7 ± 0.8	86.6 ± 0.7	98.4 ± 0.2
96	11.9 ± 0.8	86.7 ± 0.7	98.7 ± 0.2
120	12.0 ± 0.8	86.9 ± 0.7	98.9 ± 0.2
144	12.1 ± 0.8	87.0 ± 0.7	99.1 ± 0.2
168	12.2 ± 0.9	87.2 ± 0.6	99.4 ± 0.2
Carcass (168 hr)			1.7 ± 0.3

Data are expressed as the mean values ± S.E.M. of three animals.
– : Not determined

(Excerpted from Applicant's submission)

Following a single oral administration of ^{14}C-E7080 to rats, ^{14}C-E7080-related radioactivity reached its maximum levels in most tissues at 30 min post-dose with the exception of the cecum, large intestine, and skin, where it peaked at 6 hrs post-dose. Distribution of ^{14}C-E7080 was highest in the liver, adrenal gland, stomach, and small intestine (~1 to 3-fold higher than in the plasma) at 30 min post-dose and in the cecum

and large intestine (~12-fold higher than in the plasma) at 6 hrs post-dose. Radioactivity concentrations in the cerebrum, cerebellum, medulla oblongata, spinal cord, and sciatic nerve were lower than that in plasma; the tissue:plasma ratios at 30 min post-dose were 0.01, 0.02, 0.02, 0.02, and 0.1, respectively. Further, the distribution of radioactivity to the brain was ≤ 0.02% of dosed radioactivity, suggesting very low distribution to the brain. Distribution of radioactivity to the skeletal muscle, liver, skin, and blood was 20.48%, 10.17%, 8.67%, and 9.92% of dosed radioactivity, respectively (data not shown). Mean tissue radioactivity concentrations decreased with time, and reached <1% of their maximum levels by 336 hrs post-dose. The half-life of radioactivity was the longest in the eyeball, urinary bladder, sciatic nerve, mandibular lymph node, aorta, and trachea (5.5, 6.5, 7.3, 12.8, 17.6, and 19.6 days, respectively) compared to plasma (0.7 days; data not shown). The distribution of radioactivity to the membrane fraction of red blood cells was 15.7%, 27.6%, and 7% at 24, 72, and 168 hrs post-dose, respectively.

Table 17: Tissue Distribution following a single oral dose of ^{14}C-E7080 to Rats

	Concentration of radioactivity (µg eq. of E7080 /g or mL)			Concentration of radioactivity (µg eq. of E7080 /g or mL)		
Tissue	30 min	6 hr	24 hr	72 hr	168 hr	336 hr
Plasma	6.2822 ± 0.7091 (1.00)	0.9355 ± 0.1720 (1.00)	0.0252 ± 0.0073 (1.00)	0.0034 ± 0.0002 (1.00)	N.D.	N.D.
Blood	3.9249 ± 0.3066 (0.62)	1.0810 ± 0.1142 (1.16)	0.1521 ± 0.0123 (6.04)	0.0195 ± 0.0031 (5.74)	0.0051 ± 0.0007	0.0014 ± 0.0002
Red blood cells	1.8800 ± 0.0431 (0.30)	1.3092 ± 0.1368 (1.40)	0.3393 ± 0.0301 (13.46)	0.0413 ± 0.0068 (12.15)	0.0154 ± 0.0023	0.0041 ± 0.0001
Cerebrum	0.0879 ± 0.0039 (0.01)	0.0219 ± 0.0031 (0.02)	0.0019 ± 0.0003 (0.08)	N.D.	N.D.	N.D.
Cerebellum	0.1014 ± 0.0030 (0.02)	0.0271 ± 0.0073 (0.03)	0.0022 ± 0.0002 (0.09)	0.0007 ± 0.0000 (0.21)	N.D.	N.D.
Pituitary gland	2.4358 ± 0.3244 (0.39)	0.4882 ± 0.1281 (0.52)	0.0553 ± 0.0128 (2.19)	0.0131 ± 0.0009 (3.85)	N.D.	N.D.
Medulla oblongata	0.0947 ± 0.0149 (0.02)	0.0294 ± 0.0103 (0.03)	0.0027 ± 0.0005 (0.11)	0.0010 ± 0.0002 (0.29)	N.D.	N.D.
Spinal cord	0.1120 ± 0.0151 (0.02)	0.0278 ± 0.0083 (0.03)	0.0032 ± 0.0004 (0.13)	0.0008 ± 0.0001 (0.24)	N.D.	N.D.
Eyeball	0.2680 ± 0.0064 (0.04)	0.0805 ± 0.0127 (0.09)	0.0113 ± 0.0022 (0.45)	0.0030 ± 0.0001 (0.88)	0.0012 ± 0.0002	0.0007 ± 0.0000
Harderian gland	2.2314 ± 0.1589 (0.36)	0.4887 ± 0.1308 (0.52)	0.0443 ± 0.0133 (1.76)	0.0098 ± 0.0003 (2.88)	0.0034 ± 0.0001	0.0010 ± 0.0002
Thyroid gland	2.3892 ± 0.1552 (0.38)	0.3845 ± 0.0535 (0.41)	0.0469 ± 0.0155 (1.86)	0.0108 ± 0.0011 (3.18)	N.D.	N.D.
Mandibular gland	2.2746 ± 0.0926 (0.36)	0.4674 ± 0.0617 (0.50)	0.0428 ± 0.0076 (1.70)	0.0134 ± 0.0013 (3.94)	0.0053 ± 0.0003	0.0029 ± 0.0011
Mandibular lymph node	1.6083 ± 0.0800 (0.26)	0.4531 ± 0.0580 (0.48)	0.0408 ± 0.0068 (1.62)	0.0105 ± 0.0017 (3.09)	0.0045 ± 0.0008	0.0052 ± 0.0016
Trachea	1.8398 ± 0.2086 (0.29)	0.4037 ± 0.0762 (0.43)	0.0236 ± 0.0059 (0.94)	0.0151 ± 0.0049 (4.44)	0.0057 ± 0.0006	0.0089 ± 0.0007
Thymus	1.1440 ± 0.0650 (0.18)	0.3586 ± 0.0438 (0.38)	0.0442 ± 0.0070 (1.75)	0.0061 ± 0.0006 (1.79)	0.0012 ± 0.0001	0.0005 ± 0.0003
Heart	2.1757 ± 0.0812 (0.35)	0.4974 ± 0.0781 (0.53)	0.0626 ± 0.0102 (2.48)	0.0214 ± 0.0024 (6.29)	0.0110 ± 0.0011	0.0051 ± 0.0003
Lung	2.6317 ± 0.0904 (0.42)	0.6606 ± 0.0856 (0.71)	0.0620 ± 0.0102 (2.46)	0.0127 ± 0.0009 (3.74)	0.0044 ± 0.0005	0.0020 ± 0.0002
Liver	9.4302 ± 0.1784 (1.50)	1.7627 ± 0.3350 (1.88)	0.1135 ± 0.0144 (4.50)	0.0419 ± 0.0040 (12.32)	0.0167 ± 0.0021	0.0030 ± 0.0003
Kidney	4.1212 ± 0.1772 (0.66)	1.1953 ± 0.2137 (1.28)	0.1656 ± 0.0337 (6.57)	0.0531 ± 0.0062 (15.62)	0.0196 ± 0.0026	0.0056 ± 0.0004

	Concentration of radioactivity (µg eq. of E7080 /g or mL)			Concentration of radioactivity (µg eq. of E7080 /g or mL)		
Tissue	30 min	6 hr	24 hr	72 hr	168 hr	336 hr
Renal cortex	4.5629 ± 0.1257 (0.73)	1.4525 ± 0.2623 (1.55)	0.2065 ± 0.0394 (8.19)	0.0598 ± 0.0060 (17.59)	0.0233 ± 0.0022	0.0065 ± 0.0006
Renal medulla	3.6204 ± 0.5212 (0.58)	0.9611 ± 0.2378 (1.03)	0.1269 ± 0.0169 (5.04)	0.0298 ± 0.0077 (8.76)	0.0089 ± 0.0009	0.0031 ± 0.0004
Adrenal gland	8.2681 ± 0.3869 (1.32)	1.7729 ± 0.2012 (1.90)	0.0975 ± 0.0233 (3.87)	0.0178 ± 0.0016 (5.24)	0.0051 ± 0.0004	N.D.
Spleen	1.8214 ± 0.0665 (0.29)	0.5630 ± 0.0656 (0.60)	0.0737 ± 0.0073 (2.92)	0.0146 ± 0.0012 (4.29)	0.0038 ± 0.0007	0.0027 ± 0.0013
Pancreas	1.9621 ± 0.0611 (0.31)	0.4297 ± 0.0476 (0.46)	0.0480 ± 0.0082 (1.90)	0.0133 ± 0.0013 (3.91)	0.0046 ± 0.0008	0.0019 ± 0.0004
Fat	1.2336 ± 0.0767 (0.20)	0.1388 ± 0.0128 (0.15)	0.0096 ± 0.0015 (0.38)	0.0023 ± 0.0001 (0.68)	0.0016 ± 0.0005	N.D.
Brown fat	2.0945 ± 0.0915 (0.33)	0.4284 ± 0.0477 (0.46)	0.0416 ± 0.0083 (1.65)	0.0119 ± 0.0007 (3.50)	0.0043 ± 0.0007	0.0018 ± 0.0004
Skeletal muscle	1.2165 ± 0.0103 (0.19)	0.2940 ± 0.0457 (0.31)	0.0767 ± 0.0097 (3.04)	0.0242 ± 0.0027 (7.12)	0.0105 ± 0.0008	0.0053 ± 0.0004
Sciatic nerve	0.6410 ± 0.0748 (0.10)	0.1500 ± 0.0095 (0.16)	0.0172 ± 0.0025 (0.68)	0.0065 ± 0.0005 (1.91)	N.D.	0.0023 ± 0.0013
Skin	1.4709 ± 0.0591 (0.23)	1.4833 ± 0.3938 (1.59)	1.2048 ± 0.3979 (47.81)	0.8004 ± 0.1981 (235.41)	0.0366 ± 0.0041	0.0064 ± 0.0009
Bone marrow	1.9837 ± 0.0248 (0.32)	0.5914 ± 0.0858 (0.63)	0.0774 ± 0.0124 (3.07)	0.0126 ± 0.0008 (3.71)	N.D.	N.D.
Vein	2.0420 ± 0.4689 (0.33)	0.4227 ± 0.0694 (0.45)	0.0329 ± 0.0061 (1.31)	0.0075 ± 0.0007 (2.21)	0.0056 ± 0.0030	N.D.
Aorta	3.8884 ± 0.2816 (0.62)	0.7795 ± 0.0981 (0.83)	0.0468 ± 0.0123 (1.86)	0.0134 ± 0.0025 (3.94)	0.0108 ± 0.0018	0.0086 ± 0.0009
Testis	0.3097 ± 0.0121 (0.05)	0.2323 ± 0.0206 (0.25)	0.0511 ± 0.0047 (2.03)	0.0130 ± 0.0014 (3.82)	0.0032 ± 0.0001	0.0007 ± 0.0004
Seminal vesicle	0.8613 ± 0.1457 (0.14)	0.3261 ± 0.0644 (0.35)	0.0330 ± 0.0072 (1.31)	0.0101 ± 0.0009 (2.97)	0.0049 ± 0.0001	0.0024 ± 0.0003
Prostate gland	1.3713 ± 0.1048 (0.22)	0.3870 ± 0.0464 (0.41)	0.0367 ± 0.0077 (1.46)	0.0067 ± 0.0004 (1.97)	0.0019 ± 0.0001	0.0009 ± 0.0004
Urinary bladder	1.9716 ± 0.3412 (0.31)	1.0829 ± 0.1353 (1.16)	0.0913 ± 0.0271 (3.62)	0.0156 ± 0.0020 (4.59)	0.0053 ± 0.0005	0.0043 ± 0.0013
Stomach	7.4548 ± 1.5174 (1.19)	0.7373 ± 0.1008 (0.79)	0.0544 ± 0.0040 (2.16)	0.0155 ± 0.0026 (4.56)	0.0052 ± 0.0003	0.0019 ± 0.0004
Small intestine	16.2803 ± 5.8580 (2.59)	0.7880 ± 0.0504 (0.84)	0.0888 ± 0.0197 (3.52)	0.0266 ± 0.0045 (7.82)	0.0126 ± 0.0019	0.0030 ± 0.0005
Cecum	2.0283 ± 0.1228 (0.32)	12.0265 ± 4.5251 (12.86)	0.1286 ± 0.0304 (5.10)	0.0186 ± 0.0003 (5.47)	0.0069 ± 0.0008	0.0018 ± 0.0003
Large intestine	1.8072 ± 0.1775 (0.29)	10.8630 ± 7.8365 (11.61)	0.0720 ± 0.0059 (2.86)	0.0147 ± 0.0016 (4.32)	0.0052 ± 0.0006	0.0019 ± 0.0006

Data are expressed as mean ± S.E.M. of 3 rats; figures in parentheses are expressed as the ratio of concentration in tissue relative to plasma.
(Excerpted from Applicant's submission)

AE-4151-G: Pharmacokinetic Study of E7080: Absorption, Distribution, and Excretion of Radioactivity after a Single Oral Administration of ^{14}C-E7080 to Monkeys

Methods

Drug, lot, SA, RP:	^{14}C-E7080, CP-3028, 3.951 MBq/mg, ≥ 98.7%
Dose, frequency, ROA, dose volume:	3 mg/kg (8.106 MBq/kg), once, oral (administered into stomach using oral catheter and syringe), 3 mL/kg
Formulation/Vehicle:	3% 0.1 mol/L hydrogen chloride in distilled water
Species/Strain:	3 yr old fasted male cynomolgus monkeys
Number/Group:	3/group
Sample collection:	Blood: 5, 15, 30 min and 1, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hr post-dose Urine: 0-6, 6-12, 12-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hr post-dose Feces: 0-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hr post-dose

The Applicant examined the absorption, distribution, and excretion of lenvatinib following a single oral administration of 3 mg/kg ^{14}C-E7080 to fasted male monkeys; radioactivity was measured by LSC.

Table 18: Pharmacokinetic Parameters following a single oral dose of ^{14}C-E7080 to Monkeys

t_{max} (mode)			6 hr [4-6 hr]	4 hr [4-6 hr]	6 hr [4-6 hr]
C_{max} (µg eq./mL)			0.5367 ± 0.0854	0.6821 ± 0.1176	0.4855 ± 0.0087
apparent $t_{1/2}$	(6 or 8-24 hr)	(hr)	11.3 ± 2.6	8.90 ± 1.81	13.1 ± 3.1
	(24-72 hr)	(day)	1.18 ± 0.19	1.15 ± 0.19	1.34 ± 0.29
	(72-168 hr)	(day)	3.19 ± 0.35	3.43 ± 0.41	4.27 ± 1.03
AUC		(0-168 hr)	11.64 ± 1.14	12.25 ± 0.94	11.55 ± 1.39
(µg eq.·hr/mL)		(0-∞)	13.22 ± 1.27	13.86 ± 1.00	14.16 ± 1.28

Data are expressed as the mean values ± S.E.M. of three animals.
Figures in brackets are represented the range of individual data t_{max}.

(Excerpted from Applicant's submission)

Following a single oral administration of ^{14}C-E7080 to monkeys, fecal and urinary excretion of radioactivity was 72.8% and 17.2% of the radioactivity dose, respectively. 90% of radioactivity was recovered in the excreta by 7 days post-dosing, and very little residual radioactivity remained in the large intestines and cage (0.7% and 1.1%, respectively).

Table 19: Excretion of Radioactivity following a single oral dose of ^{14}C-E7080 to Monkeys

	Excretion of radioactivity (% of dose)		
Time (hr)	Urine	Feces	Total
0 - 6	2.8 ± 1.0	–	–
12	6.1 ± 1.7	–	–
24	9.5 ± 1.9	14.6 ± 7.1	24.1 ± 9.0
48	12.9 ± 1.0	52.0 ± 17.0	64.9 ± 17.3
72	15.2 ± 1.2	64.3 ± 10.7	79.5 ± 10.1
96	16.1 ± 1.5	68.5 ± 8.2	84.6 ± 7.1
120	16.6 ± 1.6	70.4 ± 6.9	87.0 ± 5.5
144	16.9 ± 1.7	71.4 ± 6.3	88.3 ± 4.8
168	17.2 ± 1.7	72.8 ± 5.2	90.0 ± 3.6
Gastric contents (168 hr)			0.0 ± 0.0
Small intestine contents (168 hr)			0.0 ± 0.0
Large intestine contents (168 hr)			0.7 ± 0.5
Cage washing (168 hr)			1.1 ± 0.3

Data are expressed as the mean values ± S.E.M. of three animals.
– : Not determined

(Excerpted from Applicant's submission)

Following a single oral administration of ^{14}C-E7080 to monkeys, ^{14}C-E7080-related radioactivity reached its maximum levels in most tissues at 4 hr post-dose with the exception of the cornea, iris, choroid, lens, sclera, and large intestine, where it peaked at 24 hrs post-dose. Tissue distribution of ^{14}C-E7080 was widespread and highest in the bile in gall bladder, urine in bladder, gall bladder, liver, and choroid; tissue:plasma ratios at 4 hrs post-dose were 556.73, 57.85, 49.26, 25.18, and 24.16, respectively. Radioactivity concentrations in the cerebrum, cerebellum, medulla oblongata, and spinal cord were lower than in plasma, with tissue:plasma ratios ≤ 0.14 at 4 hrs post-dose; the percentage of distribution of radioactivity to the brain was ≤ 0.06% of dosed radioactivity (data not shown). Distribution of radioactivity to the liver, bile in gall bladder, and skeletal muscle was 10.77%, 9.04%, and 5.05% of dosed radioactivity, respectively. Mean tissue radioactivity concentrations decreased with time, but 68% and 60% of

maximum radioactivity was still present in the choroid and iris 168 hr post-dose, respectively, correlating with long radioactivity half-lives of 10.8 and 8.3 days. The half-life of radioactivity in the skin, ciliary body, and sclera was 7.7, 6.3, and 5.6 days, respectively, compared to that in the plasma (2.4 days; data not shown), indicating low elimination in melanin-containing tissues. The distribution of radioactivity to the membrane fraction of red blood cells was 3.2% and 7.7% at 24 and 168 hrs post-dose, respectively.

Table 20: Tissue Distribution following a single oral dose of ^{14}C-E7080 to Monkeys

	Concentration of radioactivity (µg eq. of E7080 /g or mL)		
Tissue	4 hr	24 hr	168 hr
Plasma	0.5486 ± 0.1318 (1.00)	0.0709 ± 0.0045 (1.00)	0.0126 ± 0.0025 (1.00)
Blood	0.4518 ± 0.0958 (0.82)	0.0759 ± 0.0024 (1.07)	0.0143 ± 0.0027 (1.13)
Red blood cells	0.2654 ± 0.0563 (0.48)	0.0873 ± 0.0012 (1.23)	0.0146 ± 0.0036 (1.16)
Cerebrum	0.0795 ± 0.0163 (0.14)	0.0307 ± 0.0034 (0.43)	0.0061 ± 0.0008 (0.48)
Cerebellum	0.0583 ± 0.0163 (0.11)	0.0197 ± 0.0007 (0.28)	0.0040 ± 0.0005 (0.32)
Pituitary gland	0.5843 ± 0.1147 (1.07)	0.1658 ± 0.0148 (2.34)	0.0335 ± 0.0074 (2.66)
Medulla oblongata	0.0715 ± 0.0140 (0.13)	0.0319 ± 0.0023 (0.45)	0.0048 ± 0.0007 (0.38)
Spinal cord	0.0580 ± 0.0144 (0.11)	0.0232 ± 0.0012 (0.33)	0.0031 ± 0.0005 (0.25)
Aqueous humor	0.0045 ± 0.0025 (0.01)	0.0039 ± 0.0008 (0.06)	N.D.
Cornea	0.0742 ± 0.0328 (0.14)	0.1106 ± 0.0161 (1.56)	0.0128 ± 0.0021 (1.02)
Lens	0.0350 ± 0.0166 (0.06)	0.0363 ± 0.0131 (0.51)	0.0062 ± 0.0002 (0.49)
Iris	4.2805 ± 2.5767 (7.80)	4.4804 ± 1.2647 (63.19)	2.7091 ± 0.8395 (215.01)
Ciliary body	7.9855 ± 2.9469 (14.56)	5.7832 ± 1.2554 (81.57)	2.9924 ± 0.9553 (237.49)
Vitreous body	0.0092 ± 0.0032 (0.02)	0.0068 ± 0.0027 (0.10)	N.D.
Retina	0.6890 ± 0.1327 (1.26)	0.3282 ± 0.0388 (4.63)	0.0834 ± 0.0205 (6.62)
Choroid	13.2559 ± 5.4122 (24.16)	17.9497 ± 1.4579 (253.17)	12.2241 ± 2.7030 (970.17)
Sclera	0.2575 ± 0.1031 (0.47)	0.2776 ± 0.0365 (3.92)	0.1327 ± 0.0185 (10.53)
Optic nerve	0.0976 ± 0.0243 (0.18)	0.0403 ± 0.0045 (0.57)	0.0065 ± 0.0014 (0.52)
Thyroid gland	0.8574 ± 0.2089 (1.56)	0.1323 ± 0.0236 (1.87)	0.0364 ± 0.0130 (2.89)

Mandibular gland	0.8340 ± 0.1535 (1.52)	0.2700 ± 0.0150 (3.81)	0.0527 ± 0.0106 (4.18)
Mandibular lymph node	0.4492 ± 0.0915 (0.82)	0.1186 ± 0.0136 (1.67)	0.0200 ± 0.0036 (1.59)
Trachea	0.3955 ± 0.1365 (0.72)	0.0828 ± 0.0179 (1.17)	0.0148 ± 0.0032 (1.17)
Thymus	0.4024 ± 0.0900 (0.73)	0.1079 ± 0.0060 (1.52)	0.0229 ± 0.0060 (1.82)
Heart	0.5553 ± 0.0908 (1.01)	0.1444 ± 0.0041 (2.04)	0.0355 ± 0.0040 (2.82)
Lung	0.9329 ± 0.1957 (1.70)	0.1539 ± 0.0128 (2.17)	0.0330 ± 0.0118 (2.62)
Liver	13.8131 ± 2.0040 (25.18)	3.1585 ± 0.2584 (44.55)	0.6891 ± 0.0791 (54.69)
Kidney	5.1618 ± 1.0222 (9.41)	1.6438 ± 0.1261 (23.18)	0.1777 ± 0.0292 (14.10)
Renal cortex	5.5457 ± 1.0152 (10.11)	1.7347 ± 0.1398 (24.47)	0.2900 ± 0.0898 (23.02)
Renal medulla	2.2815 ± 0.6359 (4.16)	0.4226 ± 0.0258 (5.96)	0.0939 ± 0.0428 (7.45)
Adrenal gland	0.7663 ± 0.0920 (1.40)	0.2571 ± 0.0273 (3.63)	0.0367 ± 0.0098 (2.91)
Spleen	0.6548 ± 0.1339 (1.19)	0.1572 ± 0.0142 (2.22)	0.0297 ± 0.0058 (2.36)
Pancreas	1.2477 ± 0.3110 (2.27)	0.2728 ± 0.0048 (3.85)	0.0534 ± 0.0090 (4.24)
Fat	0.2209 ± 0.0822 (0.40)	0.0458 ± 0.0134 (0.65)	0.0233 ± 0.0020 (1.85)
Skeletal muscle	0.3667 ± 0.0800 (0.67)	0.1384 ± 0.0143 (1.95)	0.0542 ± 0.0050 (4.30)
Sciatic nerve	0.1746 ± 0.0439 (0.32)	0.0579 ± 0.0048 (0.82)	0.0174 ± 0.0016 (1.38)
Skin	0.6294 ± 0.3018 (1.15)	0.4283 ± 0.0955 (6.04)	0.2490 ± 0.0261 (19.76)
Bone marrow	0.3019 ± 0.0652 (0.55)	0.0644 ± 0.0088 (0.91)	0.0157 ± 0.0071 (1.25)
Vein	0.4404 ± 0.1932 (0.80)	0.0871 ± 0.0121 (1.23)	0.0145 ± 0.0005 (1.15)
Aorta	0.8899 ± 0.3450 (1.62)	0.1465 ± 0.0145 (2.07)	0.0205 ± 0.0038 (1.63)
Gall bladder	27.0256 ± 17.0154 (49.26)	4.7009 ± 1.7265 (66.30)	0.1257 ± 0.0229 (9.98)
Bile in gall bladder	305.4232 ± 186.6361 (556.73)	79.2728 ± 17.5784 (1118.09)	2.1141 ± 0.9789 (167.79)
Testis	0.3923 ± 0.0983 (0.72)	0.1103 ± 0.0086 (1.56)	0.0178 ± 0.0045 (1.41)
Prostate gland	0.6453 ± 0.2169 (1.18)	0.1498 ± 0.0386 (2.11)	0.0470 ± 0.0091 (3.73)
Urinary bladder	1.0554 ± 0.3864 (1.92)	0.1451 ± 0.0386 (2.05)	0.0184 ± 0.0038 (1.46)
Urine in bladder	31.7376 ± 7.5630 (57.85)	1.7285 ± 1.2001 (24.38)	0.1711 ± 0.0488 (13.58)
Stomach	1.2962 ± 0.1966 (2.36)	0.1639 ± 0.0118 (2.31)	0.0252 ± 0.0070 (2.00)
Small intestine	0.9423 ± 0.1170 (1.72)	0.2382 ± 0.0489 (3.36)	0.0425 ± 0.0043 (3.37)
Cecum	4.4915 ± 1.0810 (8.19)	0.3287 ± 0.0588 (4.64)	0.0417 ± 0.0059 (3.31)
Large intestine	0.6440 ± 0.1423 (1.17)	0.7907 ± 0.4249 (11.15)	0.0370 ± 0.0045 (2.94)

Data are expressed as the mean values ± S.E.M. of three animals.
Figures in parentheses are expressed as the ratio of concentration in tissue relative to plasma.
N.D. : Not detected

(Excerpted from Applicant's submission)

AE-6750-G: E7080: Placental Transfer and Transfer into Milk after a Single Oral Administration of ^{14}C-E7080 in Rats

Methods

Drug, lot #, % purity: ^{14}C-E7080, CP-3765-2, >99%

Dose, ROA: 3 mg/kg (12.8 MBq/kg), oral

Dose Volume: Placental transfer: 10 mL/kg
Transfer into milk: 5 mL/kg
Formulation/Vehicle: 3 mmol/LDK378 hydrochloric acid in water
Species/Strain: Female Sprague Dawley rats
Number/Sex/Group: 3 rats/group; Groups 1-6: Placental transfer, Group 7: Transfer into milk

Study group	Route	Dosage	Day of pregnancy at dosing	Sampling time	Replicate sample	Number of animals used
Group 1	po	3 mg/kg	Day 13	30 min	Single sample/ tissue	3 animals/group (total 18 animals)
Group 2				6 h		
Group 3				24 h		
Group 4			Day 18	30 min		
Group 5				6 h		
Group 6				24 h		

Study group	Route	Dosage	Assay sample	Replicate sample	Number of animals used
Group 7	po	3 mg/kg	Plasma and milk	Single sample /time point	3 animals/group

The Applicant investigated placental transfer, transfer into milk, and metabolic profiling following a single 3 mg/kg oral dose of ^{14}C-E7080 to pregnant or lactating rats. Radioactive concentrations in maternal and fetal tissues were determined 30 min, 6 hr, and 24 hr after single oral administration of ^{14}C-E7080 to fasted rats on Days 13 or 18 of pregnancy. Radioactive concentrations in milk and plasma were determined 1, 2, 4, 8, 12, and 24 hrs after a single oral administration of ^{14}C-E7080 to lactating non-fasted rats on postnatal Day 9, and PK parameters were determined. E7080 and its metabolites were analyzed using radio-high performance liquid chromatography (HPLC).

Following a single oral administration of ^{14}C-E7080 to rats on Days 13 or 18 of pregnancy, ^{14}C-E7080-related radioactivity was distributed at the highest levels to maternal blood, liver, adrenal gland, and kidney. Radioactive concentrations in tissues, blood, and plasma were generally highest at 30 min post-dose and decreased with time, with the exception of the fetal membrane and amniotic fluid, which achieved maximum radioactive concentrations at 6 hrs post-dose. There was low distribution of ^{14}C-E7080 to the cerebrum (≤0.02% of dosed radioactivity). Radioactivity concentrations in maternal and fetal tissues were lower than that in maternal plasma at 30 min post-dose on Days 13 and 18, except for the maternal liver and adrenal gland.

At 30 min following administration of ^{14}C-E7080 on Days 13 or 18 of pregnancy, the radioactive concentrations in the placenta, fetal membrane, amniotic fluid, fetus, fetal blood/plasma, and fetal tissues were 25%, ≤14%, 0%, ≤2%, 2%, and ≤4%, respectively, of that in the maternal plasma (only data for Day 18 is shown below). Despite an increase in the ratio of radioactivity in these tissues relative to maternal plasma over time, at 24 hrs post-dose the radioactive concentrations in the fetus and fetal tissues were ≥ 81% and ≥ 70% lower than their maximum levels at 30 min post-dose. According to the Applicant, the percentage distribution of radioactivity to each fetus was ≤ 0.02% of dosed radioactivity, suggesting low placental transfer of ^{14}C-E7080 to the fetus.

Reference ID: 3686430

Table 21: Radioactive concentration in tissues following a single 3 mg/kg oral dose of ^{14}C-E7080 to rats on Day 18 of pregnancy

	Radioactive concentration (µg eq. of E7080/g or mL)		
Tissue	30 min	6 h	24 h
Plasma	9.0501 ± 0.2636	1.1651 ± 0.1206	0.0480 ± 0.0072
Blood	5.9101 ± 0.1888	1.3783 ± 0.1055	0.2604 ± 0.0359
Cerebrum	0.1067 ± 0.0058	0.0276 ± 0.0033	0.0036 ± 0.0005
Heart	2.7683 ± 0.1171	0.6474 ± 0.0421	0.1086 ± 0.0121
Lung	3.3331 ± 0.2307	0.7951 ± 0.0523	0.1075 ± 0.0140
Liver	8.6131 ± 0.6721	1.7971 ± 0.1100	0.1680 ± 0.0282
Kidney	4.9877 ± 0.4074	1.4071 ± 0.0380	0.2667 ± 0.0466
Adrenal gland	9.8113 ± 0.6785	1.9970 ± 0.1414	0.1877 ± 0.0242
Uterus	2.0831 ± 0.2308	0.6005 ± 0.1455	0.0879 ± 0.0025
Ovary	2.4217 ± 0.0733	0.4238 ± 0.0421	0.0406 ± 0.0029
Mammary gland	3.3144 ± 0.2837	0.6313 ± 0.0465	0.1487 ± 0.0230
Placenta	2.2418 ± 0.1136	0.9986 ± 0.0508	0.2109 ± 0.0044
Fetal membrane	0.4832 ± 0.0627	0.7190 ± 0.0509	0.4652 ± 0.0591
Amniotic fluid	0.0347 ± 0.0089	0.0893 ± 0.0135	0.0319 ± 0.0111
Fetus	0.1505 ± 0.0309	0.1109 ± 0.0034	0.0293 ± 0.0085
Fetal blood	0.1790 ± 0.0298	0.1614 ± 0.0199	0.0534 ± 0.0067
Fetal plasma	0.1941 ± 0.0390	0.1093 ± 0.0186	0.0238 ± 0.0022
Fetal brain	0.0779 ± 0.0084	0.0323 ± 0.0037	0.0077 ± 0.0006
Fetal heart	0.1946 ± 0.0352	0.0955 ± 0.0057	0.0164 ± 0.0018
Fetal lung	0.2048 ± 0.0279	0.1037 ± 0.0087	0.0189 ± 0.0025
Fetal liver	0.4036 ± 0.0731	0.2600 ± 0.0180	0.0506 ± 0.0127
Fetal kidney	0.2149 ± 0.0404	0.1379 ± 0.0111	0.0467 ± 0.0102
Fetal gastro-intestine	0.1875 ± 0.0186	0.1227 ± 0.0071	0.0434 ± 0.0020

Each data represents the mean ± SEM of three animals.

(Excerpted from Applicant's submission)

Table 22: Ratio of radioactive concentration in tissues relative to maternal plasma following a single 3 mg/kg oral dose of ^{14}C-E7080 to rats on Day 18 of pregnancy

	Ratio to the radioactive concentration in plasma		
Tissue	30 min	6 h	24 h
Plasma	1.00	1.00	1.00
Blood	0.65	1.18	5.43
Cerebrum	0.01	0.02	0.08
Heart	0.31	0.56	2.26
Lung	0.37	0.68	2.24
Liver	0.95	1.54	3.50
Kidney	0.55	1.21	5.56
Adrenal gland	1.08	1.71	3.91
Uterus	0.23	0.52	1.83
Ovary	0.27	0.36	0.85
Mammary gland	0.37	0.54	3.10
Placenta	0.25	0.86	4.39
Fetal membrane	0.05	0.62	9.69
Amniotic fluid	0.00	0.08	0.66
Fetus	0.02	0.10	0.61
Fetal blood	0.02	0.14	1.11
Fetal plasma	0.02	0.09	0.50
Fetal brain	0.01	0.03	0.16
Fetal heart	0.02	0.08	0.34
Fetal lung	0.02	0.09	0.39
Fetal liver	0.04	0.22	1.05
Fetal kidney	0.02	0.12	0.97
Fetal gastro-intestine	0.02	0.11	0.90

(Excerpted from Applicant's submission)

Table 23: Percent distribution of radioactivity in tissues following a single 3 mg/kg oral dose of ^{14}C-E7080 to rats on Day 18 of pregnancy

Tissue	Radioactive Content (% of Dose)					
	Day 13 of Pregnancy			Day 18 of Pregnancy		
	30 min	6 h	24 h	30 min	6 h	24 h
Cerebrum	0.01±0.00	0.00±0.00	0.00±0.00	0.02±0.00	0.00±0.00	0.00±0.00
Heart	0.18±0.01	0.06±0.00	0.01±0.00	0.26±0.03	0.06±0.00	0.01±0.00
Lung	0.27±0.02	0.09±0.00	0.01±0.00	0.37±0.04	0.08±0.00	0.01±0.00
Liver	8.05±0.37	1.96±0.01	0.23±0.01	9.60±0.63	2.06±0.14	0.25±0.03
Kidney	0.92±0.05	0.30±0.02	0.05±0.01	1.04±0.05	0.30±0.02	0.05±0.01
Adrenal gland	0.06±0.00	0.02±0.00	0.00±0.00	0.09±0.01	0.02± 0.00	0.00±0.00
Uterus	0.52±0.02	0.21±0.03	0.02±0.00	0.77±0.10	0.21±0.04	0.04±0.00
Ovary	0.02±0.00	0.00±0.00	0.00±0.00	0.04±0.00	0.00±0.00	0.00±0.00
Fetus (Per fetus)	0.00±0.00	0.00±0.00	0.00±0.00	0.02±0.00	0.02±0.00	0.00±0.00
Fetus (All fetuses)	0.01±0.00	0.00±0.00	0.00±0.00	0.30±0.08	0.19±0.00	0.09±0.04

(Excerpted from Applicant's submission)

Following a single oral administration of ^{14}C-E7080 to lactating rats on postnatal Day 9, radioactivity was approximately 1.4 and 2 times higher in milk compared to plasma based on Cmax and AUC, respectively, indicating transfer of lenvatinib into milk. ^{14}C-E7080-related radioactivity in milk and plasma peaked at 1 hr post-dosing and decreased with time.

Table 24: Radioactive concentrations in milk and plasma following a single 3 mg/kg oral dose of ^{14}C-E7080 to lactating rats

Time (h)	Radioactive concentration (µg eq. of E7080/mL)	
	Milk	Plasma
1	5.2797 ± 0.7147 (1.44)	3.6714 ± 0.1627
2	3.5519 ± 0.6396 (1.45)	2.4528 ± 0.1597
4	2.9109 ± 0.5592 (2.09)	1.3932 ± 0.1552
8	1.8116 ± 0.5512 (2.86)	0.6341 ± 0.1462
12	1.1329 ± 0.3053 (2.73)	0.4150 ± 0.0863
24	0.1444 ± 0.0645 (3.66)	0.0395 ± 0.0058

Each data represents the mean ± SEM of three animals.

Figures in parentheses represent the ratio of concentration in milk relative to plasma.

(Excerpted from Applicant's submission)

Table 25: Pharmacokinetic parameters of radioactivity in milk and plasma following a single 3 mg/kg oral dose of ^{14}C-E7080 to lactating rats

Pharmacokinetic parameters	Milk	Plasma
C_{max} (µg eq./mL)	5.2797 ± 0.7147	3.6714 ± 0.1627
t_{max} (h)	1.0	1.0
$AUC_{(0\text{-last})}$ (µg eq.·h/mL)	36.52 ± 6.77 (0-24 h)	17.62 ± 1.40 (0-24 h)
$AUC_{(0\text{-}\infty)}$ (µg eq.·h/mL)	37.50 ± 6.82	17.85 ± 1.38
$t_{1/2}$ (h)	4.34 ± 0.60 (8-24 h)	4.02 ± 0.42 (8-24 h)

Each data represents the mean ± SEM of three animals.

Figures in parentheses represent the time ranges for calculation.

$AUC_{(0\text{-last})}$ = area under the radioactive concentration × time curve from zero to time of last measurable concentration, $AUC_{(0\text{-}\infty)}$ = area under the radioactive concentration × time curve from zero to infinity, C_{max} = maximum concentration, $t_{1/2}$ = terminal elimination half-life, t_{max} = time to reach maximum concentration following drug administration.

(Excerpted from Applicant's submission)

Radio-HPLC analysis of lenvatinib and its metabolites revealed that E7080 was the main component in maternal plasma, fetal plasma, the fetus, and milk at all of the time points tested following administration of ^{14}C-E7080, suggesting that the radioactivity distributed in fetuses and milk was primarily due to the parent compound.

B03025: E7080: Structural Analysis of E7080 Metabolites Produced by Mouse, Rat, Dog, Monkey, and Human Liver Microsomes

The Applicant evaluated the in vitro metabolism of lenvatinib using mouse, rat, dog, monkey, and human liver microsomes. The microsomes were incubated with 10 µg/mL E7080 (lot # 12112750) at 37ºC for 60 min with or without NADPH, the reactions were terminated with methanol, and the reaction mixtures were analyzed by liquid chromatography/mass spectrometry (LC/MS). Eight metabolites were identified in the liver microsomes, as summarized in Table 26.

Table 26: Lenvatinib Metabolites Generated in Mouse, Rat, Dog, Monkey, and Human Liver Microsomes

Matrix	Metabolites of E7080							
	M1	M2	M3	M4	M5	M6	M7	M8
Mouse Liver microsomes	○*	○	○	○	○	-	△	-
Rat Liver microsomes	○*	○	○	○	○	-	△	-
Dog Liver microsomes	○*	○	○	○	○	-	△	-
Monkey Liver microsomes	◎*	◎	◎	◎	○	○	○	○
Human Liver microsomes	○*	◎	○	○	○	△	△	○

◎ : major metabolites at UV-chromatogram (254 nm)
○ : observed at UV-chromatogram (254 nm)
△ : detected at mass chromatogram
- : not detected at UV- and mass chromatogram
* : derived from degradation and/or impurity

(Excerpted from Applicant's submission)

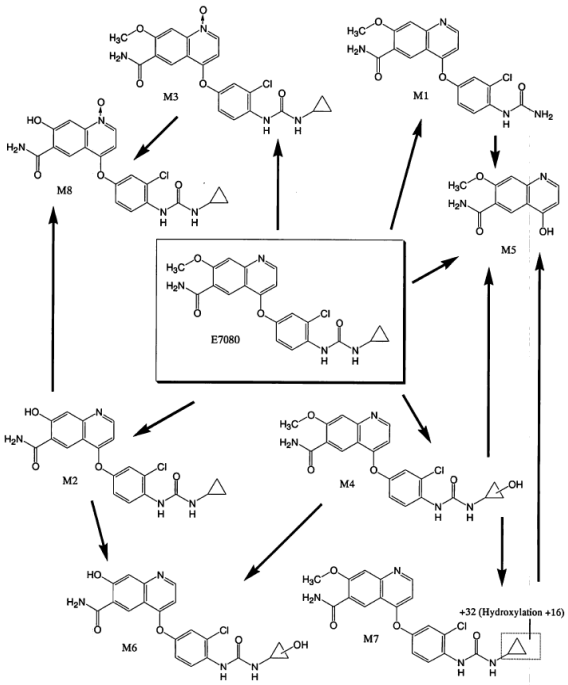
Table 27: Proposed Metabolite Structures identified in Liver Microsomes

Metabolite in this study	$[M+H]^+$	Structure Modification
M1[1)]	*m/z* 387	Decyclopropylation
M2[1)]	*m/z* 413	Demethylation
M3[1)]	*m/z* 443	*N*-Oxidation
M4[2)]	*m/z* 443	Hydroxylation
M5[2)]	*m/z* 219	O-dearylation
M6[2)]	*m/z* 429	Hydroxylation and Demethylation
M7[2)]	*m/z* 459	Hydroxylation +16
M8[2)]	*m/z* 429	*N*-Oxidation and Demethylation

(Excerpted from Applicant's submission)

Six metabolites were detected in human liver microsomes: M1, M2, M3, M4, M5, and M8. M8 was also found in monkey liver microsomes, and the other 5 metabolites were found in microsomes from all species tested. M2, the demethylated form of lenvatinib, was the major metabolite identified in human liver microsomes. The proposed metabolic pathway for lenvatinib is depicted below.

Figure 13: Proposed Metabolic Schema for Lenvatinib in Mouse, Rat, Dog, Monkey, and Human Microsomes



(Excerpted from Applicant's submission)

AE-6748-G: E7080: Metabolism in Rats and Monkeys after Single Administration of ^{14}C-E7080

Methods

Drug:	^{14}C-E7080
Lot, Specific Activity	Monkeys: CFQ40381, 4.143 MBq/mg Rats: CP-33765-2, 4.252 MBq/mg
Dose, dose volume:	Rats: 3 mg/kg (12.671 MBq/kg), 10 mL/kg Monkeys: 3 mg/kg (12.195 and 12.634 MBq/kg), 3 mL/kg
Frequency, ROA:	Once, oral (administered into stomach using oral tube/catheter and syringe)
Formulation/Vehicle:	3% 0.1 mol/L hydrogen chloride in distilled water
Species/Strain:	Fasted male Sprague Dawley rats and cynomolgus monkeys
Number/Group and Sampling:	12.2.1.1 Rat

Study group	Samples	Sampling times	Number of animals used
Group 1	Plasma, liver, kidney	4 h	3 animals/group
Group 2	Urine	0 – 6, 6 – 12, 12 – 24, and 24 – 48 h	
	Feces	0 – 24, 24 – 48, 48 – 72, and 72 – 96 h	
Group 3	Bile	0 – 12 and 12 – 24 h	
Group 3a			2 animals

12.3.1.2 Monkey Samples

Study group	Samples	Sampling times	Number of animals used
Group 1	Plasma	1, 2, 3, 4, 6, 8, 12, 24, and 48 h	3 animals/group
	Urine	0 – 48 h	
	Feces	0 – 72 h	
Group 2	Plasma, liver, kidney, urine in bladder, and bile in gall bladder	4 h	

(Excerpted from Applicant's submission)

In order to evaluate the in vivo metabolism of lenvatinib, the Applicant conducted metabolite analyses on the plasma, liver, kidney, urine, feces, and bile following a single oral administration of ^{14}C-E7080 to rats and monkeys. Radioactivity was extracted with methanol, tissue and fecal samples were treated with 2-mercaptoethanol, and metabolites were analyzed by HPLC with radioactivity detection and LC/MS. Forty-one metabolites were identified. One of the major metabolic pathways of lenvatinib was glutathione conjugation with elimination of the *O*-aryl group to form m14, leading to subsequent biotransformations. Four hours after a single oral administration of ^{14}C-E7080, the major component of rat and monkey plasma, liver, and kidney was unchanged lenvatinib. Lenvatinib was only slightly detected in rat and monkey urine, but was a major component of rat feces and bile. There were no major circulating metabolites identified in clinical studies; however, according to the Applicant, the M2 metabolite accounted for ~2.5% of lenvatinib exposure. M2 (m37) was also detected in rat and monkey feces and bile, providing nonclinical coverage for this metabolite.

Table 28: Metabolites Identified Following a Single Administration of ^{14}C-E7080 to Rats and Monkeys

Major Metabolites		% in Sample							
Metabolite Identifier	Serial Metabolite Number	Rat Plasma	Rat Liver	Rat Kidney	Monkey Plasma[a]	Monkey Liver	Monkey Kidney	Monkey Urine in Bladder	Monkey Bile in Gallbladder
		4 h	4 h	4 h	4 h	4 h	4 h	4 h	4 h
m4	me10	ND	ND	ND	ND	9.86	3.74	ND	ND
m11	NA	ND	ND	ND	ND	ND	ND	24.71	ND
m14	me40	ND	14.14	ND	ND	10.05	ND	ND	ND
m15	me41	ND	ND	6.70	ND	ND	ND	4.03	ND
m16	NA	ND	ND	ND	ND	ND	ND	19.71	38.42[b]
m33	me104, me105	ND	1.42	0.83	1.75	0.69	2.37	4.49	3.00[b]
m39 (Lenvatinib)	me116	96.40	72.08	75.16	82.04	54.87	72.09	1.10	8.89
Recovery (%)		98.30	92.44	89.05	90.37	90.52	89.75	99.75	99.87
Other Minor Metabolite Identifier Detected in the Sample		ND	m12, m30 (M1), m34 (M3)	m30 (M1), m34 (M3)	m1, m31, m38 (M3´)	m10, m29, m38 (M3´), m41	m29, m35	Many (16 metabolites)	Many (13 metabolites)

ND= not detected; (Excerpted from Applicant's submission)

Table 29: Metabolites Identified in Rat and Monkey Urine and Feces Following a Single Administration of ^{14}C-E7080 to Rats and Monkeys

Major Metabolites		% of Dose				
Metabolite Identifier	Serial Metabolite Number	Rat			Monkey	
		Urine 0-48 h	Feces 0-48 h	Bile 0-24 h	Urine 0-48 h	Feces 0-72 h
m4	me10	ND	ND	0.42	2.22	ND
m8	me19	ND	1.21	ND	ND	3.88
m10	me25	NA	NA	ND	ND	1.13

Major Metabolites		% of Dose				
Metabolite Identifier	Serial Metabolite Number	Rat			Monkey	
		Urine 0-48 h	Feces 0-48 h	Bile 0-24 h	Urine 0-48 h	Feces 0-72 h
m11	NA	NA	NA	1.36	2.03	0.61
m10 and 11	NA	ND	8.56	NA	NA	NA
m14	me40	ND	ND	10	ND	ND
m15	me41	9.15	ND	1.51	ND	ND
m39 (lenvatinib)	me116	0.45	31.01	4.65	ND	5.62
% Excretion		16.07	71.74	34.47	19.08	62.17
% Other metabolites		4.77	22.73	4.37	7.08	32.52

ND= not detected; NA= not applicable

6 General Toxicology

6.1 Single-Dose Toxicity

Single-dose studies were not reviewed.

6.2 Repeat-Dose Toxicity

S03016: E7080: A 4-Week Oral Toxicity Study in Rats

This GLP-compliant repeat-dose toxicology study in Sprague Dawley rats was originally reviewed by Wei Chen, Ph.D. in April, 2005 following submission of IND (b) (4). Ten rats/sex/group were administered 0, 10, 30, or 100 mg/kg lenvatinib (lot # 13033151) by oral gavage once daily for 4 weeks. Mortality and morbidity were observed at the 100 mg/kg dose level (600 mg/m^2) on Days 22-29. Liver toxicity (sinusoidal dilation, hepatocellular necrosis, and increased ALT and AST) was observed at lenvatinib dose levels ≥ 30 mg/kg (180 mg/m^2). Toxicities associated with VEGF blockade were observed with lenvatinib treatment, including changes in the incisors (dysplasia), teeth (white discoloration), femur (increased epiphyseal growth plate), sternum (increased epiphyseal cartilage), kidneys (glomerulopathy), testes (hypocellularity of seminiferous epithelium), epididymides (desquamated seminiferous epithelial cells), and ovaries (follicular atresia). Treatment with ≥ 30 mg/kg lenvatinib resulted in decreased mean red blood cell count, hemoglobin, and hematocrit. Additional target organs of toxicity included the GI tract, duodenum, spleen, and pituitary.

S04001: E7080: A 4-Week Oral Toxicity Study with 4-Week Recovery Period in Rats

Study report location: EDR 4.2.3.2
Conducting laboratory and location: Kawashima Research
Drug Safety Research Laboratories
Eisai Co., Ltd.
1 Takehaya, Kawashima-cho
Hashima-gun, Gifu, Japan
Date of study initiation: January 20, 2004

Reference ID: 3686430

GLP compliance:	Yes (Japan Ministry of Health and Welfare Ordinance No. 21, 1997)
QA statement:	Yes
Drug, lot #, and % purity:	E7080, 13033151, 97.7%

Methods

Doses:	0, 1, and 15 mg/kg/day
Frequency of dosing:	Once daily for 4 weeks
Route of administration:	Oral gavage
Dose volume:	2 mL/kg
Formulation/Vehicle:	PEG400
Species/Strain:	Sprague Dawley rat
Number/Sex/Group:	16/sex/group; 6/sex in the control and high dose groups for recovery cohort
Age:	8 weeks old
Weight:	244.9 to 279.4 g (M); 170.8 to 191.5 g (F)
Satellite groups:	4/sex/group for toxicokinetics (TK)

Study Summary

This GLP-compliant 4-week repeat-dose toxicology study in Sprague Dawley rats was originally reviewed by Wei Chen, Ph.D. in April, 2005 following submission of IND (b) (4). One female dosed with lenvatinib at the 15 mg/kg (90 mg/m^2) dose level was sacrificed moribund on Day 38; morbidity was related to decreased food consumption (anorexia) due to discolored/broken incisors. Toxicities associated with VEGF inhibition were observed at the 15 mg/kg dose level, including histological findings in the incisors, femur, sternum, kidney, testes, epididymides, and ovaries. GI toxicity (decreased body weight and food consumption) was also observed at the 15 mg/kg dose level. Many of the observed toxicities showed evidence of recovery, but findings in the incisors, femur, testes, and epididymides were still observed at the end of the 4-week recovery period.

The 1 mg/kg lenvatinib dose level used in this study was included in the embryofetal development study conducted in rats (#S05152), so the TK results are included below. Administration of lenvatinib at the 1 mg/kg dose level resulted in a mean $AUC_{0\text{-}24hr}$ of 8.075 µg·hr/mL on Day 27. Slight accumulation ≤ 1.4-fold was observed in the 1 mg/kg dose group on Day 27 compared to Day 1.

Table 30: Summary of Mean Lenvatinib Toxicokinetic Parameters in Rats

Dose level (mg/kg/day)	Gender	C_{max} (µg/mL)	Dose Normalized C_{max}	$AUC_{0\text{-}24\,hr}$ (µg.hr/mL)	Dose normalized AUC_{036hr}
Day 1					
1	M	1.12	1.12	4.88	4.88
15	M	29.67	1.98	99.00	6.6
1	F	2.21	2.21	7.49	7.49
15	F	47.90	3.19	146.69	9.78
Day 27					
1	M	1.35	1.35	6.99	6.99
15	M	22.26	1.48	88.42	5.89
1	F	2.75	2.75	9.16	9.16
15	F	41.45	2.76	137.15	9.14

(Table excerpted from Dr. Wei Chen's review of IND (b) (4)

S05039: E7080: A 13-Week Oral Toxicity Study in Rats

This GLP-compliant study was not reviewed because it examined the same dose levels of lenvatinib that were evaluated in the 26-week study in rats (Study #S08037), which is reviewed below in detail. There were no lenvatinib-related mortalities in the main dose groups.

Study title: E7080: A 26-Week Oral Toxicity Study in Rats

Study no.:	S08037
Study report location:	EDR 4.2.3.2
Conducting laboratory and location:	Kawashima, Drug Safety Japan Global Drug Safety Biopharmaceutical Assessments Core Function Unit, Eisai Co., Ltd. 1 Kawashimatakehaya-machi, Kakamigahara-shi, Gifu, Japan
Date of study initiation:	May 16, 2008
GLP compliance:	Yes (Japan Ministry of Health and Welfare Ordinance No. 21, 1997 and the partial revision Ordinance No. 114, 2008)
QA statement:	Yes
Drug, lot #, and % purity:	E7080, 175P2101, 98.5%

Key Study Findings

- There were 11 mortalities at the 10 mg/kg dose level. The Applicant attributed these deaths to severe duodenal (inflammation/cystic dilation accompanied by mucosal hyperplasia and medial arteriole necrosis) and renal (glomerulopathy and glomerulonephropathy) lesions. The duodenal lesions were associated with cholangitis in the liver and/or bile duct and pancreatitis with hemorrhage.
- The main target organs of toxicity included the teeth, kidney, GI tract, duodenum, stomach, pancreas, adrenals, liver, common bile duct, spleen, thymus, brain, femur, sternum, femoral and sternal marrow, ovaries, testes, and epididymides.
- Vascular lesions associated with VEGF inhibition were observed in multiple organs, including adventitial arteriole thickening in the heart.
- Proteinuria was observed at lenvatinib dose levels ≥ 2 mg/kg (12 mg/m^2).
- The severely toxic dose in 37% of rats was 10 mg/kg (60 mg/m^2); deaths occurred after 3 months of treatment.

Methods

Doses:	0, 0.4, 2, and 10 mg/kg/day
Frequency of dosing:	Once daily for 26 weeks
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	Water for injection
Species/Strain:	Sprague Dawley rats/Crl:CD(SD)

Reference ID: 3686430

Number/Sex/Group:	15/sex/group
Age:	8 weeks old
Weight:	251.3-284.6 g (M); 165.8-196.58 g (F)
Satellite groups:	4/sex/group for TK
Unique study design:	None
Deviation from study protocol:	One control TK male was mistakenly dosed with 10 mg/kg lenvatinib on Day 13, which did not affect subsequent TK examinations or the validity/integrity/interpretation of the study.

Observations and Results

Mortality: Checked at least once daily

There were 11 mortalities at the 10 mg/kg dose level between Days 84 and 147; the remaining males in this dosing group were euthanized on Day 150. One TK rat dosed with lenvatinib at the 10 mg/kg dose level was euthanized in moribund condition on Day 97 (details not provided). According to the Applicant, mortality was attributed to the progression of duodenal and renal lesions similar to those observed in the 4- and 13-week toxicology studies. In addition to the findings listed below, prolonged PT and APTT, decreased hemoglobin, hematocrit, and glucose, and increased AST, ALP, total bilirubin, and creatinine were observed in the preterm decedents.

Table 31: Rat Mortalities at the 10 mg/kg/day Lenvatinib Dose Level

Sex/Rat Number	Day of Death	Key Findings
Male/03M06	Found dead on Day 84	-Discolored/broken teeth, food left, soft stool, peri-anal staining, body weight loss **Noteworthy Postmortem Findings:** -Glomerulopathy (kidney), marked adrenal cortical necrosis, marked autolysis in the stomach, duodenum, jejunum, ileum, and cecum
Male/03M13	Euthanized moribund on Day 94	Discolored/broken teeth, food left, soft stool, peri-anal staining, body weight loss **Noteworthy Postmortem Findings:** -Distended/thickened duodenum, pancreatic edema -Cholangitis in bile duct and liver, glomerulopathy (kidney), mucosal hyperplasia and marked inflammation/cystic dilation in the duodenum, slight pancreatitis with hemorrhage
Male/03M15	Euthanized moribund on Day 129	Discolored/broken teeth, food left, peri-ocular and nose staining, chromaturia, body weight loss **Noteworthy Postmortem Findings:** -Distended duodenum, edema in pancreas -Cholangitis in liver and bile duct, glomerulonephropathy, inflammation/cystic dilation in the duodenum, moderate pancreatitis
Male/03M08	Euthanized moribund on Day 135	Discolored/broken teeth, food left, peri-anal and nose staining, soft stool, body weight loss **Noteworthy Postmortem Findings:** -Distended duodenum, dilated common bile duct -Glomerulonephropathy, medial arteriole necrosis in the stomach and duodenum, mucosal hyperplasia and marked inflammation/cystic dilation in the duodenum

Reference ID: 3686430

Sex/Rat Number	Day of Death	Key Findings
Female/03F01	Euthanized moribund on Day 135	Discolored/broken teeth, food left, loss of incisor, ano-genital staining, pale pinna, eyeball discoloration, discolored stool, few feces, body weight loss **Noteworthy Postmortem Findings:** -Distended duodenum, glomerulopathy, medial arteriole necrosis in the stomach and duodenum, marked inflammation/cystic dilation in the duodenum, sinusoidal dilation in the adrenals with hemorrhage
Male/03M02	Found dead on day 140	Discolored/broken teeth, food left, loss of incisor, body weight loss **Noteworthy Postmortem Findings:** -Distended duodenum, enlarged kidney, edema in pancreas, blackish contents in small and large intestine, bile duct dilation -Moderate cholangitis, glomerulopathy, medial arteriole necrosis in duodenum (with hemorrhage), moderate inflammation/cystic dilation in the duodenum
Male/03M09	Euthanized moribund on Day 141	Discolored/broken teeth, food left, soft/discolored stool, peri-anal and nose staining, body weight loss **Noteworthy Postmortem Findings:** -Distended duodenum, glomerulonephropathy, medial arteriole necrosis and marked inflammation/cystic dilation in the duodenum
Male/03M12	Euthanized moribund on Day 141	Discolored/broken teeth, food left, soft stool, loss of incisor, oral region staining, body weight loss **Noteworthy Postmortem Findings:** -Distended duodenum, glomerulonephropathy, medial arterial necrosis in the stomach and duodenum, marked inflammation/cystic dilation in the duodenum
Male/03M14	Euthanized moribund on Day 147	Discolored/broken teeth, food left, pale pinna, discolored stool, eyeball discoloration, body weight loss **Noteworthy Postmortem Findings:** -Distended duodenum, watery contents in cecum, discolored kidney, pancreatic edema -Adventitial arteriole thickening in the heart, marked glomerulonephropathy and medial arteriole necrosis in the kidney, medial arteriole necrosis in the stomach and duodenum, moderate inflammation/cystic dilation in the duodenum, sinusoidal dilation in the adrenals with hemorrhage, slight malacia in the cerebrum
Female/03F02	Euthanized moribund on Day 147	Discolored/broken teeth, food left, incisor loss, eyeball discoloration, ano-genital and facial staining, soft stool, body weight loss **Noteworthy Postmortem Findings:** -Distended duodenum, glomerulopathy, medial arteriole necrosis in the stomach and duodenum, marked inflammation/cystic dilation in the duodenum, sinusoidal dilation with hemorrhage in the adrenals
Female/03F07	Euthanized moribund on Day 147	Discolored/broken teeth, food left, peri-ocular and facial staining, eyeball discoloration, soft stool, body weight loss **Noteworthy Postmortem Findings:** -Distended duodenum, slight glomerulopathy, marked inflammation/cystic dilation in the duodenum, sinusoidal dilation with hemorrhage in the adrenals

*See Table 37 and Table 39 for additional gross pathology and histopathology findings

Clinical Signs: Observed daily

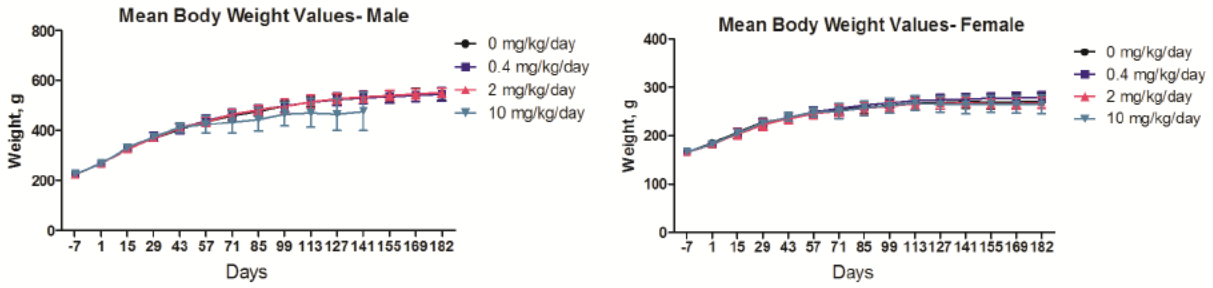
Table 32: Clinical Observations in Rats

Dose (mg/kg/day)	0		0.4		2		10	
Sex	M	F	M	F	M	F	M	F
Broken teeth	2	2	2		14	14	15	15
Discoloration of teeth				1	15	15	15	15
Loss of incisor							7	9
Soft stool							6	3
Discolored stool							2	1
Few feces								1
Peri-ocular staining			3			2		3
Peri-anal staining							4	
Nose staining						1	3	4
Eye staining						1		
Facial staining								2
Staining oral region							1	1
Staining ano-genital region								3
Pale pinna							1	1
Discoloration of eyeball							1	10
Chromaturia							1	
Hemorrhage, hindlimbs							1	

Body Weights: Recorded weekly

There was a ≤ 11% decrease in total mean body weight in males dosed with lenvatinib at the 10 mg/kg dose level compared to controls.

Figure 14: Mean Body Weight Values in Male and Female Rats

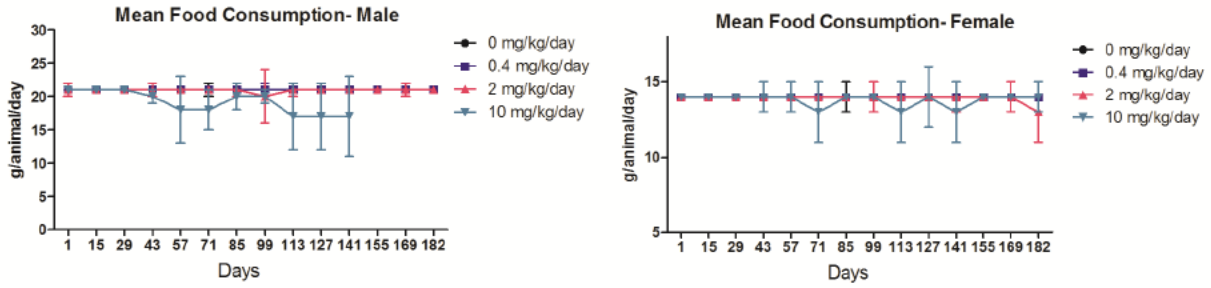


Food Consumption: Recorded weekly

Table 33: Residual Food Left for Rats

Dose (mg/kg/day)	0		0.4		2		10	
Sex	M	F	M	F	M	F	M	F
Food left	3	2	4	3	15	9	15	13

Figure 15: Food Consumption Values in Male and Female Rats



Ophthalmoscopy

Ophthalmologic examinations were performed once pre-dosing, once on Day 148 (male high dose only), and once on Day 177/178. The findings were unremarkable.

ECG: Not applicable

Hematology

Blood samples were collected on the day of necropsy for hematology and clinical chemistry.

Table 34: Changes in Hematology Compared to Control Groups after 26 Weeks

Dose (mg/kg/day)	0.4		2		10	
Sex	M	F	M	F	M	F
Red blood cells					10%↓*	
Mean corpuscular volume (MCV)	4%↑#		6%↑#		14%↑#	6%↑#
Mean corpuscular hemoglobin (MCH)			6%↑#		14%↑#	7%↑#
White blood cells	22%↑	19%↑	55%↑#	33%↑	153%↑#	76%↑#
Neutrophils	26%↑	22%↑	97%↑#	80%↑	310%↑#	226%↑#
Lymphocytes	21%↑	17%↑	46%↑#	21%↑	119%↑#	38%↑
Monocytes	31%↑	43%↑	77%↑#	114%↑#	431%↑#	271%↑#
Eosinophils		63%↑	50%↑#	50%↑#	71%↑#	50%↑
Basophils	100%↑		100%↑		400%↑#	
PT				3%↓*		7%↓#
APTT					18%↑*	

% relative to controls
*, P ≤ 0.05; #, P ≤ 0.01 versus controls

Clinical Chemistry

Table 35: Changes in Clinical Chemistry Compared to Controls after 26 Weeks

Dose (mg/kg/day)	0.4		2		10	
Sex	M	F	M	F	M	F
ALT		10%↑	20%↑	47%↑		114%↑*
AST		6%↑		23%↑	38%↓*	61%↑
Total cholesterol	16%↑	11%↑	19%↑	2%↑	122%↑#	26%↑#
Urea nitrogen	6%↑	9%↑	16%↑#	16%↑	26%↑#	21%↑*
Glucose	8%↓	11%↑	2%↓	4%↑	22%↓*	6%↑

Dose (mg/kg/day)	0.4		2		10	
Sex	M	F	M	F	M	F
Total protein			7%↓#	6%↓*	12%↓#	17%↓#
Albumin			12%↓#	13%↓*	21%↓#	25%↓#
Globulin					6%↓	9%↓#
A/G ratio	7%↓*		8%↓#	9%↓*	16%↓#	18%↓#
Total bilirubin	25%↑		12%↑		25%↓#	

% relative to controls
*, P ≤ 0.05; #, P ≤ 0.01 versus controls

Urinalysis:

Urine was collected from fasted rats on the day before necropsy. Proteinuria was observed in males dosed with ≥ 2 mg/kg lenvatinib. One female dosed with 10 mg/kg exhibited occult blood (2+) in the urine.

Table 36: Changes in Urinalysis Compared to Controls after 26 Weeks

Dose (mg/kg/day)		0.4		2		10	
	Sex	M	F	M	F	M	F
Protein	+	1	1	1		1	
	2+			2	1	2	1
	3+					4	

Gross Pathology

Surviving rats were necropsied on Days 183, 184, or 185, except surviving males dosed with 10 mg/kg were necropsied on Day 150.

Table 37: Gross Pathology Findings in Rats after 26 Weeks

Dose (mg/kg/day)		0		0.4		2		10	
Sex		M	F	M	F	M	F	M	F
# of rats examined (preterm decedents)		15	15	15	15	15	15	7 (8)	12 (3)
Adrenals	Enlarged, bilateral							(4)	2 (3)
	Discoloration							(4)	8 (1)
Abdominal cavity	Fluid, clear							(1)	
Adipose tissue	Discolored							(2)	
Cecum	Watery contents							(1)	
Common bile duct	Dilation			1		1		(3)	
Duodenum	Distension							6 (7)	8 (3)
	Nodule							(1)	
	Thickened							(1)	
Epididymides	Small, bilateral							(1)	
Ileum and large intestine	Blackish contents							(1)	
Incisor	Broken			1		8	11	2 (5)	7
	Discoloration					15	14	2 (5)	6
	Loss			2				7 (4)	8 (3)

Dose (mg/kg/day)		0		0.4		2		10	
Sex		M	F	M	F	M	F	M	F
# of rats examined (preterm decedents)		15	15	15	15	15	15	7 (8)	12 (3)
Kidney	Enlarged, bilateral Discoloration, bilateral							(1) (2)	
Liver	Adhesion					1			
Ovaries	Small Cyst								1 1
Pancreas	Edema							(4)	
Pituitary	Enlarged								1
Prostate	Small Enlarged Edema					1		(1) (1) (1)	
Seminal Vesicles	Enlarged					1			
Small intestine	Watery contents Blackish							(1) (1)	
Spleen	Small Pale discolored							(1)	(1) (2)
Stomach	Watery contents Reddish focus, glandular stomach							(1)	(2)
Subcutis	Discoloration							(1)	
Testes	Soft Small							(2) (5)	
Thymus	Small							4 (7)	(3)
Tongue	Discolored							(1)	
Trachea	Foamy material							(1)	

( ) = observed in preterm decedent

Organ Weights

Table 38: Organ Weight Changes after 26 Weeks of Treatment

Dose (mg/kg/day)		0.4		2		10	
Sex		M	F	M	F	M	F
Brain	Abs Rel to BW					 15%↑*	
Heart	Abs Rel to BW				10%↓# 9%↓*	12%↓#	12%↓# 9%↓*
Kidney	Abs Rel to BW					5%↑ 19%↑*	
Testis	Abs Rel to BW			4%↓ 5%↓		19%↓# 8%↓	
Ovaries	Abs Rel to BW				38%↑# 33%↑#		14%↑ 17%↑

% relative to controls ; Rel to BW = Relative to body weight; *, P ≤ 0.05; #, P ≤ 0.01 versus controls

Histopathology

Adequate Battery: Yes
Peer Review: Yes

Table 39: Histological Findings in Rats after 26 Weeks of Lenvatinib

	Dose (mg/kg/day)	0		0.4		2		10	
	Sex	M	F	M	F	M	F	M	F
# of rats examined (preterm decedents)		15	15	15	15	15	15	7 (8)	12 (3)
Heart	Fibrosis, focal								
	Slight			1		2			
	Adventitial thickening, arteriole, Slight							(1)	
Liver	Thickened capsule								
	Moderate					1			
	Kupffer cell hypertrophy/hyperplasia								
	Slight							(3)	1
	Pigmentation, hepatocytes, periportal								
	Slight								4
	Cholangitis								
	Slight							(1)	
	Moderate								(2)
Common bile duct	Cholangitis								
	Slight			1		1			
	Moderate							(3)	
	Arteritis								
	Slight					2			
Kidney	Glomerulopathy								
	Slight					5			11 (3)
	Moderate							5 (3)	1
	Marked							1	
	Glomerulonephropathy								
	Slight							1 (2)	
	Moderate							(2)	
	Marked							(1)	
	Basophilic tubules								
	Slight				1	1	1		
	Medial necrosis, arteriole								
	Slight						1	(1)	
	Cyst, Slight							(2)	
	Neutrophil infiltration								
	Moderate							(1)	
Incisor	Dysplasia								
	Slight					1	3		
	Moderate					14	12		
	Marked							7 (8)	12 (3)
	Inflammatory cell infiltration, pulp								
	Slight					2		4 (6)	5
	Moderate							1 (1)	1 (2)
	Inflammatory cell								

Dose (mg/kg/day)		0		0.4		2		10	
Sex		M	F	M	F	M	F	M	F
# of rats examined (preterm decedents)		15	15	15	15	15	15	7 (8)	12 (3)
	infiltration, gingiva								
	Slight					2	1	6 (3)	8 (1)
	Moderate							(1)	
	Fracture								
	Slight	1		2					
Molar	Abcess, gingiva								
	Slight					1			1
Stomach	Mucosal hyperplasia, glandular stomach								
	Slight							5 (3)	6 (1)
	Moderate							2 (4)	5 (2)
	Inflammatory cell infiltration, glandular stomach								
	Slight		1					5 (4)	9 (2)
	Moderate							1 (2)	1 (1)
	Mineralization, mucosa								
	Slight							2	
	Medial necrosis, arteriole								
	Slight							(3)	
	Erosion, Slight							(2)	(2)
	Autolysis, Marked							(1)	(1)
Duodenum	Inflammation/cystic dilation, duodenal gland								
	Moderate							1 (3)	4
	Marked							4 (4)	5 (3)
	Medial necrosis, arteriole								
	Slight							4 (4)	
	Moderate							(1)	
	Mucosal hyperplasia								
	Slight							1 (3)	3
	Autolysis, Marked							(1)	
Jejunum	Foamy cell/neutrophil accumulation								
	Slight							4	4
	Autolysis, Marked							(1)	
Ileum	Hyperplasia, crypt								
	Slight							3 (4)	5 (2)
	Lymphoid depletion								
	Slight							(1)	
	Autolysis, Marked							(1)	
Cecum	Hyperplasia, crypt								
	Slight							4 (5)	4 (1)
	Moderate							3	(1)
	Edema, submucosa								
	Slight							2 (3)	(1)
	Erosion, Slight					1	1		
	Autolysis, Marked							(1)	

	Dose (mg/kg/day)	0		0.4		2		10	
	Sex	M	F	M	F	M	F	M	F
# of rats examined (preterm decedents)		15	15	15	15	15	15	7 (8)	12 (3)
Colon	Hyperplasia, crypt								
	Slight							(2)	1 (3)
	Inflammatory cell infiltration, mucosa								
	Slight							(2)	
	Decreased goblet cells								
	Slight							(2)	
Rectum	Hyperplasia, crypt								
	Slight							(2)	1 (3)
	Decreased goblet cells								
	Slight							(3)	
Pancreas	Arteritis								
	Slight					1		(1)	
	Moderate							(1)	
	Basophilic foci								
	Slight			2					
	Atrophy								
	Slight			1					
	Moderate			1					
	Decreased zymogen granules								
	Slight							(2)	1 (1)
	Moderate							(2)	(2)
	Pancreatitis								
	Slight							(1)	
	Moderate							(1)	
	Fatty necrosis								
	Slight							(1)	
	Moderate							(1)	
Adipose tissue	Fatty necrosis								
	Slight							(1)	
	Medial necrosis, arteriole, Slight							(1)	
Pituitary	Vacuolation, basophilic cell, Slight							3 (3)	
Adrenals	Sinusoidal dilation								
	Slight					1	1	2 (1)	6
	Moderate							(4)	5 (2)
	Marked								(1)
	Cortical necrosis								
	Slight		1		1		1	1 (1)	2 (1)
	Moderate						1		6 (1)
	Marked							(3)	
Ovaries	Follicular atresia								
	Slight								1
	Moderate								11 (3)
	Cyst, Slight								1
Vagina	Mucification, Slight								(3)

	Dose (mg/kg/day)	0		0.4		2		10	
	Sex	M	F	M	F	M	F	M	F
# of rats examined (preterm decedents)		15	15	15	15	15	15	7 (8)	12 (3)
Testes	Hypocellularity, seminiferous epithelium, bilateral								
	Slight							1 (3)	
	Moderate							1	
	Marked							(1)	
	Medial necrosis, arteriole, Slight							(1)	
Epididymides	Desquamated seminiferous epithelial cells, bilateral								
	Slight							1 (3)	
	Moderate							(1)	
Prostate	# of rats examined:	15				1		7 (8)	
	Prostatitis, Moderate					1			
	Edema, Slight							(1)	
Spleen	Mineralization, trabecula								
	Slight						1	(2)	9 (2)
	Moderate							3	1 (1)
	Extramedullary hematopoiesis								
	Slight			1				(1)	
	Lymphoid depletion								
	Slight							(3)	1 (2)
	Moderate							(1)	(1)
	Medial necrosis, central arteries, Slight							(1)	
Thymus	Atrophy								
	Slight							(3)	(1)
	Moderate							(2)	(1)
	Marked								(1)
Submaxillary glands	Acinar hypertrophy								
	Slight							(5)	
Mesenteric lymph node	Histiocytosis, sinus								
	Slight							(1)	
Femur	Increased epiphyseal growth plate								
	Slight								3
	Moderate							2	2 (3)
	Marked							5 (8)	
Femoral marrow	Hypocellularity, diffuse								
	Slight							4 (4)	7
	Moderate							(1)	(3)
	Marked							(1)	
Sternum	Increased epiphyseal cartilage, Slight							3 (6)	3 (3)
Sternal marrow	Hypocellularity, diffuse								
	Slight							1 (1)	1 (1)
	Moderate							(1)	(2)
	Marked							(1)	

Dose (mg/kg/day)		0		0.4		2		10	
Sex		M	F	M	F	M	F	M	F
# of rats examined (preterm decedents)		15	15	15	15	15	15	7 (8)	12 (3)
Brain	Eosinophilic exudate, choroid plexus Slight							2 (6)	3 (2)
	Mononuclear cell infiltration, perivascular Slight								1
	Malacia, Slight							(1)	

Slight = Grade 1+, Moderate = Grade 2+, Marked = Grade 3+
( ) = observed in preterm decedent

Special Evaluation: None

Toxicokinetics

- Blood samples were collected from animals in the control group 15 min after vehicle administration on Days 1 and 181.
- Blood samples were collected 0.25, 0.5, 1, 2, 4, 8 and 24 hrs after lenvatinib administration on Day 1, and prior to dosing and 0.25, 0.5, 1, 2, 4, 8 and 24 hrs after lenvatinib administration on Day 149 (male high dose group) or Day 181.
- Lenvatinib drug exposures were generally dose proportional on Day 1; mean $AUC_{0\text{-}24hr}$ increased slightly less than dose proportionally between the 0.4 and 10 mg/kg dose groups on Day 149/181.
- Lenvatinib drug exposures were slightly higher in females (< 2-fold) compared to males.
- There was evidence of slight accumulation based on Cmax and $AUC_{0\text{-}24hr}$ on Day 149/181 compared to Day 1, except for $AUC_{0\text{-}24hr}$ in the 10 mg/kg group.

Table 40: Summary of Mean Toxicokinetic Data Following 26 weeks of Daily Lenvatinib Administration to Rats

Sex	Dose (mg/kg)	Day	C_{max} (µg/mL)	t_{max} (hr)	$AUC_{0\text{-}24hr}$ (µg·hr/mL)
Male	0.4	Day 1	0.3619 ± 0.1212	1 (0.5-1)	2.0917 ± 0.2039
		Day 181	0.5053 ± 0.1458	1.25 (0.5-4)	3.2102 ± 0.2641
	2	Day 1	2.6623 ± 0.5819	0.25 (0.25-0.5)	12.3832 ± 1.5265
		Day 181	4.0121 ± 0.8716	0.5 (0.25-0.5)	18.2235 ± 0.6711
	10	Day 1	7.6523 ± 1.4713	3 (1-8)	62.2321 ± 19.1741
		Day 149*	10.1696 ± 2.1201	1 (0.5-2)	55.6223 ± 13.4592
Female	0.4	Day 1	0.6026 ± 0.1078	0.25 (0.25-0.5)	2.3159 ± 0.2563
		Day 181	0.9497 ± 0.1747	0.25 (0.25-0.5)	3.5546 ± 0.6953
	2	Day 1	3.3249 ± 0.7181	0.38 (0.25-1)	13.2388 ± 1.0661
		Day 181	3.9652 ± 1.5404	0.5 (0.25-0.5)	17.0003 ± 2.3123
	10	Day 1	14.6644 ± 2.1798	0.38 (0.25-4)	85.2384 ± 9.6574
		Day 181	19.2626 ± 4.0611	0.38 (0.25-1)	71.1701 ± 8.8162

C_{max} and $AUC_{0\text{-}24hr}$ values represent the mean ± SD of 4 animals
(*: the mean ± SD of 3 animals, one animal had no data for death).
t_{max} values represent the median and range of 4 animals
(*: the median and range of 3 animals, one animal had no data for death).

(Excerpted from Applicant's submission)

Dosing Solution Analysis

Met pre-specified acceptance criteria.

S03077: E7080: A 4-Week Oral Toxicity Study with 4-Week Recovery Period in Dogs

This GLP-compliant repeat-dose toxicology study in beagle dogs was originally reviewed by Wei Chen, PhD in April, 2005 following submission of IND [redacted] (b) (4). Three dogs/sex/group were administered 0, 0.1, or 0.5 mg/kg lenvatinib (lot # 13033151) by oral capsule once daily for 28 days. Two dogs/sex dosed with 0 or 0.5 mg/kg lenvatinib were assessed in a 4-week recovery period. There were no mortalities. Clinical signs of GI toxicity included watery stool in dogs dosed with lenvatinib at the 0.5 mg/kg (10 mg/m^2) dose level. Histological findings at the 0.5 mg/kg dose level included arterial fibroid necrosis in the gallbladder, glomerulopathy, and lymphoid depletion/necrosis in the jejunum and ileum. Histological findings in the testes and epididymides were observed at dose levels ≥ 0.1 mg/kg (2 mg/m^2) lenvatinib.

SBL47-86: E7080: A 4-Week Oral Toxicity Study with 4-Week Recovery Period in Monkeys

This GLP-compliant repeat-dose toxicology study in cynomolgus monkeys was originally reviewed by Wei Chen, Ph.D. in April, 2005 following submission of IND [redacted] (b) (4). Three dogs/sex/group were administered 0, 0.3, 3, or 30 mg/kg lenvatinib (lot # 13033151) by oral capsule once daily for 28 days. Two monkeys/sex dosed with 0, 3, or 30 mg/kg lenvatinib were assessed in a 4-week recovery period. One female dosed with lenvatinib at the 30 mg/kg dose level died on Day 21 due to body weight loss attributable to anorexia. The main organs of lenvatinib toxicity were the GI tract (soft/watery stool, decreased body weight and food consumption, and medial arterial degeneration in stomach and cecum), kidney (glomerulopathy), liver (increased ALT and AST), gallbladder (arterial fibroid necrosis), testes, brain (hemorrhage in the choroid plexus), spleen (lymphoid atrophy), and adrenals (cortical hypertrophy). These findings generally showed evidence of recovery, except for hypocellularity in the testes.

SBL47-94: E7080: A 13-Week Oral Toxicity Study in Monkeys

This GLP-compliant study was not reviewed because it examined the same dose levels of lenvatinib that were evaluated in the 39-week study in monkeys (Study #SBL038-031), which is reviewed below in detail. According to the Applicant, one female dosed with lenvatinib at the 3 mg/kg dose level was euthanized in moribund condition on Day 75. This preterm decedent exhibited anorexia, emaciation, hypothermia, watery stool, severe cachexia, and histological changes in the kidneys, duodenum, gallbladder, stomach, small intestine, and ovaries.

Study title: E7080: A 39-Week Oral Toxicity Study in Monkeys

Study no.:	SBL038-031
Study report location:	EDR 4.2.3.2
Conducting laboratory and location:	[redacted] (b) (4)

(b) (4)

Date of study initiation: August 12, 2008
GLP compliance: Yes (Japan Ministry of Health and Welfare Ordinance No. 21, 1997, Notification No. 655, 1999, and Notification No. 443, 1996)
QA statement: Yes
Drug, lot #, and % purity: E7080, 175P2101, 98.5%

Key Study Findings

- One male dosed with lenvatinib at the 3 mg/kg dose level was euthanized in moribund condition on Day 51. Lenvatinib-induced inflammatory changes in the lower large intestine in combination with severe duodenal changes were considered the cause of morbidity.
- The main target organs of toxicity included the kidney, GI tract, duodenum, gallbladder, pancreas, adrenals, pituitary, spleen, thymus, femur, choroid plexus, skeletal muscle, vagina, and ovaries.
- Proteinuria was observed at the 3 mg/kg (36 mg/m^2) dose level.

Methods

Doses: 0, 0.1, 0.5, and 3 mg/kg/day
Frequency of dosing: Once daily for 39 weeks
Route of administration: Oral gavage
Dose volume: 5 mL/kg
Formulation/Vehicle: Water for injection
Species/Strain: Cynomolgus monkeys
Number/Sex/Group: 4/sex/group
Age: 4 to 6 yrs old (M); 3 to 5 yrs old (F)
Weight: 2.75-4.5 kg (M); 2.19-3.06 kg (F)
Satellite groups: None
Unique study design: None. Statistical analysis was not conducted.
Deviation from study protocol: None that impacted study interpretation

Observations and Results

Mortality: Checked three times daily

Table 41: Monkey Mortalities

Sex/Monkey Number	Dose level	Day of Death	Key Findings*
Male/26	3 mg/kg	Euthanized moribund on Day 51	-32% body weight loss compared to baseline, 87% decrease in food consumption, anorexia, soft/watery stool, decrease in spontaneous activity, and emaciation -Hypothermia and sitting position (Day 51) **Noteworthy Postmortem Findings:** -Increased BUN and potassium

Sex/Monkey Number	Dose level	Day of Death	Key Findings*
			-Decreased sodium and chloride -Increased viscosity of the bile in the gallbladder -Histological findings in the kidneys, gallbladder (focal arterial degeneration/fibroid necrosis), choroid plexus (eosinophilic exudate and arterial fibroid necrosis), femur, duodenum (atrophy with inflammation and arterial fibroid necrosis), colon and rectum (inflammatory cell infiltration). **Cause of morbidity:** Inflammatory changes in the lower large intestine in combination with severe duodenal changes

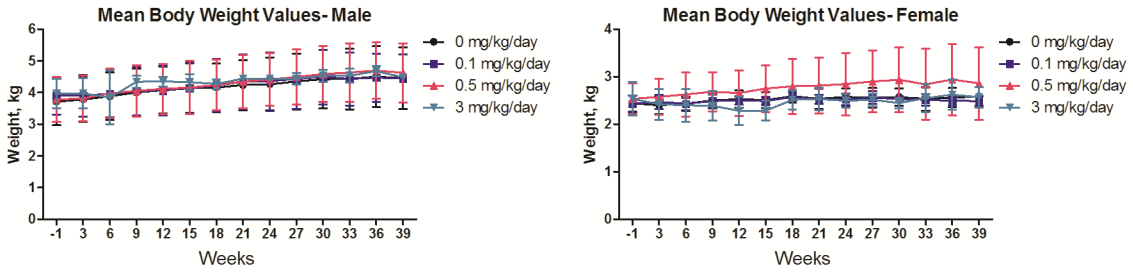
*See Table 46 for detailed histological findings

Clinical Signs: Observed three times daily

Decreased incidence of menstruation was observed in 6/8 females dosed with lenvatinib at dose levels ≥ 0.5 mg/kg. Additional clinical signs were only observed in the preterm decedent (see Table 41).

Body Weights: Recorded weekly

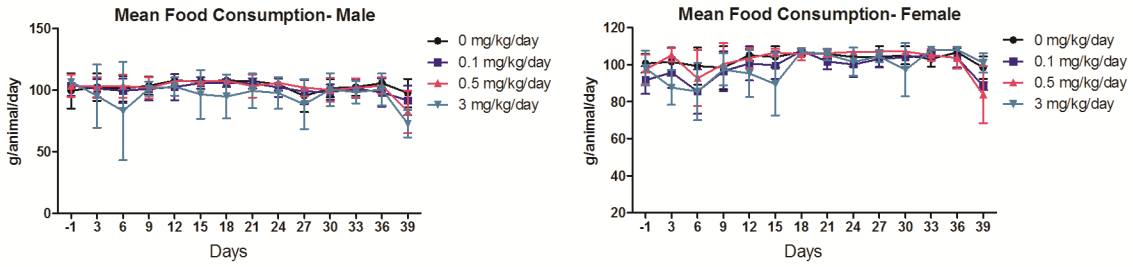
Figure 16: Mean Body Weight Values in Male and Female Monkeys



Food Consumption: Recorded daily

There was an 18% decrease in mean food consumption in males dosed with lenvatinib at the 3 mg/kg dose level compared to controls during Week 6, which was due to the extremely low food consumption exhibited by the preterm decedent (76% decrease compared to controls). There was a dose-dependent decrease in mean food consumption in lenvatinib-treated males compared to controls at the end of the study (Week 39), which reached 26% in the 3 mg/kg dose group.

Figure 17: Food Consumption Values in Male and Female Monkeys



Ophthalmoscopy

Ophthalmologic examinations were performed on Days -19 and 268. The findings were unremarkable.

ECG

Diastolic, systolic, and mean blood pressure were recorded on Days -19 and 268, and the findings were unremarkable. Electrocardiograms were recorded on Days -15 and 267 (before and 4 hrs after dosing). Only heart rate data was provided; the findings were unremarkable. Treatment with lenvatinib for 13 weeks in Study SBL47-94 did not result in QT prolongation in monkeys.

Hematology

Blood samples for hematology and clinical chemistry were collected on Days -8, 28, 87, and 273 after overnight fasting.

Table 42: Changes in Hematology Compared to Control Groups after 39 Weeks

Dose (mg/kg/day)	0.1		0.5		3	
Sex	M	F	M	F	M	F
White blood cells			17%↑	10%↑	44%↑	22%↑
Hematocrit	5%↑	5%↑	6%↑	10%↑	15%↑	13%↑
Hemoglobin	6%↑	5%↑	6%↑	12%↑	16%↑	145%↑

% relative to controls

Clinical Chemistry

Table 43: Changes in Clinical Chemistry Compared to Controls after 39 Weeks

Dose (mg/kg/day)	0.1		0.5		3	
Sex	M	F	M	F	M	F
Blood urea nitrogen					27%↑	10%↑
Creatinine	11%↑		15%↑	12%↑	53%↑	7%↑
Inorganic phosphorous	14%↓		20%↓		32%↓	

% relative to controls

Urinalysis:

Urine was collected on Days -13, 24, 86, and 272.

Table 44: Changes in Urinalysis Compared to Controls after 39 Weeks

Dose (mg/kg/day)	0.1		0.5		3	
Sex	M	F	M	F	M	F
Mean Protein	36%↑	15%↑^	350%↑^	24%↑	2818%↑*	20%↓

% relative to controls; ^ = No increase compared to baseline
* = One male exhibited a 7748%↑ compared to controls and a 1068% increase compared to baseline.

Gross Pathology

Table 45: Gross Pathology Findings in Monkeys after 39 Weeks

Dose (mg/kg/day)		0		0.1		0.5		3	
Sex		M	F	M	F	M	F	M	F
# of monkeys examined		4	4	4	4	4	4	4	4
Kidney	Depressed area, several, left, present					1			
Gallbladder	Increased viscosity, bile							1*	

* = observed in preterm decedent

Organ Weights

There was a dose-dependent decrease in mean absolute ovarian weight at lenvatinib dose levels ≥ 0.5 mg/kg; statistical significance was not assessed.

Histopathology

Adequate Battery: Yes

Peer Review: Yes

Table 46: Histological Findings in Monkeys after 39 Weeks of Lenvatinib

Dose (mg/kg/day)		0		0.1		0.5		3	
Sex		M	F	M	F	M	F	M	F
# of monkeys examined		4	4	4	4	4	4	4	4
Submaxillary glands	Acinar atrophy, bilateral, Slight							1*	
Parotid glands	Acinar atrophy, bilateral, Slight							1*	
Stomach	Granuloma, parasitic	1			1			2*	
Duodenum	Atrophy, duodenal glands								
	Slight								3
	Moderate							1*	
	Villus atrophy								
	Slight							1*	
	Hyperplasia, crypt								
	Slight							2*	4
	Arterial fibrinoid necrosis, Slight							1*	
Colon and rectum	Inflammatory cell infiltration, mucosa								
	Slight							1*	
Esophagus	Erosion, Slight						1		
Pancreas	Decreased zymogen granules								
	Slight							1	1
	Moderate							1*	
	Ectopic spleen			1					

	Dose (mg/kg/day)	0		0.1		0.5		3	
	Sex	M	F	M	F	M	F	M	F
	# of monkeys examined	4	4	4	4	4	4	4	4
Gallbladder	Inflammatory cell infiltration, submucosa								
	Slight							2*	1
	Focal arterial degeneration/fibroid necrosis								
	Slight							1*	1
	Moderate							1	
Kidney	Glomerulopathy								
	Slight					3		3*	2
	Moderate							1	1
	Fibrosis, focal								
	Slight					1			
	Inflammatory cell infiltration, focal								
	Slight						1		1
Prostate	Inflammatory cell infiltration, focal								
	Slight			1		1		1	
Brain	Eosinophilic exudate, choroid plexus								
	Slight							4*	1
	Arterial fibrinoid necrosis, choroid plexus								
	Slight							1	
	Moderate							1*	
Femur	Increased epiphyseal growth plate, Slight					1		2*	4
Bone marrow	Hypocellularity								
	Slight							1*	
Mesenteric lymph node, spleen, and thymus	Lymphoid depletion								
	Slight							1*	
Pituitary	Cyst	1						2	
	Vacuolation, basophilic cell								
	Slight						1		2
	Moderate						1		2
Adrenals	Cortical hypertrophy, focal, Slight			1			1	1	
	Cortical hypertrophy, bilateral, Slight							1*	
	Mononuclear cell infiltration, Slight								1
Skeletal muscle	Parasite			2	1	1	1		
	Degeneration/necrosis, Muscle fiber, Slight				1				1
Ovary	Follicular atresia								
	Slight						1		4

Dose (mg/kg/day)		0		0.1		0.5		3	
Sex		M	F	M	F	M	F	M	F
# of monkeys examined		4	4	4	4	4	4	4	4
Vagina	Atrophy, epithelium Slight								1
Eye	Retinal dysplasia Slight								1

Slight = Grade 1+, Moderate = Grade 2+, Marked = Grade 3+
* = observed in preterm decedent

Special Evaluation: None

Toxicokinetics

- Blood samples were collected 1, 2, 4, 8, and 24 hrs after lenvatinib administration on Day 1, and prior to dosing and 1, 2, 4, 8 and 24 hrs after lenvatinib administration on Days 91 and 273.
- Lenvatinib drug exposures generally increased more than dose proportionally on Days 1 and 273, except for mean $AUC_{0\text{-}24hr}$ in females on Day 273.
- There were no significant gender differences.
- The mean Tmax ranged from 1-4 hrs.
- There was evidence of slight accumulation (≤ 2.7-fold) based on Cmax and $AUC_{0\text{-}24hr}$ on Day 273 compared to Day 1, except for the 3 mg/kg dosing group in females.

Table 47: Summary of Mean Toxicokinetic Data Following 39 weeks of Daily Lenvatinib Administration to Monkeys

Dose (mg/kg)	Day	Male C_{max} (µg/mL)	Male $AUC_{0\text{-}24hr}$ (µg·hr/mL)	Female C_{max} (µg/mL)	Female $AUC_{0\text{-}24hr}$ (µg·hr/mL)
0.1	Day 1	0.0125 ± 0.0023	0.0884 ± 0.0343	0.0204 ± 0.0111	0.1500 ± 0.0942
	Day 91	0.0236 ± 0.0028	0.1748 ± 0.0243	0.0224 ± 0.0102	0.1917 ± 0.0702
	Day 273	0.0332 ± 0.0133	0.2051 ± 0.0952	0.0388 ± 0.0096	0.2649 ± 0.0399
0.5	Day 1	0.1731 ± 0.0485	1.0527 ± 0.2125	0.1630 ± 0.0652	0.9731 ± 0.2572
	Day 91	0.1652 ± 0.0733	1.0513 ± 0.3230	0.1254 ± 0.0454	0.8449 ± 0.1689
	Day 273	0.2904 ± 0.0367	1.5418 ± 0.3605	0.2310 ± 0.0328	1.2944 ± 0.0946
3.0	Day 1	1.3318 ± 0.3388	7.6061 ± 1.6584	1.9223 ± 0.5754	8.2213 ± 2.7475
	Day 91	1.4328 ± 0.3053*	8.8307 ± 2.7719*	1.3483 ± 0.7848	7.4213 ± 3.4277
	Day 273	1.8855 ± 0.2178*	11.1901 ± 1.8730*	1.7407 ± 0.1198	8.3662 ± 1.6454

C_{max} and $AUC_{0\text{-}24hr}$ values represent the mean ± S.D. of 4 animals
(*: the mean ± S.D. of 3 animals, one animal had no data because of euthanasia).

(Excerpted from Applicant's submission)

Dosing Solution Analysis

Met pre-specified acceptance criteria.

7 Genetic Toxicology

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

Study title: E7080: Reverse Mutation Assay in Bacteria

Study no.:	S03007
Study report location:	EDR 4.2.3.3.1
Conducting laboratory and location:	Kawashima Research, Drug Safety Research Laboratories, Eisai Co., Ltd. 1 Takehaya, Kawashima-cho, Hashima-gun, Gifu, Japan
Date of study initiation:	April 9, 2003
GLP compliance:	Yes (Japan Ministry of Health and Welfare Ordinance No. 21, 1997)
QA statement:	Yes
Drug, lot #, and % purity:	E7080, 13012251, 99%

Key Study Findings: E7080 was not mutagenic in the in vitro Ames assay in the presence or absence of metabolic activation.

Methods

Strains:	*Salmonella typhimurium* tester strains TA100, TA1535, TA98, and TA1537; *Escherichia coli* strain WP2 *uvrA* (pKM101)
Concentrations in definitive study:	0, 313, 625, 1250, 2500, and 5000 µg/plate in the presence and absence of metabolic activation (S9 mix)
Basis of concentration selection:	A range finding test was conducted using 39 to 5000 µg/plate. Cytotoxicity was not observed in any of the bacterial strains in the presence or absence of S9, so concentrations of 313 to 5000 µg/plate were chosen for the main test.
Negative control:	Dimethyl sulfoxide (DMSO)
Positive control:	TA100, TA98 and WP2 in the absence of S9: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (AF2); TA1535 in the absence of S9: sodium azide (SA); TA1537 in the absence of S9: 9-Aminoacridine hydrochloride (9AA); all strains in the presence of S9: 2-Aminoanthracene (2AA)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	The pre-incubation method was used. E7080 or control solutions in the presence and absence of S9 mix were incubated at 37º C for ~48 hrs; 3 plates per dose were used. The number of revertants that

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appeared on each plate was counted automatically or manually. Cytotoxicity was determined by a reduction in the number of revertants, a clearing, or diminution of the background lawn.

Evaluation Criteria: Mutagenic = number of revertants is significantly greater than the negative control, and there is a significant dose-related increase at non-cytotoxic doses.

Study Validity

The study was determined to be valid because: 1) an adequate number of bacteria strains were used in this study; 2) appropriate E7080 concentrations were tested; 3) no bacterial contamination was observed; 4) positive controls produced expected results consistent with historical data.

Results

Cytotoxicity was not observed in any of the bacterial strains at concentrations up to 5000 µg/plate in the presence or absence of S9 mix. There was no statistically significant increase in the number of TA100, TA1535, TA98, TA1537 or WP2 *uvrA* (pKM101) revertants following incubation with up to 5000 µg E7080 per plate in the presence or absence of S9 mix compared to controls.

Table 48: In Vitro Mutagenicity of E7080 in the Main Ames Assay

		No. of revertants (mean ± S.D. for 3 plates)				
Without metabolic activation						
	Dose (µg/plate)	TA100	TA1535	TA98	TA1537	WP2 *uvrA* (pKM101)
Control	0	117 ± 13.9	14 ± 4.0	23 ± 1.5	9 ± 2.0	87 ± 9.2
E7080	313	113 ± 9.5	13 ± 5.3	21 ± 7.0	12 ± 2.5	75 ± 1.2
	625	87 ± 5.6 [P]	8 ± 3.1 [P]	20 ± 0.6 [P]	8 ± 5.5 [P]	88 ± 9.3 [P]
	1250	87 ± 16.6 [P]	8 ± 2.6 [P]	24 ± 7.4 [P]	9 ± 3.5 [P]	89 ± 7.9 [P]
	2500	97 ± 22.2 [P]	7 ± 2.5 [P]	20 ± 4.4 [P]	12 ± 2.1 [P]	80 ± 2.1 [P]
	5000	89 ± 10.0 [P]	6 ± 2.0 [P]	23 ± 2.0 [P]	8 ± 2.6 [P]	82 ± 5.7 [P]
AF2	0.005	NT	NT	NT	NT	932 ± 45.6
	0.01	430 ± 54.1	NT	NT	NT	NT
	0.1	NT	NT	713 ± 22.9	NT	NT
9AA	80	NT	NT	NT	565 ± 169.8	NT
SA	0.5	NT	516 ± 15.6	NT	NT	NT
With metabolic activation						
	Dose (µg/plate)	TA100	TA1535	TA98	TA1537	WP2 *uvrA* (pKM101)
Control	0	129 ± 11.2	12 ± 3.8	30 ± 6.4	12 ± 3.0	96 ± 7.8
E7080	313	121 ± 16.3	12 ± 0.6	30 ± 5.0	14 ± 0.6	87 ± 4.4
	625	87 ± 13.7 [P]	8 ± 2.1 [P]	23 ± 6.7 [P]	12 ± 3.2 [P]	104 ± 8.5 [P]
	1250	94 ± 11.5 [P]	10 ± 4.7 [P]	24 ± 4.0 [P]	12 ± 1.5 [P]	98 ± 16.3 [P]
	2500	92 ± 4.0 [P]	8 ± 1.5 [P]	28 ± 4.2 [P]	10 ± 3.2 [P]	113 ± 12.5 [P]
	5000	95 ± 17.1 [P]	7 ± 1.5 [P]	31 ± 2.6 [P]	7 ± 3.5 [P]	97 ± 12.7 [P]
2AA	0.5	NT	NT	585 ± 53.8	NT	NT
	1	1423 ± 48.6	NT	NT	NT	NT
	2	NT	290 ± 21.5	NT	278 ± 6.0	1591 ± 99.2

S.D.: standard deviation, NT: not tested, [P]: Precipitation,
AF2: 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide, 9AA: 9-aminoacridine hydrochloride, SA: sodium azide,
2AA: 2-aminoanthracene.

(Excerpted from Applicant's submission)

7.2 *In Vitro* Assays in Mammalian Cells

Study title: E7080: Mouse Lymphoma TK Assay

Study no.: S03008
Study report location: EDR 4.2.3.3.1
Conducting laboratory and location: Kawashima Research, Drug Safety Research Laboratories, Eisai Co., Ltd. 1 Takehaya, Kawashima-cho, Hashima-gun, Gifu, Japan
Date of study initiation: April 22, 2003
GLP compliance: Yes (Japan Ministry of Health and Welfare Ordinance No. 21, 1997)
QA statement: Yes
Drug, lot #, and % purity: E7080, 13012251, 99%

Key Study Findings: E7080 was not mutagenic or clastogenic in mouse lymphoma L5178Y $TK^{+/-}$ cells following 3 hrs of treatment in the presence or absence of S9 activation, or following 24 hrs of treatment in the absence of S9 activation.

Methods

Cell line: L5178Y $TK^{+/-}$ mouse lymphoma

Concentrations in definitive study:

Method	S9 mix	Compound	Treatment concentrations (μg/mL)
Short treatment method for 3 hours	with S9 mix	Control E7080 CP	0 12.5, 25, 50, 100, 200 2
	without S9 mix	Control E7080 MMS	0 12.5, 25, 50, 100, 200 20
Continuous treatment method for 24 hours	without S9 mix	Control E7080 MMS	0 5, 10, 20, 22.5, 25, 27.5 5

Basis of concentration selection: A range finding test was conducted at lenvatinib concentrations ranging from 1.56 to 100 μg/mL in the presence and absence of S9 mix (3 hrs) and in the absence of S9 mix (24 hrs). Following 3 hrs of treatment, precipitation was observed at 100 μg/mL in the presence of S9; cytotoxicity was not observed up to 100 μg/mL. Following 24 hrs of treatment, cytotoxicity was observed in the absence of S9 and a ≤20% decrease in survival was observed at concentrations ≥25 μg/mL.
Negative control: DMSO
Positive control: + S9: cyclophosphamide monohydrate (CP)
- S9: methyl methanesulfonate (MMS)
Formulation/Vehicle: DMSO
Incubation & sampling time: A mouse lymphoma tk assay was conducted using a 3 hr treatment method in the presence and absence of S9 mix, and a continuous 24 hr treatment method in the

	absence of S9 mix. Cell cultures were tested in duplicate. Treatment was followed by two days of treatment-free cell growth to allow $tk^{-/-}$ mutation expression. Cells were then transferred into RPMI 20 media in microtitre plates +/- trifluorothymidine for 12 days. Cytotoxicity was assessed based on relative survival (RS) or relative total growth (RTG), and mutation frequency was quantitated by comparing colony growth in selective medium versus plating efficiency in non-selective medium.
Evaluation Criteria:	Mutagenic = mutation frequency at one or more treatment concentrations is significantly greater than that of the negative control ($P < 0.05$) and related to increased concentrations.

Study Validity

The study was determined to be valid because: 1) mutation frequencies in the positive and negative controls produced expected results consistent with historical data; 2) plating efficiencies on Days 0 and 2 for the negative control cultures were within an acceptable range; 3) RS (Day 0) or RTG for the high treatment concentration was 20% or lower when neither 5 mg/mL nor 10 mmol/L was selected and no precipitation was observed.

Results

Using the 3 hr treatment method, precipitation was observed at concentrations ≥100 µg/mL and 200 µg/mL in the presence and absence of S9 mix, respectively. There was no statistically significant increase in mutant frequencies following 3 hrs of treatment with E7080 in the presence or absence of S9 mix compared to controls. There was also no statistically significant increase in mutant frequencies following 24 hrs of continuous treatment with E7080 in the absence of S9 mix compared to controls.

Reference ID: 3686430

Table 49: In Vitro Mutagenicity of E7080 in the Mouse Lymphoma Assay

Short treatment method for 3 h with S9 mix

Compound	Concentration (μg/mL)	%RS (Day 0)	RTG	MF ($\times 10^{-6}$cells)	% of SC
Control	0	100	100	97	26
E7080	12.5	98	127	79	28
	25	91	104	93	33
	50	75	70	115	16
	100 [P]	80	62	111	29
	200 [P]	NM	NM	NM	NM
CP	2	59	46	812	43

Short treatment method for 3 h without S9 mix

Compound	Concentration (μg/mL)	%RS (Day 0)	RTG	MF ($\times 10^{-6}$cells)	% of SC
Control	0	100	100	117	24
E7080	12.5	95	95	79	31
	25	94	84	101	23
	50	59	48	109	22
	100	57	64	109	31
	200 [P]	65	37	120	29
MMS	20	84	43	1040	40

Continuous treatment method for 24 h without S9 mix

Compound	Concentration (μg/mL)	%RS (Day 0)	RTG	MF ($\times 10^{-6}$cells)	% of SC
Control	0	100	100	114	29
E7080	5	92	92	117	33
	10	77	67	127	34
	20	35	29	106	32
	22.5	23	20	106	39
	25	9	10	NM	NM
	27.5	8	6	NM	NM
MMS	5	62	67	564	35

%RS = % Relative survival; RTG = Relative total growth; MF = Mutant frequency.
% of SC = % of small colony; [P] precipitate; NM = Not measured.
CP = Cyclophosphamide monohydrate; MMS = Methyl methanesulfonate.

(Excerpted from Applicant's submission)

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

Study title: E7080: Micronucleus Assay in Rats Following Oral Administration

Study no:	S05032
Study report location:	EDR 4.2.3.3.2
Conducting laboratory and location:	Kawashima Research, Drug Safety Research Laboratories, Eisai Co., Ltd. 1 Kawashimatakehaya-machi, Kakamigahara-shi, Gifu, Japan
Date of study initiation:	March 24, 2005
GLP compliance:	Yes (Japan Ministry of Health and Welfare Ordinance No. 21, 1997)
QA statement:	Yes
Drug, lot #, and % purity:	E7080, 13033151, 97.7%

Key Study Findings: Administration of E7080 at dose levels up to 2000 mg/kg was not clastogenic in an in vivo rat bone marrow micronucleus assay.

Methods

Doses in definitive study:	0, 500, 1000, and 2000 mg/kg
Frequency of dosing:	Once
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	75% polyethylene gycol (PEG 400) in water

Species/Strain:	8 wk old male Sprague Dawley rats
Number/Sex/Group:	5 males/group
Satellite groups:	None
Basis of dose selection:	Mortality was not observed in Study # S04094 by 48 hrs after a single oral dose of 500, 1000, or 2000 mg/kg.
Negative control:	PEG 400
Positive control:	Cyclophosphamide
Study Design:	Rats were killed 24 or 48 hrs after drug administration; femoral bone marrow cells were smeared on slides and stained with acridine orange solution. 2000 polychromatic erythrocytes (PCE) per animal were counted using a fluorescence microscope. The ratio of PCE to total erythrocytes was calculated by examining 200 total erythrocytes/animal. Dose analysis, mortality, clinical signs, body weights, and food consumption were examined.
Evaluation Criteria:	Positive = incidence of micronucleated PCE (MNPCE) in the treatment group is significantly greater than that of the negative control ($P < 0.05$), and there is a significant dose response.

Study Validity

The study was determined to be valid because: 1) the incidences of MNPCE in the negative and positive controls were consistent with historical data; 2) the species and number of rats/group were acceptable; 3) there were no significant gender effects in Study #S04094, so the use of only males was acceptable; 4) dosing solution analysis confirmed the drug concentrations were on target. Of note, exposure data was not provided in Study #S04094.

Results

There were no mortalities, abnormal clinical signs, or E7080-related changes in mean body weight or food consumption reported. Although increases in the mean percent of MNPCE were observed 24 hrs and/or 48 hrs after administration of E7080 compared to controls, these increases were not dose-dependent. Further, there was no statistically significant increase in the mean incidence of MNPCE following a single dose of up to 2000 mg/kg E7080 compared to controls. There was a statistically significant decrease in the ratio of PCE to total erythrocytes 48 hrs after administration of 500 or 2000 mg/kg E7080 compared to controls, which confirmed adequate exposure of bone marrow to E7080.

Reference ID: 3686430

Table 50: Micronucleus Assay Results Following E7080 Administration in Rats

Sampling time: 24 hours						Sampling time: 48 hours					
Compound	Animal No.	No. of MNPCE (/2000 PCE)	MNPCE (%)	No. of PCE (/200 E)	PCE (%)	Compound	Animal No.	No. of MNPCE (/2000 PCE)	MNPCE (%)	No. of PCE (/200 E)	PCE (%)
Control	00M01	0	0.00	116	58.0	Control	00M06	3	0.15	141	70.5
	00M02	4	0.20	128	64.0		00M07	2	0.10	111	55.5
	00M03	2	0.10	145	72.5		00M08	3	0.15	136	68.0
	00M04	4	0.20	130	65.0		00M09	2	0.10	147	73.5
	00M05	1	0.05	158	79.0		00M10	0	0.00	135	67.5
	Mean		0.11		67.7		Mean		0.10		67.0 ##
E7080 500 mg/kg	01M11	4	0.20	107	53.5	E7080 500 mg/kg	01M16	4	0.20	89	44.5
	01M12	5	0.25	104	52.0		01M17	5	0.25	98	49.0
	01M13	4	0.20	128	64.0		01M18	2	0.10	92	46.0
	01M14	1	0.05	104	52.0		01M19	2	0.10	118	59.0
	01M15	4	0.20	111	55.5		01M20	4	0.20	106	53.0
	Mean		0.18		55.4		Mean		0.17		50.3 *
E7080 1000 mg/kg	02M21	1	0.05	107	53.5	E7080 1000 mg/kg	02M26	1	0.05	99	49.5
	02M22	1	0.05	140	70.0		02M27	2	0.10	99	49.5
	02M23	2	0.10	102	51.0		02M28	3	0.15	70	35.0
	02M24	5	0.25	106	53.0		02M29	3	0.15	92	46.0
	02M25	4	0.20	136	68.0		02M30	1	0.05	135	67.5
	Mean		0.13		59.1		Mean		0.10		49.5
E7080 2000 mg/kg	03M31	4	0.20	102	51.0	E7080 2000 mg/kg	03M36	3	0.15	82	41.0
	03M32	2	0.10	128	64.0		03M37	2	0.10	90	45.0
	03M33	4	0.20	83	41.5		03M38	3	0.15	93	46.5
	03M34	4	0.20	98	49.0		03M39	3	0.15	94	47.0
	03M35	2	0.10	136	68.0		03M40	5	0.25	120	60.0
	Mean		0.16		54.7		Mean		0.16		47.9 *
CP 10 mg/kg	04M41	40	2.00	81	40.5						
	04M42	17	0.85	88	44.0						
	04M43	25	1.25	102	51.0						
	04M44	24	1.20	110	55.0						
	04M45	25	1.25	122	61.0						
	Mean		1.31		50.3						

MNPCE = micronucleated polychromatic erythrocytes; PCE = polychromatic erythrocytes; E = erythrocytes.
Significant difference compared to control, *P<0.05 (Steel's test).
Significant trend, ##P<0.01 (Jonckheere's test).
CP = cyclophosphamide.

(Excerpted from Applicant's submission)

8 Carcinogenicity

Carcinogenicity studies were not conducted with lenvatinib.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Fertility and early embryonic development studies were not conducted with lenvatinib.

9.2 Embryonic Fetal Development

Study title: E7080: An Oral Embryo-Fetal Development Study in Rats

Study no.: S05152
Study report location: EDR 4.2.3.5.2
Conducting laboratory and location: Kawashima Research
Drug Safety Research Laboratories
Eisai Col Ltd.
1 Kawashimatakehaya-machi,
Kakamigahara-shi, Gifu, Japan
Date of study initiation: December 9, 2005
GLP compliance: Yes (Japan Ministry of Health and

	Welfare Ordinance No. 21, 1997)
QA statement:	Yes
Drug, lot #, and % purity:	E7080, 13033151, 97.7%

Key Study Findings

- Lenvatinib was embryotoxic, fetotoxic, and has teratogenic potential in rats.
- Lenvatinib treatment resulted in increased post-implantation loss due to early fetal resorptions and a decreased number of live fetuses per litter at the 1 mg/kg (6 mg/m^2) dose level.
- Lenvatinib induced a dose-dependent decrease in mean fetal body weight in rats.
- Lenvatinib induced delayed fetal ossifications, fetal external and visceral anomalies, and fetal skeletal anomalies/variations.
- Maternal toxicity was demonstrated by decreased mean gestational body weight, body weight gain, and food consumption, at the 1 mg/kg (6 mg/m^2) dose level.
- The NOAEL for maternal toxicity was 0.3 mg/kg (1.8 mg/m^2) lenvatinib.
- A fetal NOAEL could not be established.

Methods

Doses:	0, 0.1, 0.3, and 1 mg/kg
Frequency of dosing:	Once daily from Gestation Days (GD) 6 to 17
Dose volume:	10 mL/kg
Route of administration:	Oral gavage (gastric tube)
Formulation/Vehicle:	Water for injection
Species/Strain:	Sprague-Dawley rats
Age/weight:	10-11 weeks old on Day 0 of pregnancy/ 219.5-301.7 g
Number/Sex/Group:	20 mated females/group
Satellite groups:	None
Study design:	• Evaluation of lenvatinib on embryo-fetal development following daily oral dosing from implantation to closure of hard palate. • Females were mated with breeder male rats; Day 0 of gestation (GD 0) was the day of mating confirmation (copulatory plug).
Deviation from study protocol:	None that impacted study interpretation.

Dosage Justification: According to the Applicant, total post-implantation loss with early fetal resorption was observed in all dams dosed with lenvatinib at dose levels ≥ 2 mg/kg in the dose range finding study in pregnant rats (Study #S05104). There were no reported changes at the 0.2 mg/kg dose level. As a result, 1 mg/kg was chosen as the high dose in this study.

Table 51: Antemortem Study Evaluations in Rats

Observation	Time/description of assessment
Mortality	At least once daily
Clinical Observations	GD 0 and at least once daily from GD 6 to 20

Reference ID: 3686430

Observation	Time/description of assessment
Body weights	GD 0 and daily from GD 6 to 20
Food consumption	Daily from GD 6 to 20

Table 52: Postmortem Study Evaluations in Rats

Day of Cesarean section	Day 20 of pregnancy
Maternal necropsy	Day 20 of pregnancy; thoracoabdominal organs were observed grossly.
Uterine examination^	• Number of implantation sites • Live/dead fetuses • Early/late resorptions • Number of corpora lutea • Gross observation of placenta and amniotic fluid
Fetal examination	• Gender of fetus recorded • Body weights recorded • Examined for external malformations • Approximately half of the fetuses from each litter underwent either visceral or skeletal examination* • Fetuses with external findings saved in neutral-buffered 10% formalin

*Visceral and skeletal examinations were only performed at dose levels ≤ 0.3 mg/kg because all live fetuses at 1 mg/kg were stored due to external findings.
^ Uterine weights were not measured.

Observations and Results

Mortality

There were no early deaths during the study.

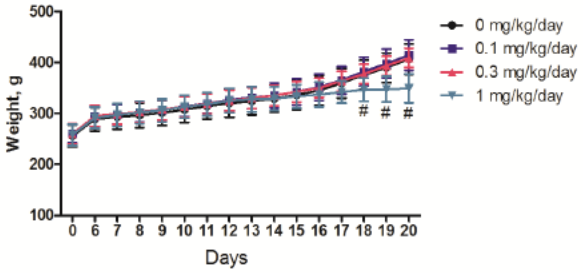
Clinical Signs

One rat dosed with lenvatinib at the 1 mg/kg dose level exhibited vaginal hemorrhage on GD 18. According to the Applicant, this was associated with fetal resorption.

Body Weight

There was a statistically significant decrease in mean gestational body weight in rats dosed with lenvatinib at the 1 mg/kg dose level compared to controls on GD 18, GD 19, and GD 20 (8%, 11%, and 14%, respectively). There was a statistically significant decrease in mean gestational body weight gain in rats dosed with 1 mg/kg lenvatinib compared to controls during GD 6 to GD 17 and during GD 17 to GD 20 (28% and 84%, respectively).

Figure 18: Mean Maternal Gestational Body Weight of Female Rats

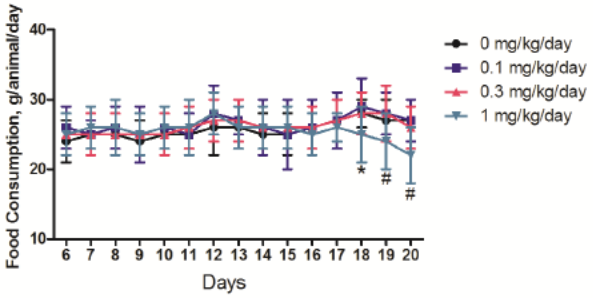


#, P ≤ 0.01 versus controls

Feed Consumption

There was a statistically significant ≤19% decrease in mean maternal food consumption in rats dosed with lenvatinib at the 1 mg/kg dose level compared to controls on GD 18 to GD 20.

Figure 19: Mean Maternal Food Consumption of Female Rats



*, P ≤ 0.05 versus controls; #, P ≤ 0.01 versus controls

Toxicokinetics: Not assessed

Dosing Solution Analysis

Met pre-specified acceptance criteria.

Necropsy

Unremarkable. Uterine findings are shown in Table 53.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

Table 53: Cesarean Section Observations in Rats

Dose (mg/kg)	0	0.1	0.3	1
# mated females	20	20	20	20
# pregnant (%)	19 (95)	19 (95)	19 (95)	18 (90)
# dams with live fetuses	19	19	19	10
Mean # corpora lutea	285	295	284	276
Mean # implantations	264	277	272	264
Mean % pre-implantation loss	6	6.1	4.4	4.3
Mean # post-implantation loss (%)	10 (3.8)	15 (5.3)	20 (7.2)	217 (82.2)#
Mean # dead (%)	0 (0)	0 (0)	0 (0)	0 (0)

Dose (mg/kg)	0	0.1	0.3	1
# mated females	20	20	20	20
Mean # late resorptions (%) Mean # early resorptions (%)	0 (0) 10 (3.8)	0 (0) 15 (5.3)	0 (0) 20 (7.2)	0 (0) 217 (82.2)#
Mean # live fetuses	254	262	252	47#
Mean fetal body weight (g)	3.69	3.59	3.41#	2.94#
Mean fetal sex ratio (Male:Female) Mean fetal sex ratio (% males)	124:130 49	130:132 48.7	117:135 47.6	18:29 49.1

#, P ≤ 0.01 versus controls; Mean % was calculated on a litter basis

Offspring (Malformations, Variations, etc.)

Table 54: Fetal Anomalies, Malformations, and Variations in Rats

Dose (mg/kg)		0	0.1	0.3	1
# of dams		19	19	19	10
External anomalies: # of fetuses affected (%)					
# fetuses examined		254	262	252	47
# fetuses with anomalies		0 (0)	1 (0.4)	21 (8.2)#	47 (100)#
Local edema (parietal)				12 (4.7)*	46 (90)#
Cryptophthalmia				2 (0.8)	1 (3.3)
Mandibular macrognathia			1 (0.4)	1 (0.4)	
Acaudate					2 (13.3)
Bent tail				1 (0.4)	1 (0.8)
Kinked tail				3 (1.2)	6 (20.2)#
Short tail				8 (3.2)	17 (43.2)#
Visceral anomalies: # of fetuses affected (%)					
# fetuses examined		122	125	114	0
# fetuses with anomalies			2 (1.8)	1 (1.3)	N/A
Membranous ventricular septum defect			1 (0.9)		N/A
Retroesophageal aortic arch			1 (0.9)		N/A
Retroesophageal subclavian artery				1 (1.3)	N/A
Skeletal anomalies: # of fetuses affected (%)					
# fetuses examined		132	136	117	0
# litters with anomalies		12 (63.2)	13 (68.4)	17 (89.5)	N/A
# fetuses with anomalies		27 (21.3)	25 (18.5)	69 (58.1)#	N/A
Carpal bone	Fused carpal bone	15 (12.5)	5 (3.8)	4 (.3.9)	N/A
Rib	Discontinuous rib cartilage	11 (8.1)	8 (6)	26 (21.7)*	N/A
Cervical vertebrae	Incomplete ossification of cervical arch Fused cervical transverse process		2 (1.5) 1 (0.8)		N/A
Thoracic centrum	Hemicentric Split cartilage	 2 (1.5)	 13 (9.6)*	6 (5)* 51 (43.5)#	N/A
Lumbar centrum	Split cartilage			1 (0.9)	N/A
Lumbar vertebra	Absent			1 (1.1)	N/A
Sacral vertebra	Absent			1 (1.1)	N/A

Dose (mg/kg)		0	0.1	0.3	1
Caudal centrum	Fused			2 (1.6)	N/A
	Misshapen			2 (1.6)	N/A
Skeletal variations: # of fetuses affected (%)					
# litters with variations		10 (52.6)	14 (73.7)	19 (100)	N/A
# fetuses with variations		19 (14.9)	38 (28.4)	67 (57.7)#	N/A
Cervical rib				1 (0.8)	N/A
Asymmetrical sternebra			2 (1.5)	2 (1.8)	N/A
Dumbbell-shaped vertebral centrum		1 (0.9)	4 (2.9)	7 (5.8)*	N/A
14th rib		18 (14)	26 (19.5)	50 (43)#	N/A
Split vertebral centrum			9 (6.6)*	36 (30.6)#	N/A
Ossifications: # of fetuses affected (%)					
# of fetuses examined		132	136	117	0
# fetuses with ossifications		132 (100)	136 (100)	116 (98.7)	N/A
# of fetuses with ossification of the:					
6th sternebra		62 (46.2)	48 (33.9)	33 (27.1)	N/A
Supraoccipital bone		131 (99.3)	136 (100)	113 (95.3)	N/A
Odontoid process		49 (37.6)	35 (25.9)	12 (11.1)#	N/A
7th cervical body		84 (61.3)	76 (55.8)	49 (40.1)*	N/A
2nd sternebra		132 (100)	135 (99.2)	113 (95.2)	N/A
Hyoid bone		124 (94.7)	130 (95.1)	112 (95.1)	N/A
Sacral and caudal vertebrae ossifications		7.6	7.5	6.7#	N/A
Left metacarpal		3.5	3.3	3#	
Right metacarpal		3.5	3.4	3#	
Left metatarsal		3.9	3.8	3.1*	
Right metatarsal		3.9	3.8	3*	

*, P ≤ 0.05 versus controls; #, P ≤ 0.01 versus controls; Mean % was calculated on a litter basis
The historical control range of anomalies for the test facility were not provided.

Study title: E7080: An Oral Embryo-Fetal Development Study in Rabbits

Study no.: S06009
Study report location: EDR 4.2.3.5.2
Conducting laboratory and location: Kawashima Research
Drug Safety Research Laboratories
Eisai Col Ltd.
1 Kawashimatakehaya-machi,
Kakamigahara-shi, Gifu, Japan
Date of study initiation: February 3, 2006
GLP compliance: Yes (Japan Ministry of Health and Welfare Ordinance No. 21, 1997)
QA statement: Yes
Drug, lot #, and % purity: E7080, 13033151, 97.7%

Key Study Findings

- Lenvatinib was an abortifacient, embryotoxic, and fetotoxic in rabbits.

- Lenvatinib treatment resulted in increased post-implantation loss due to early fetal resorptions and a decreased number of live fetuses per litter at the 0.5 mg/kg (6 mg/m^2) dose level.
- 10/11 females dosed with 0.5 mg/kg lenvatinib exhibited complete resorption of litters.
- Lenvatinib induced fetal external, visceral, and skeletal anomalies.
- Maternal toxicity was demonstrated by decreased mean gestational body weight, body weight gains, and food consumption at the 0.5 mg/kg dose level.
- The NOAEL for maternal toxicity was 0.1 mg/kg (1.2 mg/m^2) lenvatinib.
- A fetal NOAEL could not be established.

Methods

Doses:	0, 0.03, 0.1, and 0.5 mg/kg
Frequency of dosing:	Once daily from GD 6 to GD 18
Dose volume:	5 mL/kg
Route of administration:	Oral gavage (gastric tube)
Formulation/Vehicle:	Water for injection
Species/Strain:	New Zealand White rabbits/Kbl:NZW
Age/weight:	21-23 weeks old on Day 0 of pregnancy/ 3.27-3.78 kg
Number/Sex/Group:	20 inseminated females/group
Satellite groups:	None
Study design:	• Evaluation of lenvatinib on embryo-fetal development following daily oral dosing from GD 6 to GD 18. • Females were artificially inseminated with diluted fresh male semen after treatment with gonadotropin. GD 0 was the day of insemination.
Deviation from study protocol:	None that impacted study interpretation.

Dosage Justification: According to the Applicant, in dose range finding studies in pregnant rabbits, complete resorption of all implantations was observed in rabbits dosed with lenvatinib at dose levels ≥ 0.8 mg/kg (Study #S05063), and slight increases in post-implantation losses were observed at the 0.1 mg/kg and 0.3 mg/kg dose levels (Study #S05119). As a result, 0.5 mg/kg was chosen as the high dose in this study.

Table 55: Antemortem Study Evaluations in Rabbits

Observation	Time/description of assessment
Mortality	At least once daily
Clinical Observations	GD 0 and at least once daily from GD 6 to 29
Body weights	GD 0, 6 to 19, 21, 23, 25, 27, and 29
Food consumption	GD 6 to 19, 21, 23, 25, 27, and 29.

Reference ID: 3686430

Table 56: Postmortem Study Evaluations in Rabbits

Day of Cesarean section	Day 29 of pregnancy
Maternal necropsy	Day 29 of pregnancy; thoracoabdominal organs were observed grossly. No histopathology was performed.
Aborted Animals	• Weighed, euthanized, and necropsied • Thoracoabdominal organs • Number of implantation sites
Uterine examination^	• Number of implantation sites • Live/dead fetuses • Early/late resorptions • Number of corpora lutea • Gross observation of placenta and amniotic fluid
Fetal examination	• Gender of fetus recorded • Body weights recorded • Examined for external malformations • Visceral examination • Skeletal examination for anomalies, variations, and ossification patterns • Fetuses with external findings saved in neutral-buffered 10% formalin

^ Uterine weights were not measured.

Observations and Results

Mortality

There were no early maternal deaths during the study. Seven rabbits dosed with lenvatinib at the 0.5 mg/kg dose level had abortions between Days 20 and 22 of pregnancy.

Clinical Signs

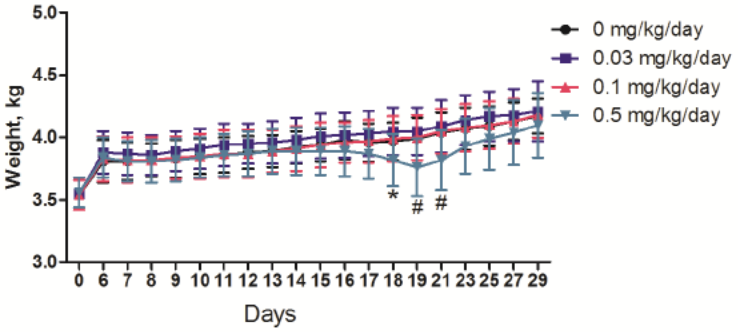
Twelve females dosed with lenvatinib at the 0.5 mg/kg dose level exhibited hemorrhage (bloodstains inside/underneath their cages) post-dosing (between GD 18 and GD 24). This finding was observed in 6/7 rabbits who had abortions.

Body Weight

There was a statistically significant ≤ 6% decrease in mean gestational body weight in rabbits dosed with lenvatinib at the 0.5 mg/kg dose level compared to controls on GD 18-21, which showed evidence of recovery. There was a statistically significant 113% decrease in mean gestational body weight gain in rabbits dosed with 0.5 mg/kg lenvatinib compared to controls during GD 6 to GD 18, which showed evidence of recovery during GD 18 to 29.

Reference ID: 3686430

Figure 20: Mean Maternal Gestational Body Weight of Female Rabbits

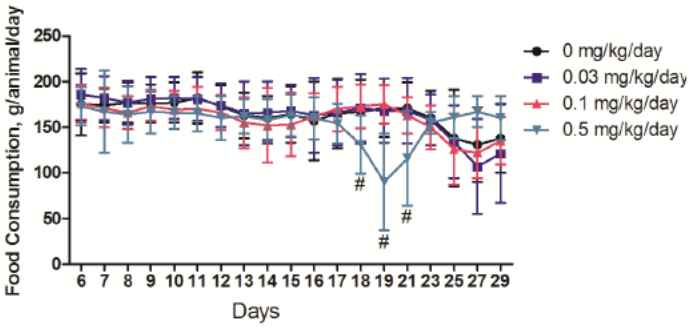


*, P ≤ 0.05 versus controls; #, P ≤ 0.01 versus controls

Feed Consumption

There was a statistically significant ≤ 47% decrease in mean maternal food consumption in rabbits dosed with lenvatinib at the 0.5 mg/kg dose level compared to controls on GD 18 to GD 21, which showed evidence of recovery.

Figure 21: Mean Maternal Food Consumption of Female Rabbits



*, P ≤ 0.05 versus controls; #, P ≤ 0.01 versus controls

Toxicokinetics: Not assessed

Dosing Solution Analysis

Met pre-specified acceptance criteria.

Necropsy

Unremarkable. Uterine findings are shown in Table 57.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

Table 57: Cesarean Section Observations in Rabbits

Dose (mg/kg)	0	0.03	0.1	0.5
# rabbits examined	19^	20^	17^	11^^
# dams with live fetuses	19	20	17	1
Mean # corpora lutea	154	176	146	80
Mean # implantations	126	157	125	72
Mean % pre-implantation loss	20.6	10.7	15.8	12

Dose (mg/kg)	0	0.03	0.1	0.5
# rabbits examined	19^	20^	17^	11^^
Mean # post-implantation loss (%)	4 (3.6)	11 (6.8)	8 (7.1)	67 (94.3)#
Mean # dead (%)	0	1 (0.6)	3 (2.7)	0
Mean # late resorptions (%)	2 (1.3)	2 (1.1)	1 (0.7)	0
Mean # early resorptions (%)	2 (2.3)	8 (5.1)	4 (3.8)	67 (94.3)#
Mean # live fetuses per litter	122	146	117	5#
Mean fetal body weight (g)	44.99	43.68	43.56	42.7
Mean fetal sex ratio (Male:Female)	70:52	65:81	56:61	1:4
Mean fetal sex ratio (% males)	55.5	44.1*	47.6	20

^ : represents the number of pregnant rabbits; ^^ : there were 18 pregnant rabbits, but 7 had abortions
*, P ≤ 0.05 versus controls; #, P ≤ 0.01 versus controls; Mean % was calculated on a litter basis

Offspring (Malformations, Variations, etc.)

Table 58: Fetal Anomalies, Malformations, and Variations in Rabbits

Dose (mg/kg)		0	0.03	0.1	0.5
# of dams		19	20	17	1
External anomalies: # of fetuses affected (%)					
# fetuses examined		122	146	117	5
# fetuses with anomalies		0	0	0	1 (20)#
Short tail		0	0	0	1 (20)#
Visceral anomalies: # of fetuses affected (%)					
# fetuses examined		122	146	117	4
# fetuses with anomalies		0	5 (4.2)	1 (0.7)	2 (50)#
Retroesophageal aortic arch			1 (0.6)		
Retroesophageal subclavian artery			4 (3.7)	1 (0.7)	2 (50)#
Skeletal anomalies: # of fetuses affected (%)					
# fetuses examined		122	146	117	4
# litters with anomalies		3 (15.8)	2 (10)	4 (23.5)	1 (100)
# fetuses with anomalies		3 (3.9)	3 (2.1)	4 (5.2)	1 (25)*
Carpal bone	Absent carpal bone		2 (1.1)		
Rib	Fused			2 (1.7)	1 (25)#
Thoracic centrum	Fused cartilage Hemicentric			1 (1) 1 (1)	
Thoracic vertebra	Thoracic hemivertebra	1 (0.7)			1 (25)#
Lumbar arch	Misshapen				1 (25)#
Lumbar vertebra	Absent			1 (0.5)	
Caudal centrum	Misaligned			1 (1)	
Skeletal variations: # of fetuses affected (%)					
# litters with variations		19 (100)	20 (100)	17 (100)	1 (100)
# fetuses with variations		82 (68.3)	108 (74.4)	72 (59.1)	4 (100)
13th rib		77 (62.9)	106 (73.2)	68 (56.1)	4 (100)
Asymmetrical sternebra				1 (1)	
Dumbbell-shaped vertebral centrum		6 (5.9)	5 (2.9)	9 (5.8)	1 (25)
Extra lumbar vertebra		21 (17.2)	21 (13.3)	24 (21.7)	3 (75)
Accessory parietal bone		1 (1.3)	1 (0.6)	2 (3.7)	

Dose (mg/kg)	0	0.03	0.1	0.5
Ossifications: # of fetuses affected (%)				
# of fetuses examined	122	146	117	4
# fetuses with ossifications	122 (100)	146 (100)	116 (99.3)	4 (100)
# of fetuses with ossifications of the:				
Distal femoral epiphysis (left)	93 (77.7)	117 (81.7)	75 (69.1)	3 (75)
Proximal tibial epiphysis (left)	620(49)	70 (50.7)	6 (55.3)	1 (25)
Proximal tibial epiphysis (right)	63 (54)	66 (47)	62 (56)	1 (25)

*, P ≤ 0.05 versus controls; #, P ≤ 0.01 versus controls; Mean % was calculated on a litter basis
The historical control range of anomalies for the test facility were not provided.

9.3 Prenatal and Postnatal Development

A prenatal/postnatal development study has not been conducted and will not be required.

J12412: E7080: A 2-Week Oral Repeated Dose Range Finding Study with 2-Week Recovery Period in Juvenile Rats

This non-GLP study was not reviewed in detail. In this study, lenvatinib was administered orally once daily for 2 weeks to Sprague Dawley rats at dose levels of 0, 0.2, 0.4, 1, or 5 mg/kg starting at postnatal day (PND) 7, or at dose levels of 0, 0.4, 1, 5, or 25 mg/kg starting at PND 21. When dosing was initiated on PND 7, lenvatinib induced mortality at dose levels ≥ 1 mg/kg (6 mg/m^2; approximately 0.8 to 3.5 times the clinical exposure at the recommended dose), which was attributed to severe intestinal toxicity sometimes accompanied by peritonitis. Treatment with lenvatinib resulted in growth suppression and secondary changes in bone measurements. Treatment with lenvatinib from PND 21 to PND 35 did not result in mortality at dose levels up to 25 mg/kg (150 mg/m^2).

Study title: E7080: A 8-Week Repeated Oral Dose Toxicity Study with 4-Week Recovery Period in Juvenile Rats

Study no.:	J13193
Study report location:	EDR 4.2.3.5.4
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 22, 2013
GLP compliance:	Yes (Japan Ministry of Health and Welfare Ordinance No. 21, 1997 and Ordinance No. 114, 2008; OECD Principles of Good Laboratory Practice)
QA statement:	Yes
Drug, lot #, and % purity:	E7080, 111P1101, 100.3%

Key Study Findings

- There were 13 mortalities at the 10 mg/kg dose level attributed to primary duodenal lesions and associated GI complications. Mortality occurred earlier in juvenile rats compared to adult rats treated at the same dose levels.
- The toxicologic profile of lenvatinib was generally similar between juvenile and adult rats.
- Treatment with lenvatinib at dose levels ≥ 2 mg/kg resulted in severe growth retardation (decreased body weight and food consumption), which correlated with a decrease in the width and/or length of the femur and tibia at dose levels ≥ 2 mg/kg, delayed physical development (prepuce separation and vaginal opening at the 10 mg/kg dose level), and reproductive organ immaturity.
- The severely toxic dose was 10 mg/kg (60 mg/m^2). The LOAEL was 0.4 mg/kg (2.4 mg/m^2).

Methods

Doses:	0, 0.4, 2, and 10 mg/kg/day
Frequency of dosing:	Once daily for 8 weeks starting on PND 21
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	Water for injection
Species/Strain:	Sprague Dawley rats/Crl:CD(SD)
Number/Sex/Group:	10/sex/group; 6/sex in the 0, 2, and 10 mg/kg groups for recovery
Age:	21 days old
Weight:	38.5-64.2 g (M); 25.2-63.1 g (F)
Satellite groups:	TK: 8/sex in the control group; 16/sex in the 0.4, 2, and 10 mg/kg groups
Unique study design:	Growth and development assessments and bone measurement
Deviation from study protocol:	None that impacted study interpretation

Observations and Results

Mortality: Checked once daily

There were 13 mortalities at the 10 mg/kg dose level between Days 26 and 51. Mortality was attributed to primary duodenal lesions and associated complications, including GI lesions and occasional bacterial infection. In addition to the findings listed below, the preterm decedents exhibited decreased body weight and food consumption, increased hemoglobin, hematocrit, neutrophils, AST, ALT, alkaline phosphatase, BUN, and cholesterol, and decreased platelets and reticulocytes. Marked autolysis of various GI organs was observed in all of the rats that were found dead. One TK rat dosed with 10 mg/kg was found dead on Day 39; details were not provided.

Reference ID: 3686430

Table 59: Juvenile Rat Mortalities at the 10 mg/kg/day Lenvatinib Dose Level

Sex/Rat Number	Day of Death	Key Findings*
Male/1404	Found Dead on Day 31	Diarrhea, soiled perineal region **Noteworthy Postmortem Findings:** -Duodenal dilatation, yellow adrenals -Glomerulopathy, mineralization, mucosal hyperplasia, and inflammatory cell infiltration in the stomach, marked inflammation/cystic dilatation in the duodenum
Male/1408	Found Dead on Day 34	Diarrhea, soiled perineal region, crushing of teeth **Noteworthy Postmortem Findings:** -Duodenal dilatation -Glomerulopathy, mineralization, mucosal hyperplasia, and inflammatory cell infiltration in the stomach, moderate inflammation/cystic dilatation in the duodenum
Female/2414	Found Dead on Day 51	Crushing of teeth, nasal hemorrhage **Noteworthy Postmortem Findings:** -Duodenum dilatation, enlarged heart and kidney -Mineralization in the stomach, marked inflammation/cystic dilatation in the duodenum, septic thrombosis in the left atrium with secondary myocardial hypertrophy, mineralization of the meninges (spinal cord), foamy cell accumulation in lung
Female/2416	Found Dead on Day 26	Diarrhea, staining around mouth, dirty nose, soiled perineal region **Noteworthy Postmortem Findings:** -Duodenum dilatation, red spot on stomach -Glomerulopathy, mineralization, inflammatory cell infiltration, and hemorrhage in the stomach, marked inflammation/cystic dilatation in the duodenum
Male/1405	Euthanized Moribund on Day 32	Crushing of teeth **Noteworthy Postmortem Findings:** -Duodenal dilatation -Glomerulopathy, mineralization, mucosal hyperplasia, and inflammatory cell infiltration in the stomach, mucosal hyperplasia and marked inflammation/cystic dilatation in the duodenum, crypt hyperplasia in the cecum
Male/1406	Euthanized Moribund on 32	Diarrhea, soiled perineal region, crushing of teeth **Noteworthy Postmortem Findings:** -Dark red lungs -Glomerulopathy, mucosal hyperplasia in the stomach, marked inflammation/cystic dilatation in the duodenum, edema in the cecum, alveolar hemorrhage in the lung
Male/1409	Euthanized Moribund on 32	Soiled perineal region, crushing of teeth, decreased respiration **Noteworthy Postmortem Findings:** -Glomerulopathy, mineralization, mucosal hyperplasia, and inflammatory cell infiltration in the stomach, mucosal hyperplasia and moderate inflammation/cystic dilatation in the duodenum, edema in the cecum
Male/1416	Euthanized Moribund on 32	Diarrhea, crushing of teeth, staining around mouth, dirty nose, soiled perineal region **Noteworthy Postmortem Findings:** -Duodenum dilatation, red spot on stomach -Glomerulopathy, mineralization, mucosal hyperplasia, and

Sex/Rat Number	Day of Death	Key Findings*
		inflammatory cell infiltration in the stomach, marked inflammation/cystic dilatation in the duodenum, edema in the cecum, decreased zymogen granules in the pancreas
Female/2402	Euthanized Moribund on 32	Diarrhea, crushing of teeth **Noteworthy Postmortem Findings:** -Duodenum dilatation -Glomerulopathy, mineralization, mucosal hyperplasia, and inflammatory cell infiltration in the stomach, marked inflammation/cystic dilatation in the duodenum, edema in the cecum, mineralization in the meninges (spinal cord)
Female/2403	Euthanized Moribund on 32	Crushing of teeth **Noteworthy Postmortem Findings:** -Moderate glomerulopathy, mucosal hyperplasia in the stomach, mucosal hyperplasia and moderate inflammation/cystic dilatation in the duodenum
Female/2404	Euthanized Moribund on 42	Diarrhea, crushing of teeth, soiled perineal region, loss of locomotor activity **Noteworthy Postmortem Findings:** -Duodenum dilatation -Glomerulopathy, mineralization, mucosal hyperplasia, and inflammatory cell infiltration in the stomach, marked inflammation/cystic dilatation in the duodenum, edema in the cecum, decreased zymogens in the pancreas
Female/2408	Euthanized Moribund on 32	Diarrhea, crushing of teeth **Noteworthy Postmortem Findings:** -Duodenum dilatation -Moderate glomerulopathy, mucosal hyperplasia and inflammatory cell infiltration in the stomach, mucosal hyperplasia and moderate inflammation/cystic dilatation in the duodenum
Female/2411	Euthanized Moribund on 32	Diarrhea, crushing of teeth, soiled perineal region, loss of locomotor activity **Noteworthy Postmortem Findings:** -Red focus on stomach -Glomerulopathy, mucosal hyperplasia, inflammatory cell infiltration, and moderate hemorrhage in the stomach, marked inflammation/cystic dilatation in the duodenum, edema in the cecum

*See Table 63 and Table 65 for additional gross pathology and histopathology findings

Clinical Signs: Observed twice daily

There was a dose-dependent increase in teeth crushing (e.g. broken teeth) in rats dosed with lenvatinib. Lenvatinib induced clinical signs of GI toxicity, including diarrhea, soiled perineal region, and blackish stool, which showed partial evidence of recovery.

Table 60: Clinical Observations in Juvenile Rats

Dose (mg/kg/day)	0		0.4		2		10	
Sex	M	F	M	F	M	F	M	F
Crushing of teeth^					7,3R	4,1R	15*	15*
Staining around mouth							2*	1*
Dirty nose							1*	1*

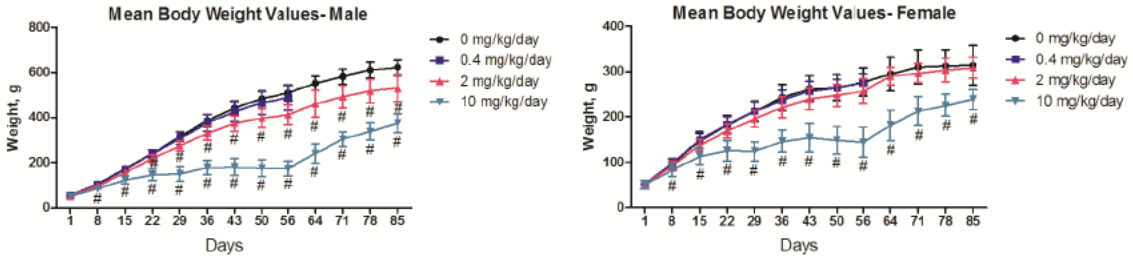
Dose (mg/kg/day)	0		0.4		2		10	
Sex	M	F	M	F	M	F	M	F
Nasal hemorrhage								1*
Paleness							1	
Decreased respiration							1*	
Soiled perineal region							15*,2R	8*
Loss of locomotor activity							1	2*
Diarrhea							14*,2R	13*,2R
Blackish stool								2

R = Recovery Cohort; ^ i.e. broken teeth

Body Weights: Recorded twice weekly

There was a dose-dependent decrease in mean body weight in rats dosed with lenvatinib compared to controls, which reached statistical significance in males dosed with ≥ 2 mg/kg lenvatinib and females dosed with 10 mg/kg lenvatinib. The decrease in mean body weight at the 10 mg/kg dose level was ≤ 66% and ≤ 48% in males and females, respectively, compared to controls. Mean body weight increased during the recovery period, but was still lower than that of controls on Day 85.

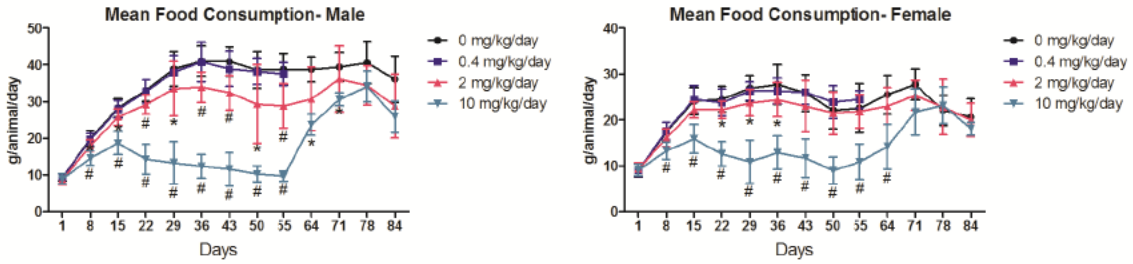
Figure 22: Mean Body Weight Values in Juvenile Rats



Food Consumption: Recorded twice weekly

There was a dose-dependent, statistically significant ≤ 75% decrease in mean food consumption in rats dosed with lenvatinib at dose levels ≥ 2 mg/kg compared to controls, which showed evidence of partial recovery.

Figure 23: Food Consumption Values in Juvenile Rats



Ophthalmoscopy

Ophthalmologic examinations were performed during Weeks 8 and 12. The findings were unremarkable.

ECG: Not applicable

Hematology

Blood samples for hematology and clinical chemistry were collected during Week 8 for the main groups and Week 12 for the recovery groups.

Table 61: Changes in Hematology Compared to Control Groups after 8 Weeks

Dose (mg/kg/day)	0.4		2		10	
Sex	M	F	M	F	M	F
Red blood cells					16%↓# 15%↓R#	8%↓ 5%↓R
Hemoglobin			9%↑# 1%↑R	7%↑# 1%↑R	7%↓ 2%↓R	
% Hematocrit			7%↑# 1%↑R	6%↑# 1%↑R	9%↓# 2%↓R	
MCV			3%↑* 4%↑R*	5%↑# 2%↑R	10%↑* 15%↑R#	15%↑* 6%↑R#
MCH			6%↑# 4%↑R*	6%↑# 2%↑R	12%↑# 15%↑R#	8%↑# 5%↑R
Reticulocytes			31%↓# 1%↑R		72%↑ 3%↑R	113%↑ 1%↑R
White blood cells	27%↑	30%↑	43%↑* 7%↑R	90%↑# 24%↑R	45%↓ 9%↓R	17%↑ 51%↑R
Neutrophils	68%↑*	52%↑	151%↑* 8%↓R	172%↑# 24%↑R	96%↑ 5%↑R	252%↑* 139%↑R
Lymphocytes	22%↑	25%↑	27%↑ 11%↑R	74%↑# 22%↑R	66%↓* 12%↓R	32%↓ 32%↑R
Basophils					50%↓* 0%↓R	100%↓ ND
Monocytes	24%↑	80%↑	53%↑ 18%↓R	140%↑# 20%↑R		380%↑* 60%↑R
Large unstained cells	17%↑	33%↑	33%↑ 12%↓R	200%↑* 0%↑R	50%↓ 25%↓R	300%↑ 33%↑R

% relative to controls
R = Recovery cohort; ND = not determinable
*, P ≤ 0.05; #, P ≤ 0.01 versus controls

Clinical Chemistry

Table 62: Changes in Clinical Chemistry Compared to Controls after 8 Weeks

Dose (mg/kg/day)	0.4		2		10	
Sex	M	F	M	F	M	F
ALT	23%↑#	19%↑	75%↑# 11%↓R	54%↑# 2%↑R	205%↑# 1%↓R	396%↑* 121%↑R^
AST	11%↑		24%↑ 13%↓R	16%↑ 3%↓R	38%↑ 11%↓R	112%↑ 32%↑R
Alkaline Phosphatase	12%↑		36%↑# 15%↑R	43%↑* 22%↑R	10%↓ 52%↑R#	112%↑* 61%↑R
Glucose					34%↓# 6%↓R	
BUN	13%↑		33%↑# 8%↑R	10%↑ 16%↑R	207%↑# 5%↑R	79%↑# 16%↑R

Dose (mg/kg/day)	0.4		2		10	
Sex	M	F	M	F	M	F
Creatinine						20%↑# 18%↓R#
Cholesterol	22%↑	16%↑	13%↑ 10%↑R	21%↑ 7%↓R	139%↑# 3%↑R	77%↑# 7%↑R
Triglycerides						261%↑* 22%↓R
Total protein			5%↓ 3%↓R		26%↓# 10%↓R	22%↓# 13%↓R*
Albumin			12%↓# 4%↓R		29%↓# 12%↓R	31%↓# 21%↓R#
Globulin					24%↓# 11%↓R	15%↓# 6%↓R
A/G ratio					9%↓ 0%↓R	16%↓# 12%↓R
Calcium			6%↓* 2%↓R		15%↓# 5%↓R*	
Potassium	9%↑*	5%↑	15%↑# 2%↑R	15%↑# 8%↑R	6%↑ 1%↑R	17%↑* 11%↑R
Chloride					3%↑* 1%↑R	

% relative to controls
R = Recovery cohort; ^ = SD larger than mean value
*, P ≤ 0.05; #, P ≤ 0.01 versus controls

Urinalysis:

Urine was collected during Week 8 for the main groups and Week 12 for the recovery groups. Proteinuria was observed in four males and one female dosed with lenvatinib at the 10 mg/kg dose level. Two out of 10 rats in the 10 mg/kg dose group still exhibited proteinuria following the 4-week recovery period.

Gross Pathology

Surviving rats were necropsied at Weeks 8 and 12 (recovery).

Table 63: Gross Pathology Findings in Juvenile Rats after 8 Weeks

Dose (mg/kg/day)		0		0.4		2		10	
Sex		M	F	M	F	M	F	M	F
# of rats examined (preterm decedents)		10,6R	10,6R	10	10	10,6R	10,6R	5(6),5R	4(7),5R
Adrenals	Enlargement, bilateral							1 (2)	(2)
	Discoloration, red, bilateral								(1)
	Discoloration, yellow, bilateral							(1)	
Coagulating glands	Small, bilateral							2	
Common bile duct	Dilatation								2
Duodenum	Dilatation							5 (4)	4 (5)

Dose (mg/kg/day)		0		0.4		2		10	
Sex		M	F	M	F	M	F	M	F
# of rats examined (preterm decedents)		10,6R	10,6R	10	10	10,6R	10,6R	5(6),5R	4(7),5R
Epididymides	Nodule, white, Small, left							1,1R 1R	
Incisors	Discoloration, white					4	1R	4(6),3R	4(7),2R
Kidneys	Discoloration, pale, bilateral Enlargement, bilateral Adhesion to thickened renal capsule, left							1 1R	 (1)
Liver	Adhesion between left medial and lateral lobes Adhesion to pleura and adipose tissue around pancreas Discoloration, yellowish white, left medial and lateral lobe Area, yellowish white							 1R	1 1 1
Seminal vesicles	Small, bilateral							2	
Stomach	Red spot, glandular stomach Red focus, glandular stomach							1 (1)	 (1)
Testes	Soft, left Small, left					1		 1R	
Thymus	Small							1 (2)	2
Heart	Enlargement								(1)
Lung with bronchi	Dark red area, two, left lobe							(1)	

( ) = observed in preterm decedent; R = Recovery cohort

Organ Weights

Table 64: Organ Weight Changes after 8 Weeks of Treatment

Dose (mg/kg/day)		0.4		2		10	
Sex		M	F	M	F	M	F
Brain	Absolute			8%↓R*		11%↓# 9%↓R#	10%↓# 1%↓R
	Rel to BW			24%↑# 11%↑R	15%↑# 5%↑R	173%↑# 58%↑R#	92%↑* 29%↑R#

Dose (mg/kg/day)		0.4		2		10	
	Sex	M	F	M	F	M	F
Thyroid	Absolute	6%↓		8%↓ 6%↓R		29%↓# 0.3%↓R	
	Rel to BW		12%↑	15%↑ 11%↑R	18%↑ 1%↓R	118%↑# 73%↑R*	127%↑* 37%↑R#
Heart	Absolute			18%↓# 13%↓R*	11%↓* 3%↓R	56%↓# 23%↓R#	43%↓# 12%↓R
	Rel to BW					35%↑# 33%↑R#	24%↑ 16%↑R#
Liver	Absolute	10%↓		30%↓# 15%↓R	15%↓# 6%↓R	64%↓# 35%↓R*	36%↓# 6%↓R
	Rel to BW					10%↑ 12%↑R	40%↑ 27%↑R*
Kidney	Absolute			17%↓# 13%↓R*		50%↓# 27%↓R#	36%↓# 13%↓R
	Rel to BW					52%↑# 25%↑R#	38%↑# 14%↑R
Adrenal	Absolute	10%↑		9%↑ 9%↓R		47%↑# 1%↑R	9%↑ 7%↑R
	Rel to BW	15%↑		39%↑# 8%↑R		352%↑# 73%↑R#	137%↑* 42%↑R#
Testis	Absolute					30%↓# 17%↓R#	
	Rel to BW	8%↑		25%↑# 11%↑R		115%↑# 42%↑R#	
Ovary	Absolute						64%↓# 10%↓R
	Rel to BW				23%↑* 2%↓R		25%↓ 19%↑R

% relative to controls; Rel to BW = relative to body weight; R = Recovery cohort
*, P ≤ 0.05; #, P ≤ 0.01 versus controls

Histopathology

Adequate Battery: Yes
Peer Review: Yes

Table 65: Histological Findings in Juvenile Rats after 8 Weeks of Lenvatinib

Dose (mg/kg/day)		0		0.4		2		10	
	Sex	M	F	M	F	M	F	M	F
# of rats examined (preterm decedents)		10,6R	10,6R	10,0R	10	10,6R	10,6R	5(6),5R	4(7),5R
Incisor	Dysplasia								
	Slight					1	9		
	Moderate					9	1		(3)
	Marked							5(6)	4(4)
	Inflammatory cell infiltration, pulp								
	Slight					1	1	1(3)	(1)
	Moderate					1		4(2)	3(3)
	Inflammatory cell infiltration, gingiva								
	Slight						1	4(1)	3(2)
	Moderate					1		(1)	1(1)

Dose (mg/kg/day)		0		0.4		2		10	
Sex		M	F	M	F	M	F	M	F
# of rats examined (preterm decedents)		10,6R	10,6R	10,0R	10	10,6R	10,6R	5(6),5R	4(7),5R
	Dysplasia, distal remnant								
	Slight					4R	6R	3R	5R
	Moderate							1R	
Molar	Inflammatory cell infiltration, pulp								
	Slight							1R	1
Femur	Increased epiphyseal growth plate/articular cartilage								
	Slight					5	2		
	Moderate					4			(2)
	Marked							5(6)	4(5)
	Remodeling/regeneration, epiphyseal growth plate/articular cartilage								
	Slight					3R		3R	3R
	Moderate							2R	2R
Sternum	Increased epiphyseal cartilage, Slight							3(6)	2(6)
Femoral marrow	Hypocellularity, focal								
	Slight					1		3(3)	2(4)
	Moderate							2(1)	(2)
	Marked							(2)	
	Hyperplasia, granulocytic cell, Slight								1
Sternal marrow	Hypocellularity								
	Slight							2(3)	1(1)
	Moderate							1(2)	(3)
	Hyperplasia, granulocytic cell, Slight								1
Kidney	Glomerulopathy								
	Slight					1		2(4),4R	2(4)
	Moderate							2(2)	2(2),1R
	Glomerulonephropathy								
	Marked							1	(1)
	Arteritis, Slight							1	
	Fibrosis, focal, Slight			1	1	1	2	1R	
	Cyst, medulla								1
	Thickened capsule								
	Slight							1R	
Adrenals	Sinusoidal dilatation								
	Slight								1(2)
	Moderate							3(2)	2(1)
	Cortical necrosis								
	Moderate							1(2)	(1)
	Marked							2(3)	(3)
	Focal cortical necrosis, residual								
	Slight							2R	
	Moderate							1R	1R
Brain	Eosinophilic exudate, choroid plexus								

Dose (mg/kg/day)		0		0.4		2		10	
Sex		M	F	M	F	M	F	M	F
# of rats examined (preterm decedents)		10,6R	10,6R	10,0R	10	10,6R	10,6R	5(6),5R	4(7),5R
	Slight							1	2
	Arterial fibrinoid necrosis, choroid plexus, Slight								1(2)
Stomach	Mineralization, mucosa								
	Slight							3(3),2R	(2)
	Moderate							1(2)	(3)
	Mucosal hyperplasia, glandular stomach								
	Slight							4(6)	3(5)
	Moderate							1	1
	Inflammatory cell infiltration, glandular stomach, Slight							5(5)	4(5)
	Hemorrhage, lamina propria, focal								
	Slight							1(1)	1(1)
	Moderate								(1)
Duodenum	Inflammation/cystic dilatation, duodenal glands							(2)	(2)
	Moderate							5(4)	4(5)
	Marked								
	Mucosal hyperplasia							3(2)	3(2)
	Slight								
	Interstitial fibrosis, residual, duodenal glands							5R	5R
	Slight								
Jejunum	Marked autolysis							(2)	(2)
Ileum	Marked autolysis							(1)	(1)
Cecum	Edema, submucosa								
	Slight							3(3)	2(3)
	Hyperplasia, crypt								
	Slight							1(1)	1
	Inflammatory cell infiltration, mucosa								
	Slight					1		1R	1R
	Marked autolysis							(2)	(2)
Colon	Edema, submucosa								
	Slight							1	
	Marked autolysis								(1)
Rectum	Marked autolysis								(1)
	Hemorrhage, focal, Slight								1R
Liver	Abscess, Marked								1
	Cholangitis, Slight								1
	Focal necrosis, hepatocytes, Slight				1		2		
	Granuloma, Moderate							1R	1R
	Mononuclear cell infiltration, periportal								
	Slight							1R	5R
Common bile	# of rats examined:	0	0	0	0	0	0	0	2

Dose (mg/kg/day)		0		0.4		2		10	
Sex		M	F	M	F	M	F	M	F
# of rats examined (preterm decedents)		10,6R	10,6R	10,0R	10	10,6R	10,6R	5(6),5R	4(7),5R
duct	Cholangitis, Marked								2
Heart	Arteritis, Slight							1	
	Thrombosis, Slight								(1)
	Myocardial hypertrophy Slight								(1)
Lung	Hemorrhage, alveolar, focal, Slight							(1)	
	Foamy cell accumulation, alveolar, focal, Moderate								(1)
Thymus	Lymphoid depletion								
	Slight					1		1(1)	2(3)
	Moderate							1(1)	1(2)
Spleen	Extramedullary hematopoiesis								
	Slight							3	(1)
	Moderate								1
	Lymphoid depletion								
	Slight							1(1)	1(1)
	Arteritis, Slight							1(1)	
Mesenteric lymph node	Lymphoid depletion Slight							2(1)	1(1)
Submaxillary lymph node	Lymphoid depletion Slight							(1)	2(1)
Pancreas	Decreased zymogen granules, Slight							2(3)	(4)
	Edema, Slight							3	2
Submaxillary glands	Single cell necrosis/apoptosis Slight							(2)	(1)
Thyroids	Marked autolysis							(1)	(1)
Urinary bladder	Marked autolysis								(1)
Testes	# of rats examined:	10,6R	0	0	0	1	0	5(6),5R	0
	Atrophy, seminiferous tubules, unilateral								
	Marked					1		1R	
	Immature							(1)	
Epididymides	# of rats examined:	10	0	0	0	1	0	5(6),5R	0
	Decreased spermatozoa, unilateral								
	Marked					1		1R	
	Spermatic granuloma, unilateral								
	Slight							1,1R	
	Moderate							1R	
	Immature							(6)	
Seminal vesicles	Immature							(4)	
Coagulating glands	Immature							(4)	
Ovaries	Immature								4(7)

Dose (mg/kg/day)		0		0.4		2		10	
	Sex	M	F	M	F	M	F	M	F
# of rats examined (preterm decedents)		10,6R	10,6R	10,0R	10	10,6R	10,6R	5(6),5R	4(7),5R
Spinal cord	# of rats examined:	10,6R	10					5(6),5R	4(7)
	Mineralization, meninges Slight							1R	(2)

Slight = Grade 1+, Moderate = Grade 2+, Marked = Grade 3+
( ) = observed in preterm decedent; R = Recovery cohort

Special Evaluation:

Bone Measurement:

- Length and width of the right tibia and femur were measured using a caliper.
- There was a statistically significant dose-dependent decrease in the width and/or length of the femur and tibia at dose levels ≥ 2 mg/kg (related to growth retardation and/or histologic changes in bone). Bone width, but not length, showed some evidence of recovery.

Table 66: Bone Measurement Changes after 8 Weeks of Treatment

Dose (mg/kg/day)		0.4		2		10	
	Sex	M	F	M	F	M	F
Femur	Length			8%↓# 8%↓R#	5%↓# 4%↓R*	25%↓# 27%↓R#	22%↓# 22%↓R#
	Width					18%↓# 3%↑R	9%↓* 7%↑R
Tibia	Length			6%↓# 7%↓R*	5%↓# 4%↓R*	25%↓# 26%↓R#	20%↓# 19%↓R#
	Width			14%↓# 3%↓R		32%↓# 21%↓R#	18%↓# 15%↓R#

% relative to controls; R = Recovery cohort
*, P ≤ 0.05; #, P ≤ 0.01 versus controls

Growth and Development

Physical Development

- Vaginal opening: Observed daily starting on PND 26
- Preputial separation: Observed daily starting on PND 35
- There was a statistically significant delay in prepuce separation and vaginal opening in males and females dosed with lenvatinib at the 10 mg/kg dose level, respectively, compared to controls. This was likely secondary to decreased body weights.

Table 67: Postnatal Day of Prepuce Separation and Vaginal Opening

Dose (mg/kg/day)	0	0.4	2	10
Prepuce separation (male)	48.3 ± 2.2	48.2 ± 3.3	49.5 ± 2.5	59.2 ± 7.2#
Vaginal opening (female)	30.6 ± 1.7	30.8 ± 1.2	31.3 ± 2.0	35.9 ± 3.5#

Mean PND ± S.D.
#, P ≤ 0.01 versus controls

Functional Development: Pinna, pupillary, corneal, auditory and deep pain reflex were evaluated during Weeks 8 and 12 (recovery). Findings were unremarkable.

Behavioral Observations

- A Morris water maze test to assess learning and memory was conducted during Weeks 7 and 12 (recovery); rats in the 10 mg/kg dosing group were excluded due to bad general condition. There were three rounds of training and three records of evaluation; additional details of the test were not provided.
  - The Applicant did not indicate swim speeds.
  - During the third record of evaluation, there was a statistically significant 210% increase in the length of time it took the rats to arrive at a goal in males dosed with 2 mg/kg lenvatinib (27.9 seconds) compared to controls (9 seconds). Taking into account all three records of evaluation, the mean time of arriving at a goal was slightly increased in a dose-dependent manner in males dosed with ≥ 0.4 mg/kg lenvatinib; however, these differences were not statistically significant compared to controls. Findings in females were unremarkable, but the high dose was not assessed for either sex.
- Open field test: assessed time for first move and number of square ambulation, standing up (rearing), face grooming, body grooming, urination, and defication during Weeks 8 and 12 (recovery).

Table 68: Summary of Open Field Test Observations at Week 8

Dose (mg/kg/day)	0.4		2		10	
Sex	M	F	M	F	M	F
Time for first move (sec)					154%↑# 78%↑R	57%↑ 39%↓R
No. of square ambulation	8%↓		16%↓ 3%↑R		39%↓# 23%↑R#	29%↓# 2%↓R
No. of stand up (rearing)			18%↓# 21%↑R		69%↓# 32%↓R^	51%↓# 3%↓R

% relative to controls; R = Recovery cohort
^ = Mean influenced by one rat with a very high value; other rats exhibited decreased standing up compared to controls
#, P ≤ 0.01 versus controls

Toxicokinetics

- Blood samples were collected 0.5, 1, 2, 4, 8 and 24 hrs after lenvatinib administration on Day 1, and prior to dosing and 0.5, 1, 2, 4, 8 and 24 hrs after lenvatinib administration on Day 56.
- Lenvatinib drug exposures were generally dose proportional.
- There were no significant gender effects on Day 1. On Day 56, Cmax at all dose levels was ~1.4- to 1.6-fold higher in females compared to males.
- There was no evidence of accumulation on Day 56 compared to Day 1, except for findings of higher Cmax in females at the 0.4 mg/kg and 2 mg/kg dose levels.

Table 69: Summary of Mean Toxicokinetic Data Following 8 weeks of Daily Lenvatinib Administration to Juvenile Rats

Dose (mg/kg)	Day	Male		Female	
		C_{max} (ng/mL)	$AUC_{(0-24h)}$ (ng·h/mL)	C_{max} (ng/mL)	$AUC_{(0-24h)}$ (ng·h/mL)
0.4	1	259.19	1,806.13	272.16	1,956.97
	56	254.49	1,371.36	416.43	1,641.99
2	1	1,459.98	11,030.94	1,396.81	10,682.33
	56	1,556.88	8,323.30	2,234.45	8,827.68
10	1	8,341.75	52,475.56	7,535.77	60,508.84
	56	5,199.33	45,905.68	7,512.60	31,115.09

(Excerpted from Applicant's submission)

Dosing Solution Analysis

Met pre-specified acceptance criteria.

10 Special Toxicology Studies

Study title: E7080: Phototoxicity Test in Balb/c 3T3 Cells

Study no.: SBL038-070
Study report location: EDR 4.2.3.7.7
Conducting laboratory and location: (b) (4)
Date of study initiation: June 19, 2012
GLP compliance: Yes (Japan Ministry of Health and Welfare Ordinances No. 21, 1997 and No. 114, 2008)
QA statement: Yes
Drug, lot #, and % purity: E7080, 115P0901, 99.8%

Key Study Findings: E7080 exhibited no phototoxic potential under the conditions tested in the in vitro 3T3 neutral red uptake (NRU) phototoxicity test.

Methods

Concentrations: 1.17, 1.76, 2.63, 3.95, 5.93, 8.89, 13.3, and 20 µg/mL
Dose Justification: Precipitation was observed at concentrations ≥ 22 µg/mL in the dose-range finding study #T12002 (non-GLP and not reviewed). Thus, 20 µg/mL was chosen as the high dose in this study.
Vehicle/negative control: Water for injection
Positive control: Chlorpromazine hydrochloride (CPZ)
Cell line: Mouse Balb/c 3T3 cells
Study design: Photoxicity potential was measured by a relative reduction in the viability of cells exposed to lenvatinib in the presence and absence of light irradiated by a solar simulator (SOL500, Dr. Hönle AG). Cells in 96-well plates were treated with E7080 for 1 hr and then ± 5

J/cm^2 UV-A light at an intensity of 1.7 mW/cm^2 for 50 min. Cells were incubated with neutral red solution for 3 hrs, and cell viability was measured by neutral red uptake. Phototoxic potential was evaluated by the Photo Irritation Factor (PIF; compares IC_{50} values ± light) and by the Mean Photo Effect (MPE; compares 2 dose-response curves). Phototoxicity = PIF ≥ 5 or MPE ≥ 0.15; No phototoxicity = PIF < 2 or MPE < 0.1.

Study Validity

The study was determined to be valid because the following pre-specified acceptance criteria were met: 1) the absorbance of the negative control was > 0.4; 2) the cell viability of the irradiated negative control was > 80% of that of the non-irradiated negative control; 3) the mean absorbance of the negative control was within the range of background data; 4) the PIF of the positive control was within the range of background data and the OECD recommendations.

Results

The positive control CPZ was phototoxic, as expected. E7080 precipitation was observed at 20 µg/mL, so this concentration was not evaluated further. Since cytotoxicity was not observed with E7080 in the presence or absence of irradiation, IC_{50} values were not determined, and subsequently the PIF could not be calculated. The MPE for E7080 was calculated to be -0.024, indicating no phototoxicity (MPE < 0.1).

Table 70: Results of In Vitro 3T3 NRU Phototoxicity Test

Treatment condition	Dose (µg/mL)	Absorbance (540 nm)/well						Mean ± SD	Cell viability (%)	IC_{50} (µg/mL)	PIF	MPE	Judgment*3
		well 1	well 2	well 3	well 4	well 5	well 6						
Absence of irradiation	Negative control	0.8781	0.8916	0.8869	0.8394	0.8774	0.8660	0.8732 ± 0.0188	100	ND	ND	-0.024	No phototoxicity
	1.17	0.8445	0.8868	0.9135	0.8545	0.9176	0.8687	0.8809 ± 0.0304	100.9				
	1.76	0.8584	0.8277	0.8089	0.8905	0.8890	0.8468	0.8536 ± 0.0327	97.7				
	2.63	0.8798	0.8836	0.8293	0.8014	0.8702	0.8346	0.8498 ± 0.0330	97.3				
	3.95	0.8394	0.8736	0.8194	0.8235	0.8598	0.8371	0.8421 ± 0.0210	96.4				
	5.93	0.8203	0.8535	0.8248	0.8608	0.8475	0.8333	0.8400 ± 0.0163	96.2				
	8.89	0.9579	0.8318	0.8534	0.7998	0.8234	0.8204	0.8478 ± 0.0567	97.1				
	13.3	0.8016	0.8213	0.7890	0.8256	0.8250	0.8337	0.8160 ± 0.0170	93.4				
	20*2	0.8063	0.7753	0.7882	0.7984	0.7312	0.8258	0.7875 ± 0.0324	90.2				
Presence of irradiation	Negative control*1	0.8602	0.8522	0.8454	0.8546	0.8499	0.8717	0.8557 ± 0.0093	100	ND			
	1.17	0.8339	0.8569	0.8742	0.8723	0.8892	0.8555	0.8637 ± 0.0192	100.9				
	1.76	0.8601	0.8665	0.8652	0.8742	0.8767	0.8658	0.8681 ± 0.0062	101.5				
	2.63	0.8509	0.8493	0.8463	0.8650	0.8394	0.8702	0.8535 ± 0.0117	99.7				
	3.95	0.8687	0.8509	0.8451	0.8492	0.8453	0.8406	0.8500 ± 0.0099	99.3				
	5.93	0.8496	0.8263	0.8436	0.8519	0.8489	0.8204	0.8401 ± 0.0134	98.2				
	8.89	0.8333	0.8512	0.8671	0.8565	0.8752	0.8438	0.8545 ± 0.0153	99.9				
	13.3	0.8173	0.8388	0.8523	0.8284	0.8613	0.8294	0.8379 ± 0.0164	97.9				
	20*2	0.8100	0.8300	0.7968	0.8337	0.8340	0.7471	0.8086 ± 0.0336	94.5				

Negative control : Water for injection, PIF : Photo Irritation Factor, MPE : Mean Photo Effect, ND : Not determined
*1 : The cell viability of the irradiated negative control was 98.0 % of the non-irradiated negative control.
*2 : The result at 20 µg/mL were not evaluated, because test article precipitation was observed at 20 µg/mL at the end of treatment.
*3 : The result was judged from the MPE, because the IC_{50} could not be determined for either the irradiated or non-irradiated plates.

(Excerpted from Applicant's submission)

11 Integrated Summary and Safety Evaluation

Pharmacology

The Applicant investigated the pharmacologic activity of lenvatinib using a series of in vitro and in vivo assays. In vitro screening for potentially relevant kinase inhibition occurred using a panel of recombinant human protein kinases in three separate studies. Lenvatinib was most selective for the vascular endothelial growth receptors VEGFR 1, 2, and 3 (Flt-1, KDR, and Flt-4), inhibiting these receptors at IC_{50} concentrations ≤ 4 nM, and RET at an IC_{50} of 6 nM. Lenvatinib also inhibited FGFR1-4, KIT, PDGFRβ, and PDGFRα at IC_{50}s < 100nM. The Cmax of lenvatinib determined in patients at the recommended dose of 24 mg ranged from ~ 400 – 1000 nM. Considering the roles of VEGFR1-3, FGFR1-4, KIT, and RET in cellular growth and function, the anti-tumor activity of lenvatinib may result not only from inhibition of angiogenesis, but also from a direct anti-tumor effect.

The Applicant also examined the off-target activity of lenvatinib against a panel of 50 physiologically important cellular targets. Lenvatinib was most selective for the serotonin $5\text{-}HT_{1B}$ receptor, norepinephrine transporter (NET), and serotonin $5\text{-}HT_{1A}$ receptor. Incubation with the drug at a concentration of 1×10^{-5} M resulted in 58%, 50%, and 38% inhibition of these targets, respectively. The 1×10^{-5} M (10,000 nM) concentration, however, is not physiologically achievable, and when the concentration of lenvatinib was lowered 10-fold to 1×10^{-6} M, there was no significant inhibition of any off-target receptors.

The Applicant investigated the effects of lenvatinib on angiogenesis and proliferation of endothelial cells in vitro, and angiogenesis and anti-tumor activity in vivo. VEGF-driven VEGFR2 activation, endothelial cell proliferation, and vascular tube formation were analyzed in vitro using human umbilical vein endothelial cell (HUVEC) model systems. In vitro, lenvatinib blocked VEGF-driven activation of VEGFR2 as shown by decreased phosphorylation of the receptor and exhibited anti-proliferative and anti-angiogenic effects on endothelial cells all at concentrations of < 5 nM. Thus, these pharmacodynamic effects occurred at concentrations that are clinically achievable in patients treated with lenvatinib at the 24 mg dose level. The lenvatinib metabolites M1, M2, and M3 also inhibited VEGF-mediated proliferation, but at concentrations approximately 10 to 60-fold higher than the parent compound. In vivo studies using xenografts of human anaplastic thyroid cancer cell line 8305C in athymic mice, also demonstrated that daily oral treatment with lenvatinib for 14 days had a dose-dependent anti-angiogenic activity at doses ranging from 3 to 300 mg/kg, indicated by decreased microvessel density within the tumor. Following daily dosing for 14 to 28 days, lenvatinib demonstrated in vivo anti-tumor activity against various human tumor xenografts implanted in athymic mice, including five types of thyroid carcinomas (differentiated [papillary and follicular] carcinoma, anaplastic, thyroid-derived squamous cell carcinoma, and medullary thyroid carcinomas), with significantly decreased tumor volume observed in all five xenograft types in a dose-dependent manner.

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Safety Pharmacology
Both in vitro and in vivo safety pharmacology studies were conducted to assess the effects of lenvatinib on cardiovascular, behavioral, general physiological, and respiratory function. Lenvatinib significantly inhibited hERG channel tail current at concentrations ≥ 10 μM using manual patch-clamp electrophysiology on HEK293 cell transfected with hERG cDNA. The IC_{50} for hERG inhibition was 11.89 μM. Lenvatinib had no significant effects on in vitro assessment of action potential parameters in isolated heart papillary muscle from the guinea pig when used at concentrations of 1 and 10 μM. In dogs administered single oral doses of lenvatinib at 6 or 30 mg/kg, there were minor, although statistically significant, changes in cardiovascular parameters compared to vehicle treatment. In male dogs there were statistically significant decreases in heart rate (6 mg/kg) at 4 hours post dose with consequent prolongation in QRS duration at 1 and 4 hours post dose, as well as increases in blood pressure (6 and 30 mg/kg - 2 hours post dose) and QT prolongation (30 mg/kg – 2 hours post dose). QT prolongation also occurred in female dogs treated with 30 mg/kg 1 hour post dose. These changes were incidental since there were no statistically significant changes compared to pre-dose values; however, QT prolongation and hypertension have been observed in clinical trials with lenvatinib.

No significant effects were observed on behavior and general physical condition at single oral doses ≤ 100 mg/kg up to 8 hours after administration of lenvatinib. Oral doses ≤ 100 mg/kg also had no effect on respiratory function parameters measured in rats up to 8 hours after administration.

Pharmacokinetics
The Applicant evaluated the in vivo pharmacokinetics of lenvatinib following oral and intravenous administration to mice, rats, dogs, and monkeys. The oral bioavailability of lenvatinib was 64.4%, 68.7%, 70.4%, and 78.4% in mice, rats, dogs, and monkeys, respectively. Lenvatinib exhibited low clearance, a small volume of distribution in mice and rats, and a moderate level of distribution in dogs and monkeys. Tmax ranged from 0.5 to 2 hours in animals following oral administration of lenvatinib, which was slightly shorter than the 1 to 4 hr range observed in humans. The terminal elimination phase half-life in all animal species tested following a single oral lenvatinib administration ranged from 1.74 to 5.27 hrs, which was much shorter than that observed in humans (~28 hrs following single and multiple doses of lenvatinib).

The Applicant examined the absorption, distribution, and excretion of lenvatinib following a single oral administration of 3 mg/kg ^{14}C-lenvatinib to fasted male rats and cynomolgus monkeys. In this experiment ^{14}C-lenvatinib-related radioactivity reached its maximum levels in most tissues at 30 min post-dose with the exception of the cecum, large intestine, and skin, where it peaked at 6 hours post-dose. Consistent with observed toxicities in toxicology studies, distribution of ^{14}C-lenvatinib was highest in the rat liver, adrenal gland, stomach, and small intestine (~1 to 3-fold higher than in the plasma) at 30 min post-dose and in the cecum and large intestine (~12-fold higher than in the plasma) at 6 hours post-dose. ^{14}C-lenvatinib distribution to the rat cerebrum, cerebellum, medulla oblongata, and spinal cord was ≤ 2% of ^{14}C-lenvatinib in the plasma, and the distribution of radioactivity to the brain was ≤ 0.02% of total dosed

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radioactivity. Of note, ^{14}C-lenvatinib-related radioactivity in the sciatic nerve was 10% of ^{14}C-lenvatinib in the plasma. Mean tissue radioactivity concentrations decreased with time, and reached <1% of their maximum levels by 336 hours post-dose. The half-life of ^{14}C-lenvatinib-related radioactivity was 17.6 days in the aorta compared to plasma (0.7 days), potentially correlating with clinical findings of arterial thromboembolic events.

Following a single oral administration of 3 mg/kg ^{14}C-lenvatinib to monkeys, radioactivity reached its maximum levels in most tissues at 4 hours post-dose with the exception of the cornea, iris, choroid, lens, sclera, and large intestine, where it peaked at 24 hours post-dose. Tissue distribution of ^{14}C-lenvatinib was widespread and highest in the bile, urine in bladder, gall bladder, liver, and choroid compared to plasma. High distribution to the bile suggested that fecal excretion via bile is a main route of lenvatinib excretion in monkeys. ^{14}C-lenvatinib distribution to the monkey cerebrum, cerebellum, medulla oblongata, and spinal cord was ≤ 14% of ^{14}C-lenvatinib in the plasma, and the distribution of radioactivity to the brain was ≤ 0.06% of total dosed radioactivity. Taken together, these nonclinical data demonstrate that lenvatinib distribution to the brains of animals is ~2-14% of that in the plasma. This suggests that lenvatinib is able to across the blood-brain barrier, at least at low levels, although there were not any clinical signs of CNS toxicity in animals. Mean tissue radioactivity concentrations decreased with time, but elimination was slow in melanin-containing tissues, possibly due to tight binding of lenvatinib to melanin. This suggests that lenvatinib has the potential to induce ocular toxicities, although they have not been observed thus far in animals or humans. Skin toxicities such as rash have been seen in humans treated with lenvatinib, though lenvatinib did not exhibit phototoxic potential under the conditions tested in the in vitro 3T3 NRU phototoxicity test in mouse Balb/c 3T3 cells. According to the Applicant, lenvatinib exhibited high levels of protein binding in both animals (~90-98%) and humans (98-99%).

The Applicant evaluated the in vitro metabolism of lenvatinib using mouse, rat, dog, monkey, and human liver microsomes. Six metabolites were detected in human liver microsomes (M1, M2, M3, M4, M5, and M8), with the demethylated form of lenvatinib (M2) being the major metabolite. All of these metabolites were also found in mouse, rat, dog, and/or monkey liver microsomes, suggesting that all of these species are appropriate models for use in nonclinical studies. Following a single oral administration of 3 mg/kg ^{14}C-lenvatinib to male rats and monkeys, 41 lenvatinib metabolites were identified in the plasma, liver, kidney, urine, feces, and/or bile. Consistent with clinical studies, the main identified metabolic pathways were oxidative metabolism and glutathione conjugation with elimination of the *O*-aryl group. There were no major circulating metabolites identified in clinical studies; however, according to the Applicant, the M2 metabolite accounted for ~2.5% of lenvatinib exposure. In keeping with this, the major component of rat and monkey plasma following administration of ^{14}C-lenvatinib was the unchanged parent compound. M2 was not identified in vivo in rat or monkey plasma, but was found in feces and bile; the percent of M2 identified in rat feces was close to that observed in human plasma, suggesting that adequate exposure to the metabolite occurred in animals to account for potential metabolite-mediated toxicity.

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Following a single oral administration of 3 mg/kg ^{14}C-lenvatinib to male rats and cynomolgus monkeys, excretion was primarily through the fecal route. Fecal excretion accounted for ~73-87% of radioactivity in rats and monkeys, whereas urinary excretion only accounted for ~12-17%. These nonclinical results were fairly consistent with findings in the clinic, where 64% of the radioactivity dose in humans was eliminated in the feces, and 25% was eliminated in the urine. Fecal excretion in rats was mediated by biliary excretion.

General Toxicology

Lenvatinib was evaluated in GLP-compliant repeat-dose toxicology studies in Sprague Dawley rats, beagle dogs, and cynomolgus monkeys. Lenvatinib was orally administered once daily to rats at dose levels of 1, 10, 15, 30, or 100 mg/kg (6, 60, 90, 180, or 600 mg/m^2) for 4 weeks and 0.4, 2, or 10 mg/kg (2.4, 12, or 60 mg/m^2) for 13 and 26 weeks. Lenvatinib was orally administered once daily to cynomolgus monkeys at dose levels of 0.3, 3, or 30 mg/kg (3.6, 36, or 360 mg/m^2) for 4 weeks and 0.1, 0.5, or 3 mg/kg (1.2, 6, or 36 mg/m^2) for 13 and 39 weeks. Recovery was only assessed in the 4-week studies. Target organs of lenvatinib-mediated toxicity in rats and monkeys were the kidney, duodenum, stomach, pancreas, adrenals (sinusoidal dilation, cortical necrosis, and/or cortical hypertrophy), liver, spleen (lymphoid depletion), thymus (lymphoid depletion and atrophy), pituitary, choroid plexus, heart (rats), femur, teeth (rats), sternum (rats), femoral and sternal marrow (rats; hypocellularity), common bile duct (rats), gallbladder (monkeys), vagina (epithelial atrophy), ovaries, testes, and epididymides. Target organs of lenvatinib-induced toxicity in dogs following 4 weeks of lenvatinib treatment included the GI tract, gallbladder, kidney, testes, and epididymides. In general, target organs were consistent between shorter and longer term studies. Many of the lenvatinib-induced findings were attributable to the primary pharmacology of VEGF inhibition, and have been seen with other VEGF inhibitors. Lenvatinib drug exposures in the 26-week rat study were generally dose proportional on Day 1 and slightly higher in females (< 2-fold) compared to males; there was also evidence of slight accumulation. Lenvatinib drug exposures in the 39-week monkey study generally increased more than dose proportionally, there were no significant gender differences, and there was evidence of slight accumulation.

Although there were no deaths in the main study rats at doses up to 10 mg/kg in the 13-week study, lenvatinib was severely toxic in rats at the 10 mg/kg dose level (60 mg/m^2; approximately 9 to 39 times the clinical exposure by AUC at the recommended human dose of 24 mg) following 94-147 days of dosing in the 26-week study, resulting in 11 mortalities. Preterm decedents exhibited decreased body weights and food consumption, discolored/broken teeth, chromaturia, and soft stool. According to the Applicant, mortality was attributed to the progression of duodenal and renal lesions similar to those observed in the 4- and 13-week toxicology studies. These lesions appeared to increase in incidence and/or severity with longer treatment time (13 versus 26 weeks). These severe duodenal lesions (inflammation/cystic dilation accompanied by mucosal hyperplasia and medial arteriole necrosis) were complicated by infections and associated with cholangitis in the liver and bile duct, pancreatitis with hemorrhage, and other GI tract lesions. One monkey dosed with lenvatinib in the 39-week toxicology

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study at the 3 mg/kg dose level (36 mg/m^2; approximately 1.4 to 6 times the clinical exposure by AUC at the recommended human dose) was euthanized in moribund condition on Day 51; this preterm decedent exhibited anorexia, body weight loss, decreased food consumption, soft/watery stool, hypothermia, and histological findings in the kidneys, gallbladder (focal arterial degeneration/fibroid necrosis), choroid plexus (eosinophilic exudate and arterial fibroid necrosis), femur, duodenum (atrophy, inflammation, arterial fibroid necrosis), colon, and rectum. As a result, the HNSTD following 39 weeks of lenvatinib treatment in monkeys was 0.5 mg/kg (6 mg/m^2).

Vascular lesions related to the pharmacology of lenvatinib were observed in multiple rat tissues following 26 weeks of lenvatinib treatment, including arteritis in the common bile duct and pancreas and medial arteriole necrosis in the kidney, stomach, duodenum, adipose tissue, testes, and spleen. Lenvatinib-related vascular lesions observed in monkeys following 39 weeks of treatment included arteriole fibroid necrosis in the duodenum, gallbladder, and choroid plexus. The observed vascular changes in the 4-week monkey study were associated with focal hemorrhage in the intestine and gallbladder at the 30 mg/kg dose level (360 mg/m^2) and in the choroid plexus at dose levels ≥ 3 mg/kg (36 mg/m^2). Vascular changes in the 26-week rat study were associated with hemorrhage in the adrenals and duodenum at the 10 mg/kg dose level (60 mg/m^2), and hemorrhage was observed in the hindlimbs of one rat administered 10 mg/kg. There was a statistically significant decrease in the mean red blood cell count in male rats dosed with lenvatinib at the 10 mg/kg dose level compared to controls along with a dose-dependent decrease in mean total protein and albumin, further indicative of hemorrhagic loss. Consistent with nonclinical findings, hemorrhagic events including fatal intracranial hemorrhage have been observed in patients administered lenvatinib, as well as other VEGF antagonists. Eosinophilic exudate in the choroid plexus was observed following 26 and 39 weeks of lenvatinib administration in rats and monkeys at dose levels of 60 mg/m^2 and 36 mg/m^2, respectively. One rat exhibited slight focal ischemic malacia in the cerebral cortex at lenvatinib exposures approximately 9 to 39 times the clinical exposure by AUC at the recommended human dose. The Applicant considered this finding to be secondary to vascular changes in the brain, and did not result in any clinical signs of CNS toxicity in rats. Thus, although there were not any direct nonclinical indicators of the Reversible Posterior Leukoencephalopathy Syndrome (RPLS) observed in humans, the choroid plexus/brain was a target organ of lenvatinib in animals.

Adventitial arteriole thickening in the heart was observed in rats following 26 weeks of lenvatinib at the 10 mg/kg (60 mg/m^2) dose level. Additional histological findings in the heart included myocardial fibrosis and focal bacterial myocarditis in rats following 4 weeks of lenvatinib treatment at the 100 mg/kg (600 mg/m^2) dose level. Although no significant effects were observed on blood pressure, QT prolongation, or other ECG parameters at the time-points measured following treatment with lenvatinib in repeat-dose toxicology studies in dogs and monkeys at dose levels up to 10 mg/m^2 and 360 mg/m^2, respectively, QT interval prolongation and hypertension have been observed clinically and are expected with VEGF inhibition. Further, blood pressure was measured in monkeys only at the end of the toxicology study, not at Cmax, and there were

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vascular histopathological findings indicative of potential hypertension in both rats and monkeys. Finally, in a single dose safety pharmacology study there was a statistically significant increase in mean blood pressure in male dogs following administration of lenvatinib at 6 or 30 mg/kg compared to controls, but these increases were gender specific and not dose-dependent.

Slight to marked glomerulopathy was observed following 26 or 39 weeks of lenvatinib treatment in rats and monkeys at dose levels ≥ 2 mg/kg (12 mg/m^2) and ≥ 0.5 mg/kg (6 mg/m^2), respectively, and slight to marked glomerulonephropathy was observed in rats at exposures approximately 9 to 39 times the clinical exposure by AUC at the recommended human dose. These findings are related to the pharmacological activity of lenvatinib, since VEGF is involved in maintaining glomerular function. Treatment with lenvatinib for 26 or 39 weeks resulted in a dose-dependent increase in mean urea nitrogen in rats and increased mean blood urea nitrogen and creatinine in monkeys. Together these nonclinical findings are indicative of renal toxicity and correlate with clinical findings of renal impairment and failure. In addition, chromaturia was observed in one rat dosed with lenvatinib at the 10 mg/kg (60 mg/m^2) dose level, and proteinuria was observed in male rats and monkeys at exposures approximately 2.6 to 11 times and 1.6 to 7 times the clinical exposure by AUC at the recommended human dose, respectively, consistent with clinical findings of proteinuria.

Treatment with lenvatinib induced changes in bone, including increased epiphyseal growth plate in the femur of rats and monkeys and increased epiphyseal cartilage in the sternum of rats. These findings have been seen with other VEGF inhibitors, and can be attributed to the pharmacologic activity of lenvatinib. Following lenvatinib administration for 26 weeks, all rats dosed with ≥ 2 mg/kg (12 mg/m^2) lenvatinib exhibited broken/discolored teeth, and 16/30 rats exhibited loss of incisors at the 10 mg/kg dose level. Histological findings in the incisors included a dose dependent increase in the incidence and/or severity of dysplasia and inflammatory cell infiltration in rats dosed with ≥ 2 mg/kg lenvatinib. Lenvatinib-related findings in the teeth/incisors were specific to rats. The lenvatinib-induced findings in bone and teeth are likely not relevant to human adults with closed growth plates, but may adversely affect the development of pediatric patients.

Treatment with lenvatinib induced clinical signs of GI toxicity in rats, monkeys, and dogs, including decreased body weight and food consumption, anorexia, and/or soft/discolored/watery stool, which correlated with clinical findings of decreased weight/appetite and diarrhea. Lenvatinib induced extensive histological findings throughout the GI tract after 26 and 39 weeks of dosing in rats and monkeys, respectively, specifically in the duodenum (inflammation/cystic dilation, mucosal hyperplasia, medial arteriole necrosis, and atrophy), stomach (erosion), ileum (crypt hyperplasia), cecum (crypt hyperplasia, edema, and erosion), and colon and rectum (crypt hyperplasia and inflammatory cell infiltration). Events of GI perforation or fistula were seen clinically with lenvatinib. Following 4 weeks of lenvatinib administration at the 0.5 mg/kg (10 mg/m^2) dose level, lymphoid depletion/necrosis in the jejunum and ileum were observed in dogs. Histological findings in the gallbladder included inflammatory

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cell infiltration, focal arterial degeneration/fibroid necrosis, and increased bile viscosity in monkeys and arterial fibrinoid necrosis in dogs. The severe duodenal lesions observed in the 26-week rat toxicology study were associated with cholangitis in the liver and/or common bile duct at exposures approximately 9 to 39 times and 0.5 to 2 times the clinical exposure by AUC at the recommended human dose, respectively. Cholangitis was generally accompanied by hemorrhage and bacterial infection, and two preterm decedents also exhibited slight to moderate pancreatitis (with hemorrhage) at exposures approximately 9 to 39 times the clinical exposure by AUC at the recommended human dose. Cholangitis has not been observed thus far in the clinic. Bacterial colonies were observed in the liver and bile duct of these animals, but not the pancreas. There was a dose-dependent increase in white blood cells, neutrophils, and lymphocytes in rats treated with lenvatinib, consistent with infection and/or inflammation. Elevated serum amylase and lipase levels have been observed clinically in patients treated with lenvatinib; however, there was no clinically significant difference in the incidence of pancreatitis in patients treated with lenvatinib versus placebo. Pancreatitis has been observed in humans treated with the multi-kinase inhibitors sorafenib and pazopanib. Additional findings in the pancreas included edema and fatty necrosis in rats and decreased zymogen granules in rats and monkeys. The observed bacterial infections suggest that sepsis may be a safety concern in humans treated with lenvatinib. In keeping with this, sepsis has been reported as a serious adverse event in Study 303 with lenvatinib.

Consistent with the hepatotoxicity observed in patients given lenvatinib in the clinic, hepatotoxicity was evident in rats and monkeys following lenvatinib administration. Increased ALT and AST were observed in rats and monkeys after 4 weeks of lenvatinib administration at dose levels ≥ 180 mg/m^2 and 360 mg/m^2, respectively, and showed partial evidence of recovery in monkeys; increased ALT and AST have also been observed clinically. In addition to the cholangitis described above, histological findings in the liver included kupffer cell hypertrophy/hyperplasia, hepatocyte pigmentation, and sinusoidal dilation. Hepatocellular necrosis was observed in rats following 4 weeks of lenvatinib administration at dose levels ≥ 30 mg/kg (180 mg/m^2).

Genetic Toxicology

Lenvatinib was not mutagenic in the in vitro Ames Assay or in the in vitro mouse lymphoma TK assay in the presence or absence of metabolic activation. Lenvatinib was not clastogenic in an in vivo rat bone marrow micronucleus assay at dose levels up to 2000 mg/kg. Although lenvatinib was not genotoxic, 5 out of 14 impurities tested in an in vitro Ames assay were determined to have mutagenic potential. Of these, (b) (4) was present in the lenvatinib drug substance and (b) (4) was present in the drug substance and drug product. These genotoxic impurities were determined to be controlled to acceptable levels for the treatment of patients with cancer.

Reproductive and Developmental Toxicology

Fertility/embryonic development studies were not conducted with lenvatinib; however, results from the repeat-dose toxicology studies suggest that administration of lenvatinib has the potential to impair male and female reproductive function and/or fertility.

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Follicular atresia of the ovaries, hypocellularity of the seminiferous epithelium in the testes, and desquamated seminiferous epithelial cells in the epididymides were observed in rats at lenvatinib exposures approximately 9 to 39 times the clinical exposure by AUC at the recommended human dose of 24 mg. Follicular atresia of the ovaries and decreased incidence of menstruation was observed in monkeys at lenvatinib exposures approximately 0.2 to 1 times the clinical exposure by AUC at the recommended human dose. Hypocellularity of the seminiferous epithelium in the testes was observed in monkeys at lenvatinib exposures approximately 4 to 19 times the clinical exposure by AUC at the recommended human dose. Dogs exhibited hypocellularity of the seminiferous epithelium in the testes and desquamated seminiferous epithelial cells in the epididymides at lenvatinib exposures approximately 0.02 to 0.09 times the clinical exposure by AUC at the recommended human dose. Histological findings in the testes and epididymides did not show evidence of recovery in rats and/or monkeys.

The Applicant investigated placental transfer of lenvatinib following a single 3 mg/kg (18 mg/m^2) oral dose of ^{14}C-lenvatinib to rats on Days 13 or 18 of pregnancy. At 30 minutes post-dose, the radioactive concentrations in the placenta, fetal membrane, amniotic fluid, fetus, fetal blood/plasma, and fetal tissues were 25%, ≤ 14%, 0%, ≤ 2%, 2%, and ≤ 4% of that in the maternal plasma, respectively. Although lenvatinib crossed the placental barrier, the percent distribution of radioactivity to each fetus was ≤ 0.02% of dosed radioactivity, indicating relatively low placental transfer of lenvatinib to the fetus. Following a single oral administration of ^{14}C-lenvatinib to lactating rats on PND 9, radioactivity was approximately 1.4 and 2 times higher in milk compared to plasma based on Cmax and AUC, respectively, indicating transfer of lenvatinib into rat milk. Radioactivity in milk and plasma peaked at 1 hr post-dosing and decreased with time. Thus, although it is unknown whether lenvatinib is excreted in human milk, this nonclinical study suggests that administration of lenvatinib to lactating women presents an adverse risk to nursing infants, and women should be advised to avoid breast-feeding while taking lenvatinib. ^{14}C-lenvatinib-related radioactivity in maternal plasma, fetal plasma, the fetus, and milk was primarily attributed to the unchanged parent compound.

An embryofetal development study conducted in rats demonstrated that lenvatinib is embryotoxic, fetotoxic, and has teratogenic potential. Daily oral administration of lenvatinib at dose levels of 0.1, 0.3, and 1 mg/kg to pregnant rats during the period of organogenesis (GD 6-17) resulted in a statistically significant decrease in mean gestational body weight, body weight gain, and food consumption at the 1 mg/kg (6 mg/m^2) dose level compared to controls (≤ 14%, 28%, and 19%, respectively). There was a statistically significant increase in mean post-implantation loss in rats dosed with lenvatinib at the 1 mg/kg dose level (82.2%) compared to controls (3.8%) due to early fetal resorptions, resulting in a statistically significant decrease in the mean number of live fetuses per litter and the number of dams with live fetuses. Toxicokinetic parameters were not assessed in this study; however, the 1 mg/kg (6 mg/m^2) dose level resulted in a mean $AUC_{0\text{-}24\ hr}$ of 9160 ng·hr/mL in non-gravid female rats in a repeat-dose general toxicology study. Maternal toxicity was observed in rats at exposures

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approximately equal to the clinical exposure by AUC at the recommended human dose, and 0.5 times the recommended human dose based on BSA. Treatment with lenvatinib at dose levels ≥ 0.3 mg/kg (1.8 mg/m^2) resulted in a statistically significant decrease in mean fetal body weight compared to controls.

Treatment with lenvatinib resulted in a statistically significant dose-related increase in the number of fetuses with external anomalies and skeletal anomalies/variations compared to controls. Fetal external anomalies were observed at dose levels ≥ 0.3 mg/kg (1.8 mg/m^2; ~0.14 times the recommended human dose based on BSA) and included dose-related parietal edema, cryptophthalmia, and absent or abnormal tails. Since 100% of the live fetuses in the 1 mg/kg dosing group exhibited external anomalies, visceral and skeletal examinations were only performed at dose levels ≤ 0.3 mg/kg. One fetus in the 0.3 mg/kg dosing group exhibited a retroesophageal subclavian artery. Fetal skeletal anomalies and variations were observed at dose levels ≥ 0.1 mg/kg (0.6 mg/m^2, ~0.05 times the recommended human dose of 24 mg based on BSA) and included increased discontinuous rib cartilage, hemicentric thoracic centrum, split cartilage of the thoracic and lumbar centrum, absent lumbar and sacral vertebra, fused/misshapen caudal centrum, increased asymmetrical sternebra, dumbbell-shaped vertebral centrum, 14th rib, and split vertebral centrum. Delayed fetal ossification was also observed at the 0.3 mg/kg (1.8 mg/m^2) dose level. The NOAEL for maternal toxicity was 0.3 mg/kg (1.8 mg/m^2) lenvatinib, and a fetal NOAEL could not be established. Thus, lenvatinib induced fetotoxicity even in the absence of clear maternal toxicity.

Lenvatinib was also embryotoxic and fetotoxic in rabbits. Toxicokinetic parameters were not assessed in this study, so comparisons to human exposure levels could not be made. Daily oral administration of lenvatinib at dose levels of 0.03, 0.1, and 0.5 mg/kg to pregnant rabbits during the period of organogenesis (GD 6-18) resulted in a statistically significant decrease in mean gestational body weight, body weight gain, and food consumption at the 0.5 mg/kg (6 mg/m^2) dose level compared to controls (≤ 6%, 113%, and 47%, respectively), which showed evidence of recovery. Abortion occurred in 7/18 rabbits dosed with lenvatinib at the 0.5 mg/kg (6 mg/m^2) dose level between Days 20 and 22 of pregnancy. There was a statistically significant increase in mean post-implantation loss in rabbits dosed with lenvatinib at the 0.5 mg/kg dose level (94.3%) compared to controls (3.6%) due to early fetal resorptions, resulting in a statistically significant decrease in the mean number of live fetuses per litter for rabbits dosed with 0.5 mg/kg (5 fetuses) compared to controls (122 fetuses). Milder increases in post-implantation loss including dead fetuses (1 and 3 dead fetuses at the 0.03 and 0.1 mg/kg dose levels, respectively) occurred at dose levels of ≥ 0.03 mg/kg (0.36 mg/m^2). Ten out of 11 females dosed with lenvatinib at the 6 mg/m^2 dose level exhibited complete resorption of litters. A fetal visceral anomaly (retroesophageal subclavian artery) was observed at dose levels ≥ 0.03 mg/kg (0.36 mg/m^2), and one of the 5 live fetuses in the 0.5 mg/kg dose group exhibited a short tail. Fetal skeletal anomalies were observed at dose levels ≥ 0.1 mg/kg (1.2 mg/m^2), including a dose-related increase in fused ribs. One fetus in the 0.5 mg/kg dosing group exhibited thoracic hemivertebra and a misshapen lumbar arch. The NOAEL for maternal toxicity was 0.1 mg/kg (1.2 mg/m^2) lenvatinib. A fetal NOAEL could not be established. Thus, fetotoxicity was observed in

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animals at doses ≤ 0.03 times the recommended human dose based on BSA. The Applicant did not classify any of the findings in the rat or rabbit embryofetal studies as malformations. Overall, many of the lenvatinib-induced adverse effects on embryofetal development are likely due to pharmacologic inhibition of VEGF. A warning for embryofetal toxicity is recommended for the label. Based on a half-life of lenvatinib of ~20-34 hours, it is advised that females of reproductive potential use effective contraception during treatment with lenvatinib and for at least 2 weeks following cessation of treatment.

In a GLP-compliant juvenile toxicology study, lenvatinib was orally administered once daily to Sprague Dawley rats for 8 weeks starting on PND 21 (approximately equal to a human pediatric age of 2 years) at dose levels of 0.4, 2, or 10 mg/kg. Target organs of lenvatinib-induced toxicity were generally similar to those observed in adult rats and included the incisors, femur, tibia, sternum, femoral and sternal bone marrow, kidney, GI tract, adrenals, liver, common bile duct, heart, thymus, spleen, pancreas, and choroid plexus. Additional lenvatinib target organs in juvenile rats included the tibia and lungs. Similar to adult rats, many of the lenvatinib-induced findings were attributable to the primary pharmacology of VEGF inhibition. Slight thrombosis and myocardial hypertrophy in the heart and alveolar hemorrhage in the lungs were observed in juveniles, but not adults, at the 10 mg/kg dose level. Lenvatinib drug exposures were generally dose proportional in juveniles, and there was slight accumulation on Day 56 compared to Day 1 based on Cmax in females dosed with 0.4 mg/kg and 2 mg/kg lenvatinib. Mean lenvatinib exposure based on AUC was generally lower in juvenile rats than that seen at the same dose levels in adult rats.

Lenvatinib was severely toxic in juvenile rats at the 10 mg/kg dose level (approximately 6 to 24 times the clinical exposure by AUC at the recommended human dose), resulting in 13 mortalities attributed to primary duodenal lesions and associated GI complications. Treatment-related mortality was not observed in adult rats dosed with 10 mg/kg lenvatinib for 13 weeks, and mortality occurred much earlier in juvenile rats (Days 26 to 51) compared to adult rats dosed with 10 mg/kg lenvatinib in the 26-week toxicology study (Days 84 to 147). Thus, juveniles appeared to be more sensitive to lenvatinib-induced toxicity. The juvenile preterm decedents dosed with 10 mg/kg lenvatinib exhibited diarrhea, decreased body weight and food consumption, broken teeth, loss of locomotor activity, decreased respiration, and/or nasal hemorrhage. They also exhibited increased hemoglobin, hematocrit, neutrophils, AST, ALT, alkaline phosphatase, BUN, and cholesterol, and decreased platelets and reticulocytes. The LOAEL in this study was 0.4 mg/kg (2.4 mg/m^2) lenvatinib.

Lenvatinib-induced clinical signs of GI toxicity (diarrhea, soiled perineal region, and blackish stool) showed partial evidence of recovery following a 4-week recovery period. Proteinuria was observed in rats dosed with lenvatinib at the 10 mg/kg dose level, indicating renal toxicity; two out of 10 rats still exhibited proteinuria at the end of the recovery period. Histological findings observed following 4 weeks of recovery included distal remnants of incisor dysplasia, epiphyseal growth plate remodeling/regeneration in the femur, glomerulopathy, residual focal necrosis in the adrenals, mineralization in the

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stomach, residual interstitial fibrosis in the duodenum, hemorrhage in the rectum, atrophy in the testes, decreased spermatozoa in the epididymides, and mineralization of the meninges.

Treatment with lenvatinib at dose levels ≥ 2 mg/kg (approximately 1.2 to 5 times the clinical exposure by AUC at the recommended human dose) resulted in severe growth retardation characterized by significantly decreased body weight and food consumption; decreased food consumption showed partial evidence of recovery. Growth retardation was further characterized by a dose-dependent and statistically significant decrease in the width and/or length of the femur and tibia at dose levels ≥ 2 mg/kg, delayed physical development (prepuce separation and vaginal opening) at the 10 mg/kg dose level, and sexual immaturity of the testes, epididymides, seminal vesicles, and ovaries. Decreased length of the femur and tibia persisted following the 4-week recovery period. Lenvatinib did not significantly affect functional development as measured by pinna, pupillary, corneal, auditory, and deep pain reflexes. In the open field test, treatment with lenvatinib at the 10 mg/kg dose level resulted in a statistically significant delay in latency and decreased ambulation compared to controls. Decreased rearing was also observed in rats dosed with lenvatinib at dose levels ≥ 2 mg/kg; these changes showed partial evidence of recovery. Potential evidence of delayed learning indicated by longer navigation times through a water maze was also observed in males treated with lenvatinib at the 2 mg/kg dose level; animals treated at the 10 mg/kg dose level were not assessed. Overall, these data suggest that administration of lenvatinib to pediatric patients may result in growth retardation and delayed development. Pediatric patients with active growth plates are particularly at risk for adverse bone effects. Based on its mechanism of action, lenvatinib may also have severe effects on organ growth and maturation during the early postnatal development. Daily administration of lenvatinib to rats from PND 7 to PND 21 resulted in mortality at dose levels ≥ 1 mg/kg (6 mg/m^2; approximately 0.8 to 3.5 times the clinical exposure by AUC at the recommended human dose), indicating that lenvatinib administration may be severely toxic to pediatric patients less than two years old.

References

McTigue M, BM Murray, JH Chen, YL Deng, J Slowiei, and RS Kania, 2012, Molecular conformations, interactions, and properties associated with drug efficiency and clinicalperformance among VEGFR TK inhibitors, Proc Natl Acad Sci USA, 109:18281-9.

12 Appendix/Attachments

None

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

/s/

EMILY M FOX
01/13/2015

STEPHANIE L AUNGST
01/13/2015

WHITNEY S HELMS
01/13/2015

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 206947 **Applicant: Eisai Inc.** **Stamp Date: August 14, 2014**

Drug Name: Lenvatinib **NDA Type: 505(b)(1); Type 1- New Molecular Entity**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		The Applicant's proposed labeling will be reviewed during the NDA review.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		Potential impurity issues and acceptability of the Applicant's proposed specifications will be determined during the NDA review.
11	Has the applicant addressed any abuse potential issues in the submission?			Not Applicable
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not Applicable

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? __Yes__

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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

/s/

EMILY M FOX
09/25/2014

WHITNEY S HELMS
09/25/2014