

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**201532**

**PHARMACOLOGY REVIEW(S)**

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

**ADDENDUM TO SECONDARY PHARMACOLOGY/TOXICOLOGY  
NDA REVIEW AND EVALUATION**

Application number: NDA 201,532  
Supporting document/s: 1  
Applicant's letter date: March 30, 2010  
CDER stamp date: March 30, 2010  
Product: Eribulin mesylate  
Indication: Patients with breast cancer who have failed at least two chemotherapeutic regimens for locally advanced or metastatic disease.  
Applicant: Eisai Inc.  
300 Tice Blvd  
Woodcliff Lake, NJ 07677  
Review Division: Division of Biologic Oncology Products  
Reviewer: Lori E. Kotch, PhD, DABT  
Supervisor/Team Leader: **Anne M. Pilaro, PhD**  
Division Director: Patricia Keegan, MD  
Project Manager: Vaishali Jarral

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**MEMORANDUM**

**TO:** The file  
**CC:** Patricia Keegan, M.D., Director, Division of Biologic Oncology Products, Office of Oncology Drug Products (OODP), Center for Drug Evaluation and Research (CDER)

John Leighton, Ph.D., D.A.B.T., Associate Director for Pharmacology and Toxicology, OODP, CDER

**FROM:** Anne M Pilaro, Ph.D., Supervisory Toxicologist, Pharmacology/Toxicology Branch, Division of Biologic Oncology Products, OODP, CDER

**NDA #:** 201532  
**SPONSOR:** Eisai Inc.  
**PRODUCT:** Halaven™ (eribulin mesylate; E7389)  
**SUBMISSION TYPE:** original NDA application  
**DATE:** November 2, 2010

**SYNOPSIS:**

The purpose of this addendum to the original secondary nonclinical review for NDA #201532 is to document the rationale for including specific language regarding the nonclinical findings with eribulin mesylate in the final labeling for Eisai Inc.'s Halaven™ product. Following discussion of the primary review and the labeling language with the Associate Director for Pharmacology and Toxicology for the Office of Oncology Drug Products, the nonclinical discipline was requested to provide support for the use of the term "abortion" to describe the findings in the rat embryo-fetal development (EFD) study, and for basing the dose comparisons between the test animals and humans on dose body surface area, rather than scaling by exposure (i.e. area under the concentration/time curve [AUC]).

The nonclinical data in support of Halaven™ for the proposed indication were reviewed by the primary reviewer, Lori Kotch, Ph.D., D.A.B.T. In the "Key Study Findings" section of her review of the EFD study of eribulin mesylate in rats (Study # LFA00033), she reports that "*Early delivery* (emphasis added), adverse clinical signs and/or enlarged spleen were observed in dams at 0.15 mg/kg." In the body of the text of her review, Dr. Kotch documents reports in the section on maternal toxicity:

*" Maternal Observations:*

*In the 0.15 mg/kg group:*

*One dam (#7885) in the 0.15 mg/kg group delivered and was sacrificed on GD 21. This early delivery may have been related to the test article because it occurred at the highest dosage tested, and 69% of the litter was resorbed" (emphasis added).*

In my secondary review and in the labeling for Halaven™, these findings are reported as "Increased abortion..." at the highest dose level, which was approximately 0.64 times the recommended clinical dose. As per discussion with Dr. Kotch, a spontaneous abortion describes the loss of the products of conception, which are typically resorptions, dead fetuses or live fetuses (i.e. lungs are assessed to determine if fetus

took a breath = liveborn). She also confirmed that resorptions are considered to be conception products that have undergone some degree of autolysis, so not all dead fetuses are considered resorptions. Further discussion of the findings with Dr. Kotch verified that the “early delivery” reported in the sponsor’s final study report for this dam was in fact a total loss of the litter of 16 conceptuses, with 9 early resorptions and one late resorption *in utero*, and expulsion of 5 dead pups on GD21. So, in this particular case, only 69% of conception products were reported to be resorptions. Therefore, these findings support the use of the term “abortion” in both the secondary review and the labeling, as the term describes a premature expulsion of the products of conception (i.e. early delivery, but of dead rather than live but premature fetuses).

Regarding the language used in the final labeling to report the doses in the rat EFD toxicity study that resulted in the fetal losses and skeletal and soft tissue anomalies as compared to the recommended human dose, current OODP policy is to report the dose comparisons as multiples of exposure (i.e. AUC or maximum plasma concentration [ $C_{max}$ ]). However, these calculations were not possible given the design of the study, the levels and schedule of dosing, and that an error in dosing formulation resulted in the rats receiving approximately 30% lower doses than the target dose in the early portion of the study. Specifically, pregnant rats in the embryo-fetal development study received eribulin mesylate every other day during organogenesis (presumed GD8, GD10 and GD12) for a total of 3 doses, and no measurements of toxicokinetic (TK) profiles were incorporated into the study design. Extrapolation of exposure of the pregnant animals from data obtained in general toxicity studies using healthy, non-pregnant female rats was not possible either, due to differences in both schedule and dose levels tested. The doses tested in the EFD study were 0.01, 0.03, 0.1 and 0.15 mg/kg/dose given every other day for 3 doses during organogenesis; while the general toxicity studies gave weekly doses x 3 (with 14 day rest) at doses of 0.1, 0.2, or 0.25 mg/kg/dose for a single cycle, or repeated for multiple cycles at the same schedule for 6 months at doses of 0.015, 0.05, or 0.15 mg/kg/dose. Given the differences in schedules and dose levels, it was not possible or appropriate to extrapolate the TK data from the general toxicity study to the estimate potential maternal exposures in the EFD study. Therefore, following discussions with Dr. Kotch I recommended that she base the dose comparison on the actual doses administered to rats, after a conversion of the animal doses to the corresponding human equivalent dose in  $mg/m^2$ , and compare those levels to the recommended human dose in the labeled indication.

**Recommendation:** I concur with Dr. Kotch’s conclusions regarding the nonclinical findings for Halaven™ in the EFD studies, the reporting of the findings of early delivery as “abortion” in the prescribing information, and reporting the dose comparisons on a  $mg/m^2$  basis, rather than by comparison of the multiples of the AUC obtained in animals versus humans. The present document serves as documentation of the rationale for these two approaches, and no additional revision of Dr. Kotch’s review is required.

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/s/  
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M S RICCI on behalf of ANNE M PILARO  
11/08/2010  
Uploaded and signed at the request of Dr. Pilaro

## MEMORANDUM

Halaven (eribulin mesylate)

**Date:** November 7, 2010

**To:** File for NDA 201532

**From:** John K. Leighton, PhD, DABT

Associate Director for Pharmacology/Toxicology

Office of Oncology Drug Products

I have examined pharmacology/toxicology supporting review and labeling provided by Dr. Kotch and supervisory memorandum and addendum provided by Dr. Pilaro. I concur with their conclusions that Halaven may be approved for the proposed indication and that no additional pharmacology/toxicology studies are needed.

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/s/  
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JOHN K LEIGHTON  
11/06/2010

**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: NDA 201,532  
Supporting document/s: 1  
Applicant's letter date: March 30, 2010  
CDER stamp date: March 30, 2010  
Product: Eribulin mesylate  
Indication: Patients with breast cancer who have failed at least two chemotherapeutic regimens for locally advanced or metastatic disease.  
Applicant: Eisai Inc.  
300 Tice Blvd  
Woodcliff Lake, NJ 07677  
Review Division: Division of Biologic Oncology Products  
Reviewer: Lori E. Kotch, PhD, DABT  
Supervisor/Team Leader: **Anne M. Pilaro, PhD**  
Division Director: Patricia Keegan, MD  
Project Manager: Vaishali Jarral

*Template Version: December 7, 2009*

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**MEMORANDUM**

**TO:** The file  
**CC:** Patricia Keegan, M.D., Director, Division of Biologic Oncology Products, Office of Oncology Drug Products (OODP), Center for Drug Evaluation and Research (CDER)  
**FROM:** Anne M Pilaro, Ph.D., Supervisory Toxicologist, Pharmacology/Toxicology Branch, Division of Biologic Oncology Products, OODP, CDER  
**NDA #:** 201532  
**SPONSOR:** Eisai Inc.  
**PRODUCT:** Halaven™ (eribulin mesylate; E7389)  
**SUBMISSION TYPE:** original NDA application  
**DATE:** October 15, 2010

**SYNOPSIS:**

Eisai Inc. has submitted an original NDA application for their synthetic, non-taxane microtubule inhibitor, eribulin mesylate (Halaven™) for the third-line treatment of patients with locally advanced or metastatic breast cancer. Halaven™ is indicated "...for the treatment of patients with metastatic breast cancer who have previously received an anthracycline and a taxane, and at least two chemotherapeutic regimens for the treatment of metastatic disease."<sup>1</sup> Nonclinical studies investigating the pharmacology, pharmacokinetics and toxicology of eribulin mesylate in rats and dogs were submitted with the new drug application (NDA) in support of the safety of Halaven™. In the Highlights section of the label, Halaven™ is defined as a "microtubule inhibitor" for its pharmacologic class, and consistent with other microtubule inhibitors including paclitaxel and docetaxel, Halaven™ acts by inhibiting tubulin polymerization and microtubule dynamics. This inhibition results in interference with mitotic spindle formation, leading to G<sub>2</sub>/M cell cycle arrest, induction of apoptosis, and cell death. In a large scale study in patients with metastatic breast cancer who had received at least two prior chemotherapy regimens (including an anthracycline and a taxane), Halaven™ treatment prolonged overall survival by approximately 2.5 months, as compared to the control cohort of patients treated with a single chemotherapeutic agent chosen by their physician (hazard ratio 0.81 [95% confidence intervals 0.66, 0.99]).

The nonclinical data in support of Halaven™ for the proposed indication were reviewed by the primary reviewer, Lori Kotch, Ph.D. D.A.B.T., and are briefly summarized in the "Executive Summary" and "Integrated Summary and Safety Evaluation" sections of her review. Pharmacology, safety pharmacology, pharmacokinetic evaluations and toxicology studies supporting the NDA for Halaven™ were conducted *in vitro* and *in vivo*. Anti-tumor activity was assessed using *in vitro* tumor cell cultures, and *in vivo* in xenograft models with human tumor cells including MDA-MB-435 breast carcinoma. The *in vitro* and *in vivo* pharmacodynamic and anti-tumor effects of eribulin mesylate were consistent with those observed with other microtubule inhibitors. Mechanistic studies in human tumor cells cultured in the presence of eribulin mesylate showed inhibition of the growth phase of microtubules without affecting the shortening phase,

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<sup>1</sup>from the current, draft labeling language for Halaven™

and sequestration of tubulin into nonproductive aggregates with resulting G<sub>2</sub>/M cell-cycle block, disruption of mitotic spindles, and ultimately, apoptotic cell death after prolonged mitotic blockade. Cardiac safety pharmacology in HEK293 cells did not show any inhibition of HERG tail current, nor were any adverse effects on cardiac action potentials detected in isolated dog Purkinje fibers after co-incubation with eribulin mesylate. However, transient decreases in heart rate, mean arterial and systolic blood pressures, and RR interval on ECG evaluation were observed in conscious dogs following a single dose of 0.8 mg/m<sup>2</sup> eribulin mesylate (approximately 0.57-fold the human dose, when scaled by body surface area).

*In vivo* safety was determined in 1 or 6-cycle (Q weekly dosing x 3, with 14 day recovery/cycle) repeat-dose toxicity studies in beagle dogs and Sprague-Dawley rats. Toxicities associated with eribulin mesylate treatment of rats and dogs were consistent between the two species and included dose-dependent, marked to severe bone marrow hypoplasia and subsequent decreases in hematologic cell populations, atrophy of the lymphoid organs including lymph nodes and thymus, non-reversible testicular degeneration in rat and dog (at 0.21-fold and 0.64-fold the proposed clinical dose), and liver necrosis and inhibition of function (elevated ALT and AST, and cholesterol levels). At doses of eribulin mesylate greater than or equal to 1.2 mg/m<sup>2</sup>, mortalities were noted in the 29-day (1-cycle) rat study (i.e. LD<sub>20</sub>), and at all dose levels tested in the rat chronic study (STD<sub>10</sub> < 0.09 mg/m<sup>2</sup>). No lethality was observed in the 29-day or chronic dog studies, at doses up to 0.045 mg/kg (0.9 mg/m<sup>2</sup>). The testicular and hematologic findings in both species were considered related to the pharmacologic activity of eribulin mesylate, and are consistent with the toxicity profiles of other approved microtubule inhibitors.

No carcinogenicity testing was performed or considered necessary for approval of eribulin mesylate, since the indicated population is for patients with metastatic or locally advanced breast cancer that have already progressed after treatment with at least two chemotherapeutic regimens, including an anthracycline and a taxane. Eribulin mesylate was positive for genotoxicity in both the *in vitro* mouse 5178Y/TK lymphoma and *in vivo* rat micronucleus assays, as expected from its mechanism of action as a microtubule inhibitor. However, using two different lots #BLDR001 (purity 96.2%) and #194P1404 (purity 92.4%), eribulin mesylate was negative in the bacterial reverse mutation (Ames) assay. Negative findings are expected in these two Ames assays based on the mechanism of action of eribulin mesylate as a microtubule inhibitor. Additionally, these negative findings confirm that any impurities present in eribulin mesylate, or any metabolites of the parent compound generated in these assays via incubation with rat microsomal cytochrome p450 enzymes do not have the potential to cause genetic toxicity through direct interaction with DNA.

Embryofetal developmental toxicity studies conducted with eribulin mesylate do not support the safe use of Halaven™ during pregnancy. Soft tissue and skeletal malformations were observed in offspring from pregnant rats treated during organogenesis on Gestation Days 8, 10 and 12 with intravenous doses of eribulin mesylate that were approximately 0.04, 0.12, 0.42 and 0.64 times the recommended human dose, based on body surface area (mg/m<sup>2</sup>). Increased abortion and severe



Specified impurity	Proposed Acceptance Criteria (NMT)	Impurity levels in Lot 194P1404 <sup>1</sup> (used in high impurity study)	<sup>1</sup> Rat MTD is 1.2mg/m <sup>2</sup> (Proposed clinical dose 1.16X higher)	Adjusted for purity (92.4%)	Impurity levels in Batches BLDR001 <sup>2</sup> (used in pivotal studies)	<sup>2</sup> Rat MTD is 0.6mg/m <sup>2</sup> (Proposed clinical dose 2.33X higher)	Adjusted for purity (96.2%)	Impurity levels in Clinical Batch BLD003 (purity is 99.6% )
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(b) (4)

**\* Acceptance criteria for this impurity exceed qualification level.**

<sup>1</sup> A rat bridging toxicology study was conducted using a drug substance with higher impurity levels (Lot #194P1404, purity 92.4%). A dose of 1.2 mg/m<sup>2</sup> is considered the MTD dose in the rat (species used for these studies).

<sup>2</sup> A dose of 0.6mg/m<sup>2</sup> is considered the MTD (non-lethal) dose in most appropriate species (rat) in pivotal studies using lot BLDR001 (96.2% purity).

<sup>3</sup> BRL = below reportable limit (i.e. (b) (4))

**Bolded** – Represents maximum qualified level.

By Dr. Kotch’s calculations, two impurities (i.e. (b) (4)) have not been appropriately qualified by the nonclinical testing. I have recalculated the levels of these two impurities that would be expected in the labeled dose of Halaven™ of 1.4 mg/m<sup>2</sup>, if the manufactured lot of drug substance was at the maximal specifications. I then compared these levels to the maximum dose of each impurity present in the nonclinical lot #19P1401 of eribulin mesylate (the batch with the highest impurity levels) used in the 1- month rat bridging toxicology study conducted with this batch, and to the

levels in clinical lot #BRD003 used in the early clinical trials.

At the maximum specifications, a human dose of Halaven™ would contain approximately (b) (4)

By contrast, at the highest dose tested in the rat study, the animals received the equivalent dose of (b) (4)

or approximately 4.5-fold less than the level anticipated to be present in the marketed product at maximum specification. The corresponding levels of these two impurities present in clinical batch #BRD003 used in the early clinical trials are (b) (4)

Therefore, the safety of these two impurities has not been appropriately qualified by either animal or human testing, and I concur with Dr. Kotch's recommendations to request that Eisai decrease the acceptance criteria for each of these two impurities to levels that have been qualified in the animal testing.

**Recommendation:** I concur with Dr. Kotch's conclusions regarding the nonclinical findings for Halaven™, her current recommendation that the licensing application be approved for marketing, and her recommendations regarding the language for the prescribing information. Additionally, I concur with her recommendation that the maximum acceptance levels for impurities (b) (4) be decreased to levels which have been appropriately qualified by animal testing, and her language to convey this recommendation to the sponsor. A copy of Dr. Kotch's review, with supervisory sign-off, has been conveyed to the regulatory project manager for inclusion in the final action package, and has been uploaded into the DARRTS database.

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/s/

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ANNE M PILARO  
10/22/2010

**DEPARTMENT OF HEALTH AND HUMAN SERVICES  
PUBLIC HEALTH SERVICE  
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# 1 Executive Summary

## 1.1 Recommendations

### 1.1.1 Approvability

The nonclinical data used to support this NDA were sufficient to determine the pharmacologic activity of eribulin mesylate, and provided a comprehensive toxicity profile in rats and a partial toxicity profile in dogs. These also data enabled an adequate assessment of genetic and reproductive/developmental toxicity, qualification of drug substance/product and a partial identification of potential clinical toxicities. The safety and efficacy of the proposed administration of eribulin mesylate is further supported by submitted clinical data.

- There are no Pharmacology/Toxicology issues which preclude approval of the product for the requested indication.
- Recommendations on labeling have been provided within the team meetings.

### 1.1.2 Additional Non Clinical Recommendations

Based on the levels of impurities that were qualified in the nonclinical testing, Eisai should adjust the acceptance criteria for the drug substance and drug product to levels that do not exceed qualification values listed in section 2.3.2

- Drug Substance – The acceptance criteria for (b) (4) should be adjusted to (b) (4). (b) (4) should be adjusted to (b) (4).
- Drug Product – The acceptance criteria for (b) (4) should be adjusted to NMT (b) (4).

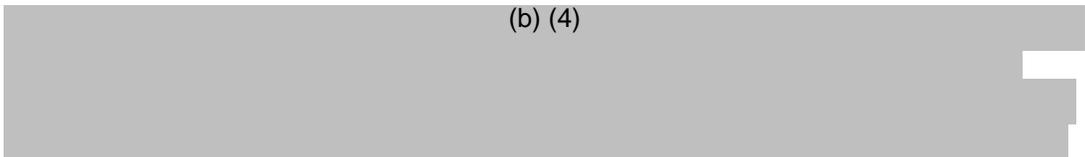
This recommendation was relayed to ONDQA on August 25, 2010, in the form of letter-ready comments to be sent to the sponsor.

### 1.1.3 Labeling – Nonclinical sections 5.3, 8.1, 8.3 and 13.1 are reviewed below.

#### 1. *Sponsor's Proposed Label:*

#### 5.3 Pregnancy - Pregnancy Category D

(b) (4)



(b) (4)

[Redacted]

**FDA Recommended Changes:**

5.3 Use in Pregnancy: There are no adequate and well-controlled studies with Halaven in pregnant women. Halaven is a microtubule inhibitor; therefore, it is expected to cause fetal harm when administered to a pregnant woman. Embryofetal toxicity and teratogenicity occurred in animals that received eribulin mesylate at approximately half of the recommended human dose, based on body surface area. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus [see *Use in Specific Populations (8.1)*]

5.4 Use in Women of Childbearing Potential

Advise women of childbearing potential to avoid becoming pregnant and to use effective contraception during treatment with Halaven [see *Use in Specific Populations (8.1)*].

**2. Sponsor Proposed Label:**

(b) (4)

[Redacted]

**FDA Recommended Changes:**

**8.1** Pregnancy Category D [see *Warnings and Precautions (5.2)*]

There are no adequate and well-controlled studies with Halaven in pregnant women. Halaven is a microtubule inhibitor; therefore, it is expected to cause fetal harm when administered to a pregnant woman. Embryofetal toxicity and teratogenicity occurred in animals that received Halaven at approximately half of the recommended human dose, based on body surface area. If this drug is used during pregnancy, or if the

patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus.

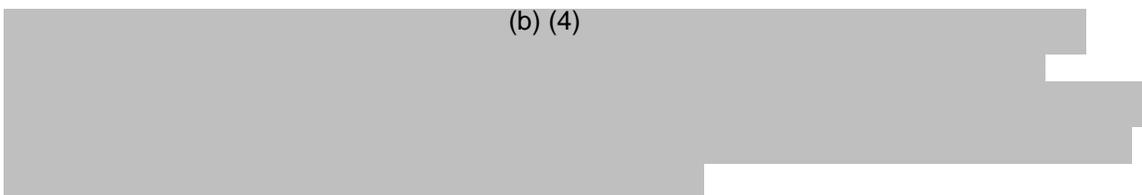
In a developmental toxicity study, pregnant rats received intravenous infusion of eribulin mesylate during organogenesis (Gestation Days 8, 10, and 12) at doses approximately 0.04, 0.12, 0.42 and 0.64 times the recommended human dose, based on body surface area ( $\text{mg}/\text{m}^2$ ). Increased abortion and severe external or soft tissue malformations were observed in offspring at doses 0.64 times the recommended human dose based on body surface area ( $\text{mg}/\text{m}^2$ ), including the absence of a lower jaw, absence of a tongue, absence of stomach and absence of spleen. Increased embryo fetal death/resorption, reduced fetal weights and minor skeletal anomalies consistent with developmental delay were also reported at or above doses of 0.42 times the recommended human dose.

Maternal toxicity of eribulin mesylate was reported in rats at or above doses of 0.42 times the recommended human dose ( $\text{mg}/\text{m}^2$ ), and included enlarged spleen, reduced maternal weight gain and decreased food consumption.

### **3. Sponsor's Proposed Label:**

#### **8.3 Nursing Mothers**

(b) (4)



#### **FDA Recommended Changes:**

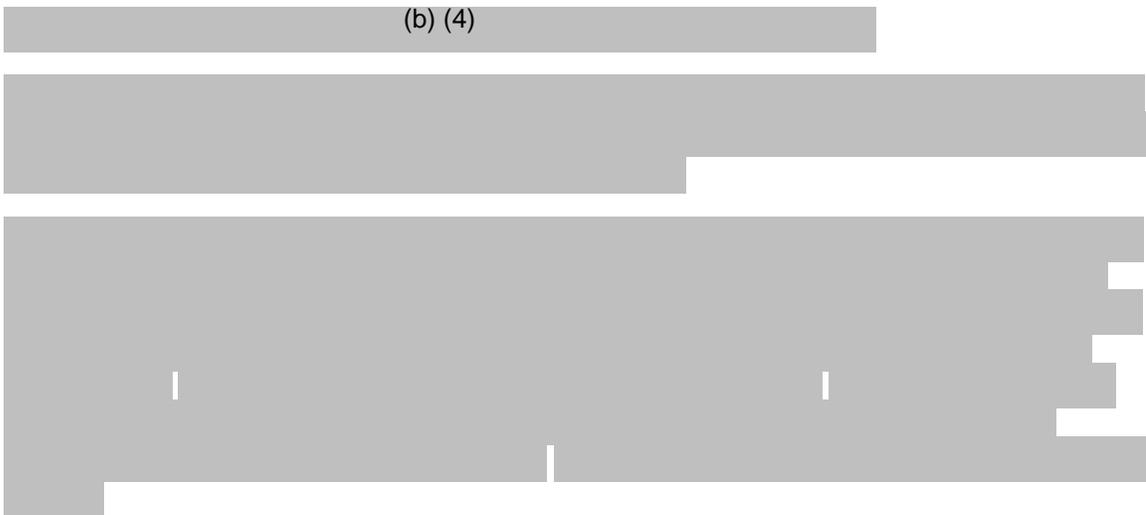
#### **8.2 Nursing Mothers**

It is not known whether Halaven is excreted into human milk. No studies in humans or animals were conducted to determine if Halaven is excreted into milk. Because many drugs are excreted into human milk and because of the potential for serious adverse reactions in human milk-fed infants from Halaven, a decision should be made whether to discontinue nursing or to discontinue Halaven taking into account the importance of the drug to the mother.

**4. Sponsor's Proposed Label:**

## 13.1 Carcinogenesis, mutagenesis, impairment of fertility

(b) (4)

A large section of the document is redacted with a grey box. The redaction covers the majority of the text under section 13.1, leaving only the section header and the 'FDA Recommended Changes' section visible.**FDA Recommended Changes:**

## 13.1 Carcinogenesis, mutagenesis, impairment of fertility

Carcinogenicity studies have not been conducted with eribulin mesylate.

Eribulin mesylate was not mutagenic in an *in vitro* bacterial reverse mutation assay (Ames test). Eribulin mesylate was positive in mouse lymphoma mutagenesis assays, and was clastogenic in an *in vivo* rat bone marrow micronucleus assay. The effects of eribulin mesylate on human fertility are unknown. Fertility studies have not been conducted with eribulin mesylate in humans or animals. However, nonclinical findings in repeated-dose dog and rat toxicology studies suggest that male fertility may be compromised by treatment with eribulin mesylate. Rats exhibited testicular toxicity (hypocellularity of seminiferous epithelium with hypospermia/aspermia) following dosing with eribulin mesylate at or above 0.42 times the recommended human dose ( $\text{mg}/\text{m}^2$ ) given once weekly for 3 weeks, or at or above 0.21 times the recommended human dose ( $\text{mg}/\text{m}^2$ ) given once weekly for 3 out of 5 weeks, repeated for 6 cycles. Testicular toxicity was also observed in dogs given 0.64 times the recommended human dose ( $\text{mg}/\text{m}^2$ ) weekly for 3 out of 5 weeks, repeated for 6 cycles.

## 1.2 Brief Discussion of Nonclinical Findings

Eribulin Mesylate (E7389) is an analogue of halichondrin B, isolated from the marine sponge *Halichondria okadai*. At effective concentrations eribulin mesylate inhibits tubulin polymerization and microtubule dynamics, thus interfering with normal mitotic spindle formation. Eribulin mesylate treatment induces G<sub>2</sub>/M cell cycle arrest, and ultimately cell death via apoptosis, after prolonged blockage of cells in mitosis. In *in vitro* studies, eribulin mesylate inhibits cell growth at sub- to low-nmol/L half-maximal inhibitory concentration (IC<sub>50</sub>) values in a wide range of established human cancer cell lines, including MDA-MB-435 breast cancer

In *safety pharmacology assessments*, treatment with 30 µM/L E7389 produced no inhibition of HERG tail current in HEK293 cells. Treatment with E7389 at concentrations up to 30µM showed no effect on cardiac action potentials in isolated dog Purkinje fibers. Infusion of intravenous E7389 for 60 min, at 0.01 or 0.04 mg/kg, transiently decreased systolic blood pressure (SBP), Diastolic blood pressure (DBP), Mean arterial pressure (MAP), and Heart rate (HR) and increased the RR interval in male and female dogs, with the effects more pronounced in males. Axonopathy of the sciatic and dorsal root ganglia was reported in mice following IV dosing three times weekly for two weeks at dose levels of ≥1.31mg/kg, although no effects of E7389 on nerve conduction velocity (NCV) or amplitude in caudal and digital nerves were noted in this same study at doses up to 1.75mg/kg/dose. Intravenous administration of E7389 to rats at 0.1 or 0.25mg/kg produced no effects on respiratory or CNS function.

*Pharmacokinetic studies* indicated that drug exposure increased with dose in rodents and dogs, and that the volume of distribution was high in all species. The high V<sub>ss</sub> correlated with a low level of observed protein binding. Species differences in protein binding of E7389 were apparent, with the highest measured in human plasma and the least in dog plasma. Clearance appeared to be moderate in all species, with a prolonged t<sub>1/2</sub>. CYP3A4 appears to be the major enzyme responsible for the metabolism of E7389 in humans, as indicated *in vitro*. After a single intravenous dose of [<sup>14</sup>C]E-814058 (acetate salt) to rats and dogs, the parent compound was the single major component observed in plasma, urine and feces. [<sup>14</sup>C]E-814058 was mainly eliminated through fecal excretion, with only 8-15% of dosed radioactivity excreted in urine of dogs and rats.

In *repeat-dose toxicity studies*, E7389 was administered intravenously to rats (slow bolus) and dogs (1-hr infusion). The primary targets of toxicity in both species were the hematopoietic organs (bone marrow, thymic and lymphoid tissue atrophy), the testes (degeneration) and the liver (necrosis, elevated AST, ALT and cholesterol); with sciatic nerve (degeneration) also noted in rats. Non-recoverable testicular effects in rat and dog (at 0.21X and 0.64X the proposed clinical dose, respectively) indicate potential for compromised male fertility. Hematologic changes related to the pharmacologic effect on bone marrow were also noted. Doses ≥1.2mg/m<sup>2</sup> (LD<sub>20</sub>) were lethal in the 29-day rat study, as were all doses administered in the rat chronic study (STD<sub>10</sub> < 0.09mg/m<sup>2</sup>). No lethality was observed in the 29-day or chronic dog studies, at doses up to 0.045mg/kg. The acute (end-of-treatment) effects of E7389 were not assessed in the 29-day dog

study, or chronic dog and rat studies, since all animals in these studies were terminated at the end of a recovery period. Accordingly, the toxicity profiles for these studies are incomplete, and the toxicities noted in these reports represent only those toxicities which persisted throughout the recovery period.

A bridging toxicology study was conducted with E7389 test article containing high impurity content (Lot No. 194P1404). Rats were administered IV doses of 0.10 or 0.20 mg/kg using the same dosing regimen in the 29-day repeat-dose rat toxicology study. The primary findings observed in this study were qualitatively similar to those observed in the rat 29-day repeat-dose toxicity study, and included bone marrow hypocellularity, thymic atrophy and testicular hypocellularity. As such, this lot will be used to qualify impurities, in addition to the toxicology lot BLDR001 used in pivotal studies.

In *genetic toxicology studies*, E7389 was found to be weakly positive in a 5178Y/TK Mouse Lymphoma Mutagenesis assay conducted using the toxicology lot (lot BLDR001), and clearly positive in a 5178Y/TK Mouse Lymphoma Mutagenesis assay conducted using a high impurity containing drug lot (lot 194P1404); indicating gene mutation/chromosomal damage and associated functional loss. E7389 was also strongly positive in the *in vivo* rat micronucleus assay, indicating its potential for induction of chromosome damage.

In an *embryofetal toxicity study*, pregnant rats received intravenous infusion of 0.06, 0.18, 0.60 or 0.90 mg/m<sup>2</sup> eribulin mesylate on gestation days 8, 10, and 12. Increased abortion and severe external or soft tissue malformations were observed in offspring at 0.90mg/m<sup>2</sup> (0.64 times the recommended human dose), and increased embryo fetal death/resorption and reduced fetal weights were reported at 0.60mg/m<sup>2</sup> (≥0.42 times the recommended human dose).

## 2 Drug Information

### 2.1 Drug –

Eribulin mesylate (Tradename – Halaven)

#### 2.1.1 CAS Registry Number (Optional)

441045-17-6 (methanesulfonate)  
253128-41-5 (free base)

#### 2.1.2 Generic Name

Eribulin mesylate, Halichondrin B analog

## 2.1.3 Code Name

E7389, NSC-707389, ER-086526, ER-086526/B1939, BOLD

## 2.1.4 Chemical Name

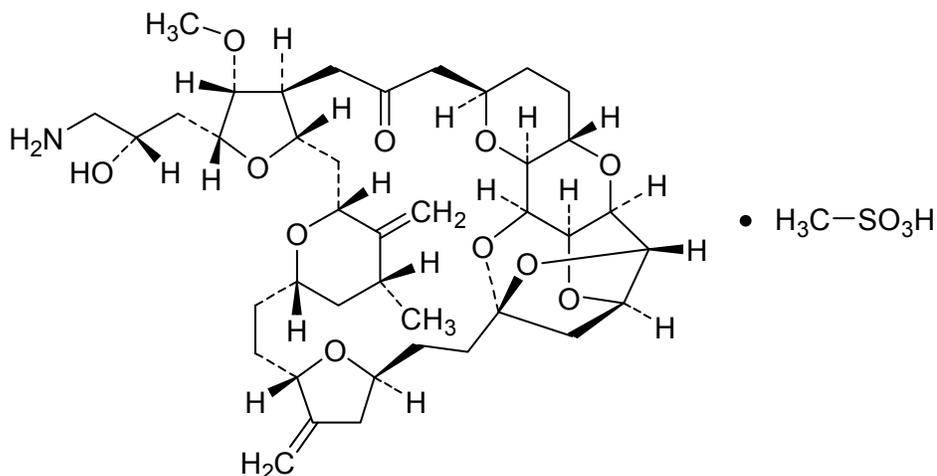
11,15:18,21:24,28-Triepoxy-7,9-ethano-12,15-methano-9*H*,15*H*-furo[3,2-*l*]furo[2',3':5,6]pyrano[4,3-*b*] [1,4]dioxacyclopentacosin-5(4*H*)-one, 2-[(2*S*)-3-amino-2-hydroxypropyl]hexacosahydro-3-methoxy-26-methyl-20,27-bis(methylene)-, (2*R*,3*R*,3*aS*,7*R*,8*aS*,9*S*,10*aR*,11*S*,12*R*,13*aR*,13*bS*,15*S*,18*S*,21*S*,24*S*,26*R*,28*R*,29*aS*)-, methanesulfonate (salt)

## 2.1.5 Molecular Formula/Molecular Weight

Molecular Formula: C<sub>40</sub>H<sub>59</sub>NO<sub>11</sub>•CH<sub>4</sub>SO<sub>3</sub>

Molecular Weight: 826.0, salt (729.9, free base)

## 2.1.6 Structure



## 2.1.7 Pharmacologic class – microtubule inhibitor

## 2.2 Relevant IND/s, NDA/s, and DMF/s

IND 67,193 (Eisai Medical Research, Inc);

IND 64,395 (DCTP/NCI, National Institutes of Health)

## 2.3 Clinical Formulation

### 2.3.1 Drug Formulation

Eribulin mesylate will be prepared as a clear, colorless aqueous solution for IV administration. Each vial will contain 1 mg of eribulin mesylate as a 0.5 mg/mL solution in ethanol:water (5:95).

Composition of eribulin mesylate Injection (as excerpted from sponsor's report):

Ingredients	Amount of ingredients/vial (with overage) <sup>a</sup>	Function	Grade
Eribulin mesylate <sup>b</sup>	(b) (4)	Active ingredient	Eisai Standard
	(b) (4)		USP, EP, JP
			NF, EP, JP
			NF, EP, JP
			USP, EP, JP

a: An overage of (b) (4) is used to ensure that the labeled content can be removed from the vial with a needle and syringe.

b: Amount expressed as (b) (4)

c: (b) (4) are used to adjust pH if necessary. Nominal amount is (b) (4) for each component. The final drug product has a pH specification of (b) (4)

### 2.3.2 Comments on Impurities:

Summary:

Acceptance criteria for the drug substance and drug product will need to be adjusted to levels that do not exceed qualification values listed in section 2.3.2.

- Drug Substance – The acceptance criteria for (b) (4) should be adjusted to (b) (4) should be adjusted to (b) (4).
- Drug Product – The acceptance criteria for (b) (4) should be adjusted to NMT (b) (4).

This recommendation was relayed to ONDQA on August 25, 2010.



(b) (4)

<sup>1</sup> A rat bridging toxicology study was conducted using a drug substance with higher impurity levels (Lot #194P1404, purity 92.4%). A dose of 1.2mg/m<sup>2</sup> is considered the MTD dose in the rat (species used for these studies).

<sup>2</sup> A dose of 0.6mg/m<sup>2</sup> is considered the MTD (non-lethal) dose in most appropriate species (rat) in pivotal studies using lot BLDR001 (96.2% purity).

<sup>3</sup> BRL = below reportable limit (i.e. < (b) (4))

**Bolded** – Represents maximum qualified level.

\* Acceptance criteria for this impurity exceed qualification level.

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*Comments:*

- The sponsor proposed to use toxicology batch 682-005E to qualify (b) (4) since impurity levels present in this batch were greater than BLDR001. Due to the lack of adequate data collected for the toxicology studies in which this lot was used, only the lots used in the pivotal nonclinical studies (lot BLDR001) or in early clinical testing (lot BLD003) will be used to qualify these impurities.
- The sponsor used a rat MTD of 1.5mg/m<sup>2</sup> to justify qualification of impurities, however this was determined to be >LD<sub>20</sub> (1.2mg/m<sup>2</sup>) found in repeat dose rat studies (using lot BLDR001). For review purposes, the dose of 0.6mg/m<sup>2</sup> will be used as the MTD for pivotal rat studies (using lot BLDR001), and a dose of 1.2mg/m<sup>2</sup> will be used as the MTD in the high impurity bridging toxicology study (using lot 194P1404), for the purpose of qualifying impurities.

**Conclusion:** Acceptance criteria for impurity (b) (4) should be adjusted to (b) (4) and for impurity (b) (4) should be adjusted to (b) (4), based on nonclinical safety data and qualification threshold listed in ICHQ3, respectively.

(b) (4) level Qualification:

- Data for (b) (4) are not available for earlier clinical and toxicology batches BLDR001 (toxicology batch) through BLD007, since the test methodology for these impurities did not exist during this period of drug development. However, subsequent testing of a more recent toxicology batch 194P1404 was done. Based on nonclinical toxicology data using Lot 194P1404, the (b) (4) levels are qualified as follows:

(b) (4)	MTD = $\geq 0.20\text{mg/kg}$ ( $1.2\text{mg/m}^2$ )	Adjust for purity (Lot 194P1404 = 92.4% purity)
	Qualified at $\leq$	
	ICH Q3C (b) (4) acceptance limit - option 2 (b) (4) ) based on dose of $1.4\text{mg/m}^2$	
	ICH Q3C (b) (4) acceptance limit - option 2 (b) (4) ) based on dose of $1.4\text{mg/m}^2$	
	ICH Q3C (b) (4) acceptance limit - option 2 (b) (4) ) based on dose of $1.4\text{mg/m}^2$	
		(b) (4)

<sup>1</sup> Acceptance limit based on nonclinical safety data (using lot 194P1404)

**Conclusion:** (b) (4) levels in eribulin mesylate are qualified by the nonclinical data at the sponsor's proposed acceptance level of NMT (b) (4) .

*Drug Product:*

**Table 4 Acceptance Criteria for Impurities in Eribulin Mesylate Injection, 1 mg/vial**

Test	Acceptance Criteria	Batch Analysis
Impurities: Specified Degradants: (b) (4)	NMT (b) (4) NMT (4) NMT NMT	All batches comply at release except batches ESG001 and ESH001 which had reported levels for (b) (4) (b) (4) respectively.
Unspecified Impurities:  Total Impurities: (b) (4)	NMT (each) NMT (total) NMT NMT (b) (4) impurity not included in total)	
		All batches on stability at the long-term storage condition comply except batch N0500441, which had a reported level for (b) (4) at the 6 month interval. <sup>a</sup>

NMT: 'Not more than'

a: The amount of (b) (4) reported for these samples was determined using previous methods for analysis of impurities in Eribulin mesylate Injection. In each case, the reported value contains a contribution from error in the integration of the (b) (4) peak because of low resolution between eribulin mesylate and (b) (4) which eluted as a rider peak on the tail of the eribulin

*Comment:* Based on nonclinical safety data and drug purity, acceptance criteria for (b) (4) should be adjusted to NMT (b) (4) (which is also consistent with qualification threshold levels listed in ICHQ3B).

## 2.4 Proposed Clinical Population and Dosing Regimen

For treatment of patients with breast cancer who have failed at least two chemotherapeutic regimens for locally advanced or metastatic disease.

Eribulin mesylate will be administered at 1.4 mg/m<sup>2</sup> (0.038 mg/kg), as an intravenous bolus (over 2 to 5 minutes); on Days 1 and 8 of every 21-day cycle.

## 2.4 Regulatory Background

Nonclinical data contained in IND 64,395 and IND 67,193 were used to support this NDA. IND 64,395 was submitted in April 2002 by NCI. Data collected under the proposed Phase I clinical study conducted by NCI, as well as additional nonclinical data were submitted by the commercial sponsor (Eisai) in support of IND 67,193.

### 3 Studies Submitted

#### 3.1 Studies Reviewed

Study #	Study Title	Reviewed	
		Yes	No
<b>Pharmacology Studies</b>			
CAIVT0101, CAIVT0107	Anti-proliferative effects against human cancer cells	x	
CAIVT0100	Effects on cell cycle	x	
CAIVT0103	Effects on tubulin polymerization	x	
CAIVT0105, CAIVT0106	Anti-proliferative effects against P-glycoprotein overexpressing Multidrug resistant human cancer cells	x	
(b) (4)			x
(b) (4)			x
MT 1335, ERI-35, ERI-74, ERI-120, ERI-121, ERI-99A, ERI-90A, ERI-14, ERI-89A, ERI-95A, ERI-97A, ERI-98A, ERI-100A, ERI-106A, ERI-111A, ERI-118	Anticancer effects against sc human cancer xenografts as single agent	x	
(b) (4)			x
<b>Safety Pharmacology</b>			
SPH03-001	Effect on hERG Tail Current	x	
SPP03-002	Effects on Action Potential Parameters	x	
SPT03-001	Effects on Blood Pressure, Heart Rate, ECG, and Body Temperature	x	
SPI03-003	Effects on Central Nervous System	x	
SPR03-003	Effects on Respiratory Function	x	
PPC-2009-01	Eribulin mesylate-induced neuropathy evaluation	x	
<b>Pharmacokinetics</b>			

DSD2002-42	Pharmacokinetics of E7389 in mice, rats and dogs.		x <sup>1</sup>
DSD2001-38	Protein binding of E7389 in mouse, rat, dog and human plasma In Vitro		x <sup>1</sup>
DSD2003-01	Identification of human hepatic metabolic pathways of E7389 In Vitro		x <sup>1</sup>
NSC-707390-D	Analytical methods development and pharmacology studies with Halichondrin B analogs		x <sup>1</sup>
NSC 707389-D	Pharmacokinetics of Halichondrin B analog following three 1-hour CIV infusions in rats		x <sup>1</sup>
NSC 707389	Pharmacokinetic and dose range-finding study of Halichondrin B analog following three 1-hour CIV infusion in beagle dogs		x <sup>1</sup>
NSC 707389	Multiple dose toxicity of Halichondrin B analog in beagle dogs		x <sup>1</sup>
G465520B	Pharmacokinetics and dose range-finding study of Halichondrin B analog following three 1-hour CIV infusion in beagle dogs		x <sup>1</sup>
DSD2001-38	Plasma Protein Binding of Eribulin Mesylate		x <sup>1</sup>
45N-0405,	Metabolism in vivo - Metabolite Identification and Profiling in Plasma, Urine, Bile and Feces in Rats	x	
45N-0406	Metabolite Identification and Profiling in Plasma, Urine and Feces in Dogs	x	
DSD2003-01	Metabolism in vitro - Metabolizing Enzymes Identification <i>in vitro</i>		x <sup>1</sup>
	(b) (4)		x
			x
DMPK2000-13	Induction/Inhibition of Drug-Metabolizing Enzymes -Inhibition of CYPs		x <sup>1</sup>
DSD2001-31	Induction/Inhibition of Drug-Metabolizing Enzymes -Inhibition of CYP3A4		x <sup>1</sup>
	(b) (4)		x
N01-CM-07110,	Excretion Urinary Excretion of Eribulin Mesylate	x	
45-0404, 6212	Excretion - Excretion of Radioactivity	x	
DDDA2008-004	Drug-Drug Interactions Inhibition of P-glycoprotein	x	
<b>Toxicology Studies</b>			
G465520A	Repeat-Dose Toxicity- Rat/ Fischer 344 (Q4D×3) – Non-GLP		x <sup>1</sup>
G465520C	Repeat-Dose Toxicity- Rat/ Fischer 344 (Q4D×3)		x <sup>1</sup>
7306	Repeat-Dose Toxicity- Rat/ Fischer 344(Q7D×3)	x	
S09043	Repeat-Dose Toxicity-Rat/Fischer 344(Q7D×3)–High Impurity study	x	
7640	Chronic Repeat-Dose Toxicity- Rat/ Fischer 344 (Q7D×3 with a 14-day recovery was repeated 6 cycles)	x	
G465520B	Repeat-Dose Toxicity- Dog/ Beagle (Q4D×3) – Non-GLP		x <sup>1</sup>
G465520D	Repeat-Dose Toxicity- Dog/Beagle (Q4D×3)		x <sup>1</sup>
6288	Repeat-Dose Toxicity- Dog/ Beagle (Q7D×3)	x	
6528	Chronic Repeat-Dose Toxicity- Dog/Beagle (Q7D×3 with a 14-day recovery was repeated 6 cycles)	x	
<b>Genetic Toxicology</b>			

(b) (4)			x
960905	E7389: Bacterial Mutation Test	x	
S09044	E7389: Reverse Mutation Assay of Impurity-containing Drug Substance in Bacteria	x	
AA37RJ.703. BTL	5178Y/TK Mouse Lymphoma Mutagenesis Assay:	x	
S09045	E7389: Mouse Lymphoma TK Assay of Impurity-containing Drug Substance	x	
960994	E7389: Rat Micronucleus Test	x	
<b>Reproductive and Developmental Toxicity</b>			
LFA00033	E7389: An Intravenous Embryo-Fetal Development Study in Rats by Intermittent Injection during the Mid-Organogenesis Period	x	
<b>Special Toxicology Studies</b>			
DSD 2001-06, DSD 2001-07, DSD 2001-08	Myelotoxicity of NSC-707389 on murine, dog and human CFU-GM progenitor cells	x	
HG-EISAI-01	Hemotoxicity assay- bone marrow mononuclear cell of mouse, dog and human	x	

<sup>1</sup> previously reviewed

### 3.2 Previous Reviews Referenced

IND 64395, 4/22/2002, W. Schmidt, Ph.D.

IND 67193, 4/23/2003, Margaret E. Brower, Ph.D.

## 4 Pharmacology

### 4.1 Primary Pharmacology

*Summary:* E7389 is a macrocyclic ketone analogue of halichondrin B, isolated from the marine sponge *Halichondria okadae*. At effective concentrations, eribulin mesylate induces G<sub>2</sub>/M cell cycle arrest without affecting progression through the G<sub>1</sub> or S cell cycle phases or the G<sub>1</sub>/S cell cycle transition point. Eribulin mesylate inhibits tubulin polymerization and microtubule dynamics (i.e., inhibition of microtubule growth, but not shortening), thereby interfering with normal mitotic spindle formation resulting in blocks within the prometaphase portion of mitosis. As assessed by both morphological and biochemical criteria, prolonged blockage of cells in mitosis by eribulin mesylate treatment leads to cell death via apoptosis. Additionally, eribulin mesylate demonstrated *in vitro* activity against cancer cells that are taxane-resistant due to  $\beta$ -tubulin mutations. In contrast, eribulin mesylate is a substrate for the P-gp drug efflux pump, and thus shows reduced *in vitro* potency against human cancer cells expressing high levels of P-gp. In *in vitro* studies, eribulin mesylate inhibits cell growth with sub- to low-nmol/L half-maximal inhibitory concentration (IC<sub>50</sub>) values in a wide range of established human cancer cell lines, including MDA-MB-435 breast cancer, HT-29, COLO 205 and DLD-1

colon cancers, H522-T1 non-small cell lung cancer (NSCLC), NCI-H82 small cell lung cancer, DU 145 and LNCaP prostate cancers, U937 histiocytic lymphoma, FaDu pharyngeal squamous cell carcinoma (head and neck cancer), A2780/1A9 ovarian cancer, MES-SA uterine sarcoma, HL-60 promyelocytic leukemia, and LOX melanoma.

In *in vivo* studies, subcutaneous (sc) or intracranial (ic) human cancer xenograft models have been used. In sc human cancer xenograft studies, anticancer effects of eribulin mesylate have been evaluated in the HT-1080 (fibrosarcoma), PANC-1 (pancreas), NCI-H82 (small cell lung), NCI-H322M and NCI-H522 (non-small cell lung), MDA-MB-435, MX-1 and UISO-BCA-1 (breast), SR475 (head and neck), U251 (glioblastoma), COLO 205 (colon), NIH:OVCAR-3 (ovary), and LOX (melanoma), human cancer models using a variety of dosing administration schedules including Q1D×5, Q2D×3 [ $\times 3$  weeks], Q4D×3, Q4D×4 and Q7D×3. In these studies, treatment with eribulin mesylate in the 0.05 to 1.70 mg/kg/day range led to significant anticancer effects at doses below the maximum tolerated dose (MTD) levels, with responses ranging from tumor growth inhibition to tumor regression and eradication of tumors. Studies comparing Q1D×5, Q2D×3 [ $\times 3$  weeks], Q4D×3 and Q7D×3 dosing schedules in the MDA-MB-435 human breast cancer xenograft model showed that maximal activity and minimal toxicity is achieved with moderately intermittent dosing schedules, such as Q2D×3 [ $\times 3$  weeks] and Q4D×3.

In ic human cancer xenograft studies, anticancer effects of eribulin mesylate have been evaluated in the U251 and SF-295 human glioblastoma models using a Q2D×3 [ $\times 3$  weeks] and Q1D×5 [ $\times 2$  weeks] dosing schedule, respectively. In the U251 human glioblastoma xenograft models, the anticancer effects of eribulin mesylate were inconclusive. On the other hand, in the SF-295 human glioblastoma xenograft models, dosing with eribulin mesylate in the 0.34 to 0.60 mg/kg range showed anticancer activity.

In *in vivo* combination studies, sub-optimal doses of eribulin mesylate led to substantial anticancer activity when given in combination with capecitabine in the MX-1 and UISO-BCA-1 human breast cancer xenograft model, with erlotinib in the NCI-H322M human lung cancer xenograft model, and with gemcitabine in the NCI-H522 human lung cancer xenograft model, respectively.

#### **Sponsor-Generated Tabular Summary of *In vivo* Pharmacology Studies Conducted:**

Type of Study	Methods	Species/ Strain	Gender, Number/ Group	Route of Admin.	Doses	Schedule/ Duration	Observations	Study Number
Anticancer effects against sc human cancer xenografts as single agent	<i>In vivo</i> human cancer xenograft study conducted in HT-1080 fibrosarcoma model in athymic mice	NCr nu/nu mice	Female, 10 mice/group	iv	Eribulin mesylate: 1.27, 1.69, 2.25, 3.0, 4.0 mg/kg  Paclitaxel: 20 mg/kg	Q4D×3	Eribulin mesylate showed potent anticancer activity against HT-1080 fibrosarcoma xenografts at and under the MTD dose (1.69 mg/kg).	MT 1335
Anticancer effects against sc human cancer xenografts as single agent	<i>In vivo</i> human cancer xenograft study conducted in PANC-1 pancreatic cancer model in athymic mice	NCr nu/nu mice	Female, 10 mice/group (6 mice/group for the treatment with 90 and 270 mg/kg gemcitabine)	iv	Eribulin mesylate: 0.4, 0.53, 0.71, 0.95, 1.27, 1.69, 2.25, 3.0, 4.0 mg/kg  Gemcitabine: 90, 180, 270 mg/kg	Q4D×3  Q3D×4	Eribulin mesylate showed potent anticancer activity against PANC-1 xenografts at and under the MTD dose (1.27 mg/kg).	ERI-35
Anticancer effects against sc human cancer xenografts as single agent	<i>In vivo</i> human cancer xenograft study conducted in NCI-H82 small cell lung cancer model in athymic mice	NCr nu/nu mice	Female, 10 mice/group	iv	Eribulin mesylate: 0.54, 0.71, 0.96, 1.27, 1.70 mg/kg  Paclitaxel: 20 mg/kg	Q4D×3  Q2D×3 [×3 weeks]	Eribulin mesylate showed potent anticancer activity against NCI-H82 xenografts at all doses tested.	ERI-74
Type of Study	Methods	Species/ Strain	Gender, Number/ Group	Route of Admin.	Doses	Schedule/ Duration	Observations	Study Number
Anticancer effects against sc human cancer xenografts as single agent	<i>In vivo</i> human cancer xenograft study conducted in NCI-H322M non-small cell lung cancer model in athymic mice	NCr nu/nu mice	Female, 10 mice/group	iv  po  ip	Eribulin mesylate: 0.2, 0.4, 0.8, 1.6 mg/kg  Erlotinib: 50, 75, 100 mg/kg  Pemetrexed: 200 mg/kg  200, 400, 600 mg/kg	Q4D×4  Q1D×14  Q1D×14  Q4D×4 or Q7D×4	Eribulin mesylate at all doses was tolerated without deaths. Erlotinib mesylate showed potent anticancer activity against NCI-H322M xenografts at doses of 0.8 and 1.6 mg/kg.	ERI-120
Anticancer effects against sc human cancer xenografts as single agent	<i>In vivo</i> human cancer xenograft study conducted in NCI-H522 non-small cell lung cancer model in athymic mice	NCr nu/nu mice	Female, 10 mice/group	iv  po  ip	Eribulin mesylate: 0.2, 0.4, 0.8, 1.6 mg/kg  Erlotinib: 50, 75, 100 mg/kg  Pemetrexed: 200 mg/kg  200, 400, 600 mg/kg?	Q4D×4  Q1D×14  Q1D×14  Q4D×4 or Q7D×4	Eribulin mesylate at all doses was tolerated without deaths, and showed potent anticancer activity against NCI-H522 xenografts.	ERI-121

Type of Study	Methods	Species/ Strain	Gender, Number/ Group	Route of Admin.	Doses	Schedule/ Duration	Observations	Study Number
Anticancer effects against sc human cancer xenografts as single agent	<i>In vivo</i> human cancer xenograft study conducted in SR475 head and neck cancer model in athymic mice	NCr nu/nu mice	Female, 10 mice/group	iv	Eribulin mesylate: 0.47, 0.63, 0.84, 1.1, 1.5 mg/kg  Paclitaxel: 20 mg/kg	Q2D×3 [×3 weeks]	Eribulin mesylate showed potent anticancer activity against SR475 xenografts at the MTD (1.1 mg/kg), and at a lower dose of 0.84 mg/kg.	<a href="#">ERI-99A</a>
Anticancer effects against sc human cancer xenografts as single agent	<i>In vivo</i> human cancer xenograft study conducted in U251 glioblastoma model in athymic mice	NCr nu/nu mice	Female, 10 mice/group	iv  ip	Eribulin mesylate: 0.45, 0.6, 0.8 mg/kg  Carmustine: 12 mg/kg	Q2D×3 [×3 weeks]  Q4D×3	Eribulin mesylate at all doses was tolerated without deaths. Eribulin mesylate showed potent anticancer activity against U251 xenografts at all doses tested.	<a href="#">ERI-90A</a>
Type of Study	Methods	Species/ Strain	Gender, Number/ Group	Route of Admin.	Doses	Schedule/ Duration	Observations	Study Number
Anticancer effects against sc human cancer xenografts as single agent; comparison of dosing schedules	<i>In vivo</i> human cancer xenograft study in MDA-MB-435 breast cancer model, comparing different dosing administration schedules	NCr nu/nu mice	Female, 10 mice/group	iv	Doses adjusted so total eribulin mesylate given in all treatments was either 9.0 mg/kg or 4.5 mg/kg	Q1D×5, Q2D×3 [×3 weeks], Q4D×3, Q7D×3	Schedule dependence was determined as follows:  Anticancer activity: Q2D×3[×3 weeks] > Q4D×3 > Q7D×3 (Q1D×5 was toxic at both dose levels)  Toxicity: Q2D×3[×3 weeks] < Q7D×3 < Q4D×3 << Q1D×5  Intermittent dosing schedules more effective and less toxic, but not possible to identify most effective dosing schedule.	<a href="#">ERI-14</a>
Type of Study	Methods	Species/ Strain	Gender, Number/ Group	Route of Admin.	Doses	Schedule/ Duration	Observations	Study Number
Anticancer effects against ic human cancer xenografts as single agent	<i>In vivo</i> human cancer xenograft study conducted in SF-295 glioblastoma model in athymic mice	NCr nu/nu mice	Female, 10 mice/group	iv  po	Eribulin mesylate: 0.22, 0.3, 0.4 mg/kg  Temozolomide: 80 mg/kg	Q1D×5 [×2 weeks]  Q4D×3	Eribulin mesylate showed anticancer activity (increase in lifespan) at a dose of 0.4 mg/kg.	<a href="#">ERI-106A</a>
Anticancer effects against ic human cancer xenografts as single agent	<i>In vivo</i> human cancer xenograft study conducted in SF-295 glioblastoma model in athymic mice	NCr nu/nu mice	Female, 10 mice/group	iv  po	Eribulin mesylate: 0.34, 0.45, 0.60 mg/kg  Temozolomide: 80 mg/kg	Q1D×5 [×2 weeks]  Q4D×3	Eribulin mesylate showed anticancer activity (increase in lifespan) at all doses tested, but lack of a dose-response.	<a href="#">ERI-111A</a>

Type of Study	Methods	Species/ Strain	Gender, Number/ Group	Route of Admin.	Doses	Schedule/ Duration	Observations	Study Number
Anticancer effects against ic human cancer xenografts as single agent	<i>In vivo</i> human cancer xenograft study conducted in U251 glioblastoma model in athymic mice	NCr nu/nu mice	Female, 10 mice/group	iv  ip	Eribulin mesylate: 0.45, 0.6, 0.8 mg/kg  Carmustine: 12 mg/kg	Q2D×3 [×3 weeks]  Q4D×3	Eribulin mesylate showed anticancer activity (increase in lifespan) at a dose of 0.6 mg/kg, but lack of a dose-response.	ERI-89A
Anticancer effects against ic human cancer xenografts as single agent	<i>In vivo</i> human cancer xenograft study conducted in U251 glioblastoma model in athymic mice	NCr nu/nu mice	Female, 10 mice/group	iv  po	Eribulin mesylate: 0.45, 0.6, 0.8 mg/kg  Temozolomide: 80 mg/kg	Q2D×3 [×3 weeks]  Q4D×3	Eribulin mesylate showed anticancer activity (increase in lifespan) at all doses tested, but lack of a dose-response.	ERI-95A
Anticancer effects against ic human cancer xenografts as single agent	<i>In vivo</i> human cancer xenograft study conducted in U251 glioblastoma model in athymic mice	NCr nu/nu mice	Female, 10 mice/group	iv  po	Eribulin mesylate: 0.6 mg/kg  Temozolomide: 80 mg/kg	Q2D×3 [×3 weeks]  Q4D×3	Eribulin mesylate did not show anticancer activity (increase in lifespan).	ERI-97A
Anticancer effects against ic human cancer xenografts as single agent	<i>In vivo</i> human cancer xenograft study conducted in U251 glioblastoma model in athymic mice	NCr nu/nu mice	Female, 10 mice/group	iv  po	Eribulin mesylate: 0.6 mg/kg  Temozolomide: 80 mg/kg	Q2D×3 [×3 weeks]  Q4D×3	Eribulin mesylate did not show anticancer activity (increase in lifespan).	ERI-98A
Anticancer effects against ic human cancer xenografts as single agent	<i>In vivo</i> human cancer xenograft study conducted in U251 glioblastoma model in athymic mice	NCr nu/nu mice	Female, 10 mice/group	iv  po	Eribulin mesylate: 0.6 mg/kg  Temozolomide: 80 mg/kg	Q2D×3 [×3 weeks]  Q4D×3	Eribulin mesylate showed anticancer activity (increase in lifespan).	ERI-100A

## 4.2 Secondary Pharmacology - none

## 4.3 Safety Pharmacology

**Overall Summary:** Treatment with 30 µM/L E7389 produced no inhibition of HERG tail current in HEK293 cells. E7389, when infused intravenously for 60 min at 0.01 or 0.04 mg/kg, transiently decreased SBP, DBP, MAP, and HR and increased the RR interval in male and female dogs, with the effects in males more pronounced than in females. Treatment with E7389 at concentrations up to 30µM showed no effect on cardiac action potentials in dog Purkinje fibers. Axonopathy of the sciatic and dorsal root ganglia was reported at doses of ≥1.31mg/kg (IV, Q2Dx3 for two weeks) in mice, although no effects of E7389 on nerve conduction velocity (NCV) or amplitude in caudal and digital nerves were noted in this study (at doses up to 1.75mg/kg). Intravenous administration of E7389 at 0.1 or 0.25mg/kg produced no effects on respiratory or CNS function in rats.

### 1.) E7389: Effects on CNS (Study SPI03-003, Quintiles)

Six male Fischer 344 rats/group were administered IV E7389 at doses of 0.1 or 0.25mg/kg and assessed for 8 hours according to parameters set forth in the Irwin testing paradigm. No remarkable effects were reported.

### 2.) E7389: Effects on Respiration Function (Study SPR03-003, Quintiles)

Six male Fischer 344 rats/group were administered IV E7389 at doses of 0.1 or 0.25mg/kg. Respiratory function (respiratory rate and tidal volume) was measured

before and at approximately 30, 60, 120, 240, and 480 minutes after administration using whole body plethysmography. No remarkable effects were reported.

3.) *E7389: Effects on HERG Tail Currents recorded from Stably Transfected HEK293 Cells (Study SPH03-001, Quintiles)*

Treatment with 30 µM/L E7389 produced no inhibition of HERG tail current in HEK293 cells stably transfected with HERG cDNA.

4.) *E7389: Effects on Blood pressure, Heart rate, ECG and Body Temperature in Conscious Dogs (Study No: SPT03-001, Quintiles):*

Methods: Approximately 20 days after implantation of the telemetry implants, one group of six dogs received doses of E7389, according to the ascending dose regimen listed below:

Day 1	Vehicle (1.5 mL/kg/h);	3 male dogs
Day 3	Vehicle (1.5 mL/kg/h);	3 female dogs
Day 8	E7389 (0.01 mg/kg);	3 male dogs
Day 10	E7389 (0.01 mg/kg);	3 female dogs
Day 15	E7389 (0.04 mg/kg);	3 male dogs
Day 17	E7389 (0.04 mg/kg);	3 female dogs

Results: Vehicle and test article were intravenously infused at a rate of 1.5 mL/kg/h to conscious dogs. Intravenous infusion (1-hr) of E7389 at 0.01 mg/kg reduced Diastolic blood pressure (10%,  $p < 0.05$ ), Mean arterial pressure (8%,  $p < 0.05$ ), Heart rate (27% at 8 hours in females) and RR Interval (+39%,  $p < 0.05$ , at 8 hours). Intravenous infusion (1hr) of E7389 at 0.04 mg/kg significantly decreased Systolic Blood Pressure (-14%,  $p < 0.001$ ), Diastolic Blood Pressure (-22%,  $p < 0.001$ ), and Mean Arterial Pressure (-18%,  $p < 0.001$ ) in males, and Heart rate (-36% males, -16% females), and increased RR interval (+44% at 60min, +14% at 30min) was observed in both sexes, as compared to vehicle controls. At 8 hours HR was still reduced 16% in females at 0.04mg/kg. There were no significant effects on core body temperature.

5.) *E7389: Effects on Action Potential Parameters (Study SPP03-002, Quintiles) – E7389 at concentrations of 1, 10, and 30 µM showed no effect on cardiac action potentials in isolated dog Purkinje fibers.*

6.) *E7389-induced neuropathy evaluation in mice: The Effects of E7389 on nerve conduction velocity (NCV), amplitude and morphology in the BALB/c Mouse (Study PPC-2009-01, Eisai Research Institute)*

E7389 administered to BALB/c female mice ( $n=10$ ) intravenously Q2Dx3 for two weeks at doses of 0.44mg/kg, 0.875mg/kg, 1.31mg/kg and 1.75mg/kg (MTD) induced no significant reduction in nerve conduction velocity or amplitude in caudal and digital nerves. Morphological changes were evident in sciatic nerve (axonopathy) and dorsal root ganglia (axonopathy, degeneration) of E7389-treated mice at doses  $\geq 0.75$  MTD (1.31 mg/kg or 3.9mg/m<sup>2</sup>).

## 5 Pharmacokinetics/ADME

*Overall Summary:* Pharmacokinetic studies indicated that drug exposure increased with dose in rodents and dogs, and that the volume of distribution was high in all species. The high  $V_{ss}$  correlated with a low level of observed protein binding. Species differences in protein binding of E7389 were apparent, with the highest measured in human plasma and the least in dog plasma. Clearance appeared to be moderate in all species, with a prolonged  $t_{1/2}$ . CYP3A4 appears to be the major enzyme responsible for the metabolism of E7389 in humans, as indicated by *in vitro* assays. After a single intravenous dose of [ $^{14}\text{C}$ ]E-814058 to rats and dogs, the parent compound was the single major component observed in plasma, urine and feces. [ $^{14}\text{C}$ ]E-814058 was mainly eliminated through fecal excretion, with only 8-15% of dosed radioactivity excreted in urine of dogs and rats.

### 5.1 PK/ADME

#### Summary of pharmacokinetic studies previously reviewed:

*Pharmacokinetic Summary for IND 67,193* (Reviewed by Margaret Brower; April 22, 2003).

1.) *Study DSD2002-42 Pharmacokinetics of E7389 in mice, rats and dogs.* Conducted by Eisai Research Institute, Wilmington, MA. And (b) (4) and completed January, 2003.

2.) *Study DSD2001-38 Protein binding of E7389 in mouse, rat, dog and human plasma in vitro.* Conducted by Eisai Research Institute, Wilmington, MA. from August to October, 2001.

3.) *Study DSD2003-01 Identification of human hepatic metabolic pathways of E7389 In Vitro.* Conducted by Eisai Research Institute, Wilmington, MA in January, 2003.

Summary (as reviewed by Margaret Brower): Studies submitted with this IND indicated that drug exposure increased with dose in rodents, especially mice, and the volume of distribution was high in all species. Clearance appeared to be moderate in all species. The sponsor indicated that the elimination of E7389, indicated as the mean residence time extrapolated to infinity ( $\text{MRT}_{inf}$ ), was relatively slow in all species. Protein binding appeared to be the least in dog plasma and the highest in human plasma; binding was similar in mice and rats. E7389 was not strongly bound to mouse, rat, dog or human plasma protein; percent binding was concentration independent. Single and q4dX3 dose administration in the rat and dog indicated similar elimination half-lives of 11h, with ~10% of the dose excreted in the urine of the rat. No data was submitted on canine excretion. CYP3A4 appears to be the major enzyme responsible for the metabolism of

E7389 in humans, as indicated *in vitro*. This is based on inhibitory effects observed from CYP3A4-specific inhibitors and correlations between the capability of E7389 metabolism and CYP3A4-specific activity using human liver microsomes. Metabolites formed by CYP3A4-mediated reactions were primarily isomeric monohydroxylates.

Pharmacokinetic and ADME studies reviewed in this NDA (201,532):

1.) *Study DSD2001-30 Pharmacokinetics of Halichondrin B Analog 707389D Following Three 1-hour CIV Infusions in Rats.*

*Methods:*

To assess the pharmacokinetics of Halichondrin B Analog 707389-D, male and female Fischer 344 rats (N=9/sex/group) were administered Halichondrin B Analog 707389-D at doses of 0.13 and 0.20mg/kg (1-hour IV infusion) on Days 1, 5 and 9 (q4Dx3). The female rats in the 0.2mg/kg group received 0.20mg/kg as a slow bolus IV dose q4Dx3, rather than as a 1-hour IV infusion.

*Mortality:*

Males: At 0.13mg/kg 2/9 males were euthanized in moribund condition on day 8. At 0.20mg/kg 4/9 males were found dead on days 7-10. Adverse clinical signs were cold to touch, hunched posture, rapid respiration, diarrhea and lethargy.

Females: At 0.13mg/kg 2/9 females were found dead on day 10. At 0.20mg/kg 3/9 females were found dead/euthanized moribund on days 6-9. Adverse clinical signs were hunched posture, rapid respiration and lethargy.

*Comment:* The doses used and exposure values reported ( $C_{max}$ ) in this study overlap with those observed in the pivotal rat study, despite a difference in dosing regimen (q4Dx3 vs. q7Dx3). As such, these data further support that a non-lethal dose of E7389 in rats is <0.13mg/kg.

*Results:* The plasma concentrations of the test agent are presented below.

Parameter	0.13mg/kg/day		0.20mg/kg/day	
	Day 1	Day 9	Day 1	Day 9
<b>Males</b>	1-hr IV infusion		1-hr IV infusion	
AUC <sub>0-∞</sub> (ng.min/mL)	5108	5429	Insufficient data	5235
C <sub>max</sub> (ng/mL)	40.0	45.5	Insufficient data	57.6
t <sub>1/2</sub> (min)	260	304	Insufficient data	261
<b>Females</b>	1-hr IV infusion		IV slow bolus (over 1 minute)	
AUC <sub>0-∞</sub> (ng.min/mL)	5414	9261	6554	8912

C <sub>max</sub> (ng/mL)	40.9	37.6	313	366
t <sub>1/2</sub> (min)	540	1034	488	435

2.) *Studies DDDP2005-090, DDDP2005-095, DDDP2005-097, DDDP2005-153 and DDDP2005-163 Pharmacokinetics of E7389 in Mice and Rats Following Single Intravenous and Oral Administrations*

**Methods:**

Male BALB-c mice (3/time point) were administered E7389 intravenously at 1 and 2 mg/kg, or via oral gavage at 3 and 10 mg/kg. Male CF-1 wild type and CF-1 P-gp deficient mice (n = 3/time point) were administered E7389 intravenously at 2 or 5.5 mg/kg orally via oral gavage. Male Sprague-Dawley rats (n = 4/dose group) were administered E7389 intravenously at 1 mg/kg, or via oral gavage at 3 and 10 mg/kg. Blood and brain specimens (only for CF-1 wild type and CF-1 P-gp deficient mice) were collected from each animal at predetermined times. Plasma and brain levels of E7389 were determined by LC/MS/MS.

**Results:**

(below Tables excerpted from Sponsor's report)

**PK parameters of E7389 following a single IV or PO administration**

Species	Dose mg/kg – Route <sup>a</sup>	C <sub>max</sub> (ng/mL)	t <sub>max</sub> (hr)	AUC <sub>0-t</sub> (hr•ng/mL)	AUC <sub>0-∞</sub> (hr•ng/mL)	t <sub>1/2</sub> (hr)	CL (L/hr/kg)	V <sub>ss</sub> (L/kg)	F (%)
Mouse	1 - IV	NA	NA	722.731	739.456	5.5	1.190	4.952	3.2 <sup>b</sup>
	2 - IV	NA	NA	1006.795	1014.542	6.8	1.735	6.909	
	3 - PO	5.858	0.083	7.467	NR	NR	NA	NA	
	10 - PO	48.496	1.0	132.750	161.159	15.4	NA	NA	
Rat	1 - IV	NA	NA	557.109	632.959	26.7	1.391	38.604	NA
	3 - PO	1.337	2.0 <sup>c</sup>	5.933	13.152	7.9	NA	NA	0.4
	10 - PO	26.028	2.0 <sup>c</sup>	136.344	171.119	21.5	NA	NA	2.4

<sup>a</sup> Dose was adjusted to the free base by multiplying by the salt factor (b) (4)

<sup>b</sup>  $F = (AUC_{0-\infty, PO(10\text{ mg})} \cdot D_{IV(2\text{ mg})}) / (AUC_{0-\infty, IV(2\text{ mg})} \cdot D_{PO(10\text{ mg})}) \cdot 100$

<sup>c</sup> Median value

NR: Not reportable due to lack of reliable data points

NA: Not applicable

**Plasma and Brain PK Parameters of E7389 in CF-1 Wild Type and P-gp Deficient Mice**

Dose mg/kg – Route <sup>a</sup>	Strain	C <sub>max</sub> <sup>b</sup> (ng/mL)	t <sub>max</sub> (hr)	AUC <sub>0-t</sub> <sup>b</sup> (hr•ng/mL)	AUC <sub>0-∞</sub> <sup>b</sup> (hr•ng/mL)	t <sub>1/2</sub> (hr)	CL (L/hr/kg)	V <sub>ss</sub> (L/kg)	F (%)
Plasma									
2.0 - IV	WT <sup>c</sup>	NA	NA	1047.573	1131.917	5.9	1.6	4.5	NA
	P-gp D <sup>d</sup>	NA	NA	1721.884	1978.849	10.2	0.9	8.8	NA
5.5 - PO	WT <sup>c</sup>	53.2	1	195.013	213.462	1.9	NA	NA	6.9
	P-gp D <sup>d</sup>	263.7	4	2401.313	2864.916	11.7	NA	NA	52.6
Brain									
2.0 - IV	WT <sup>c</sup>	45	0.08	112.170	159.414	4.7	NA	NA	0.1 <sup>e</sup>
	P-gp D	352	24	5863.331	NA	NA	NA	NA	3.4 <sup>e</sup>
5.5 - PO	WT <sup>c</sup>	5	6	NA	NA	NA	NA	NA	NA
	P-gp D	244	36	5124.750	NA	NA	NA	NA	2.1 <sup>e</sup>

<sup>a</sup> Dose was adjusted to the free base by multiplying by the salt factor (b) (4)

<sup>b</sup> Concentration unit in brain: ng/g

<sup>c</sup> CF-1 wild type mice

<sup>d</sup> CF-1 P-gp deficient mice

<sup>e</sup> Brain penetration index (BPI) = (AUC<sub>0-∞ (brain)</sub> / AUC<sub>0-∞ (plasma)</sub>)

NA: Not applicable

Summary: The PK profile of E7389 in BALB-c and CF-1 wild type mice and Sprague-Dawley rats was characterized by extensive distribution, slow to moderate clearance, slow elimination, and low oral bioavailability. Oral bioavailability and brain penetration were significantly improved in CF-1 P-gp deficient mice, suggesting that P-gp was the major factor for the low oral bioavailability and low brain penetration observed in mice.

3.) *STUDY QPS45-0403 and 45-0404 Quantitative Tissue Distribution of Drug-Related Material Using Whole- Body Autoradiography Following a Single IV Dose of [<sup>14</sup>C]E814058 (1.5 mg/kg, as the salt form) to Male Long-Evans (pigmented) and Sprague Dawley Rats (albino)*

**Methods:** [<sup>14</sup>C]E-814058 (the acetate salt used, rather than the mesylate salt used in E7389) was administered to Sprague-Dawley or Long-Evans rats (N=18) for quantitative whole body autoradiography (QWBA) and a mass balance assessment. To obtain tissue distribution data in rats using QWBA, [<sup>14</sup>C]E-814058 was administered as a single IV dose (1.5mg/kg). In addition, a mass balance study in rats was conducted to obtain the quantitative measurement of [<sup>14</sup>C]E-814058 in the excreta following doses of 1.5, 1.0 or 0.5mg/kg.

**Comment:** The concentrations of the dosing solutions for these studies were tested, and the deviation from the intended concentration ranged from -25.2 to 9.9%, so actual dose may be up to 25% less than nominal dose listed.

**Results:** Six rats were found dead or euthanized moribund within 72 hours post dose. In most tissues, peak concentration of radioactivity was observed at 5-10 min post-dose. Tissues showed limited elimination of radioactivity over the remaining time points (20 min to 73 h post-dose). The concentration-time data suggest a normal distribution after a single intravenous administration, and [<sup>14</sup>C]E814058-derived radioactivity was

widely distributed into the tissues of male rats following an IV administration of 1.5 mg/kg.

*4.) Study 45-0409 Quantitative Tissue Distribution and Human Dosimetry Predictions of Drug-Related Material Using Whole-Body Autoradiography Following a Single IV Dose of [<sup>14</sup>C]E814058 (0.75 mg/kg, as the salt form) to Male Long-Evans and Sprague Dawley Rats*

*Methods:*

- To determine the tissue distribution of drug-related material, twelve male albino rats and three male pigmented rats received a single IV administration of [<sup>14</sup>C]E814058 as a solution in 5% ethanol/ 95% saline (0.9%) at dose of 0.75 mg/kg.
- One albino rat per time point was scheduled for euthanasia at 5 and 30 minutes (min), 6 and 10 hours (h), and 1, 2, 3, 4, 5, 6, 7, and 10 days post-dose. One pigmented rat per time point was scheduled for euthanasia at 5 min, and 3 and 7 days post-dose.
- All euthanized rats were prepared QWBA.

*Results:*

- Three albino rats were sacrificed moribund.
- Following a single IV administration of [<sup>14</sup>C]E814058 at 0.75 mg/kg, drug-derived radioactivity was widely distributed to tissues through 7 days post-dose.
- The blood concentration of radioactivity in male albino rats was highest (1.176 µg equiv/g) at 5 min post-dose, declined to 0.012 µg equiv/g at 2 days post-dose and was below limit of detection by 3 days post-dose.
- Tissue distribution patterns in male albino and pigmented rats were similar, and there appeared to be no preferential association of [<sup>14</sup>C]E814058 with pigmented tissues.
- The highest tissue concentrations measured occurred in the lung (12.239 µg equiv/g), urinary bladder (7.637 µg equiv/g), renal cortex (6.439 µg equiv/g), renal medulla (5.412 µg equiv/g), spleen (2.929 µg equiv/g), thyroid (2.541 µg equiv/g), stomach (2.365 µg equiv/g), and salivary gland (2.288 µg equiv/g).
- The CNS had the lowest concentration (0.028 µg equiv/g to BQL), and the thymus retained measurable concentration levels (0.20 µg equiv/g) extending beyond 7 days post dose.
- Excessive whole body and/or individual tissue exposure of human subjects to <sup>14</sup>C is not expected to occur with an IV dose of up to 100 µCi of [<sup>14</sup>C] E814058.

*5.) Study Numbers DDDP2005-098 and DDDP2005-135 Tumor and Brain Penetration of E7389 in LOX Xenograft Mice Following Single and Multiple Intravenous Administrations*

*Methods:* Female Ncr LOX tumor-bearing nu-nu mice (n = 3/time point/dose group) were administered single intravenous doses of E7389 at 1.0 or 2.0 mg/kg, or multiple

intravenous doses at 0.5 or 1.0 mg/kg once every two days for three doses (Q2Dx3). Levels of E7389 in brain, tumor, and plasma were determined by LC/MS/MS.

*Results:* The PK profile of E7389 in LOX tumor-bearing mice was characterized by extensive distribution, moderate clearance, and moderately slow elimination. E7389 penetrated well into LOX xenograft tumors following 0.5, 1.0, or 2.0 mg/kg, with exposures approximately 20-30 times higher than that in plasma, while exposure in the brain was roughly 13-45% of that in plasma.

5.) *Study 45N-0405 [<sup>14</sup>C]E-814058 Metabolite Identification and Profiling in Plasma, Urine, Bile and Feces from SD-rats following a single intravenous administration of [<sup>14</sup>C]E-814058.*

*Methods:*

The objectives of this study were to determine the metabolic profile of [<sup>14</sup>C]E-814058-derived radioactivity and to identify the metabolites of [<sup>14</sup>C]E-814058 in plasma, urine, bile, and feces from intact and bile duct-cannulated Sprague-Dawley rats following a single intravenous dose of 0.5 mg/kg [<sup>14</sup>C]E-814058. Although higher doses were also used (1.5 and 1.0 mg/kg), lethality in these groups precluded full sample collection.

*Results:*

- No prominent metabolites of [<sup>14</sup>C]E-814058 were found in plasma, urine and feces.
- [<sup>14</sup>C]E-814058 was the dominant component accounting for the majority of the excreted radioactivity in all matrices.
- Fecal excretion appeared to be the major elimination pathway for [<sup>14</sup>C]E-814058 for intact rats. For BDC rats, bile excretion was the main elimination pathway.
- No significant gender-related differences in metabolic profiles were apparent.
- Six metabolites were identified based on LC-MS-RFD (radio flow detection) analyses.
  - M1 and M2 are minor mono-oxidation metabolites detected in bile samples and accounted for 0.78% and 0.33% of dosed radioactivity, respectively.
  - M3 was a metabolite observed in fecal samples, and accounted for 1.34%, 0.34% and 1.88% of dosed radioactivity, respectively.
  - M4 was another mono-oxidation metabolite observed in urine samples and accounted for 0.15% of dosed radioactivity.
  - M5 is tentatively assigned as a glucose conjugate metabolite and was only observed in plasma.
  - M6 was a metabolite observed in both male and female feces and showed the same molecular weight as E814058, and was likely a degradation product of [<sup>14</sup>C]E-814058.

6.) *Study 45N-0406 [<sup>14</sup>C]E-814058 Metabolite Identification and Profiling in Plasma, Urine and feces from Beagle Dogs following a Single IV administration of [<sup>14</sup>C]E-814058*

*Methods:*

To identify the metabolites and determine the metabolic profile of [<sup>14</sup>C]E-814058-derived radioactivity in plasma, urine and feces, male and female beagle dogs were administered a single intravenous dose of [<sup>14</sup>C]E-814058 at 0.08 mg equiv/kg (~4.8 µCi/kg).

*Results:*

[<sup>14</sup>C]E-814058 showed minimal metabolic changes following intravenous administration to male and female Beagle dogs. [<sup>14</sup>C]E-814058 was the major component in plasma, urine and feces. Three minor metabolites were identified based on LC-MS-RFD (radio flow detection) experiments, as follows:

- M1 is a minor metabolite accounting for 0.26% of the dosed radioactivity observed in the female urine 0-8 hour sample.
- M2 is a mono-oxidization metabolite detected in plasma (both sexes).
- M3 is a metabolite observed in urine and fecal samples, and accounted for 0.18% of dosed radioactivity in female urine, and accounted for 1.84% and 3.76% of dosed radioactivity in male and female fecal excretion, respectively. M3 showed the same molecule weight as the parent, and is likely a degradation product of [<sup>14</sup>C]E814058.

7.) *Study 6212 [14C]E-814058: A Study of Mass Balance of Radioactivity Following a Single Intravenous Administration of 14C-Labelled E-814058 to Beagle Dogs*

*Methods:* To determine the mass balance of <sup>14</sup>C-labelled E-814058 following a single intravenous (IV) bolus injection (0.08mg/kg) to Beagle dogs (3/sex/group), excreta collection was performed predose, 0-8, 8-24 hour and at 24-hour intervals for fourteen consecutive days after dosing. Serial blood sampling was performed at predose, 5, 10, 15, 25, 40 min, 1, 2, 4, 6, 8, 12, 24, 48, 72 and 96 hours postdose.

*Results:*

The maximum concentrations ( $C_{max}$ ) of radioactivity in plasma were 92.92 and 66.94 ng equiv/mL for male and female animals, respectively, and were observed ~5 min postdose. The major route of elimination of [<sup>14</sup>C]E814058-derived radioactivity was via the feces (84.4% in males, 86.9% in females). Urinary excretion of drug-derived radioactivity appears to be a secondary route of elimination for this compound accounting for 8% (males) and 10.6% (females) of the total radioactivity administered. Animals eliminated ~82% of the total radioactivity administered within 48 hours postdose. By 15 days postdose, the overall mean recovery of the dosed radioactivity in all matrices was 96% and 99% for male and female dogs, respectively, indicating that elimination was complete at this stage.

8.) *Study 45-0404 Mass Balance and Excretion of Radioactivity in SD-Rats following a Single Intravenous Bolus Dose of [14C]E-814058*

*Methods:* A mass balance study in Sprague Dawley rats was conducted to obtain the quantitative measurement of [<sup>14</sup>C]E-814058 in the excreta following doses of 1.5, 1.0 or

0.5mg/kg in Sprague-Dawley rats. A high rate of lethality precluded full collection (up to 240 hours post-dose) of samples at doses  $\geq 1.0$ mg/kg.

*Results:*

- In rats receiving a dose of 1.0mg/kg, fecal excretion was found to be the predominant route of elimination resulting in 51.6% of the total dose, and urinary excretion was 16.2%.
- In rats receiving a dose of 0.5mg/kg, fecal excretion was the predominant route of elimination of total radioactivity, accounting for mean of 64.9% of the administered dose.
- Excretion of radioactivity occurred largely within the first 48 h (averaging 49% of the dose). The urinary excretion of total radioactivity accounted for a mean of 15.6% of the administered dose.
- At 240 h, a mean of 13.2% of the dose was present in the carcasses of animals, indicating that there was retention of radioactivity in the body.

**5.2 Toxicokinetics** (included in toxicity studies)

## 6 General Toxicology

### 6.1 Single-Dose Toxicity and Dose Range-Finding studies (non-GLP):

- No single-dose toxicity studies were conducted.
- Multiple dose range-finding studies of Halichondrin B analog (NSC-707389, Batelle study #s G465520 A, B, C and D) were conducted in the rat and dog, and previously reviewed by Wendelyn J. Schmidt (IND 64,395).

*Summary of Findings (as reviewed in IND 64,395):* In a multiple dose toxicity study in rats with Q4Dx3 regime (dosed on Days 1, 5 and 9) at IV doses of 0.013, 0.13 and 0.20 mg/kg. The primary target was hematopoietic organs, and at higher doses the gonads, liver, kidneys and skeletal muscle were also affected. A dose of 0.25mg/kg was found to be lethal (LD<sub>33</sub>) in rats, and a dose of 0.20mg/kg was considered the MTD, for the purpose of establishing a clinical start dose (0.12mg/m<sup>2</sup>). In a multiple dose toxicity study in dogs with Q4Dx3 regime (dosed on Days 1, 5 and 9) at IV doses of 0.004, 0.03 and 0.04mg/kg/day, the hematopoietic and reproductive organs were the primary targets. No lethality was observed. The reviewer commented that low animals numbers in these studies (N=3/group in rat, N=1/group in dog) did not provide adequate power to fully interpret data submitted.

### 6.2.1 Repeat-Dose Toxicity

Study title: E7389: A Repeat Dose Intravenous Injection Toxicity and Toxicokinetic Study in Fischer F-344 Rats Followed by an up to 14-Day Observation Period

Study no.: 7306  
 Study report location: eCTD - module 4.2.3.2.1  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: June 14, 2004  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: E7389 (Lot No. BLDR001; Batches 16-023-NA and 17-117-NA; 96.2% purity)

#### Key Study Findings:

E7389, at nominal doses of 0.10, 0.20, and 0.25 mg/kg (actual doses up to 14.4% reduced), was administered by intravenous injection to male and female Fisher 344 rats on Days 1, 8 and 15. Resultant toxicities included:

- An LD<sub>20</sub> of 0.20mg/kg, and an MTD of 0.10mg/kg.
- Dose-dependent hypocellularity of the bone marrow and necrosis/atrophy of the thymus, which were not present in recovery groups.
- Dose-dependent changes in the testis, epididymis, and sciatic nerve at doses ≥0.20mg/kg, which persisted in recovery animals.

#### Methods

Doses: E7389 was administered at nominal doses of 0, 0.1, 0.2, and 0.25 mg/kg (see below note for study protocol deviation).  
 Frequency of dosing: Days 1, 8, and 15  
 Route of administration: Intravenously  
 Dose volume: 2.5, 1.0, 2.0 and 2.5 mL/kg body weight for control, low, mid and high dose groups, respectively  
 Formulation/Vehicle: Vehicle is 5% ethanol in 0.9% NaCl  
 Species/Strain: Rats/Fischer F-344  
 Number/Sex/Group: 10/sex/group (5/10 necropsied on Day 18 and 5/10 necropsied on Day 29 as recovery subgroup).  
 Age: 6-10 weeks old at dosing initiation  
 Weight: 134 to 184 g for males; 90 to 134 g for females  
 Satellite groups: Recovery – 5/sex/group.
 

- Toxicokinetic (TK) - 8/sex/group (4/sex used)

for Day 1 analysis, and 4/sex used for Day 8 and 15 analyses).

- Administered E7389, Lot No. BLDR002 (96.3% purity).
- Blood samples were collected at 5 min, 1, 2, 4, 8, and 24 hrs after dosing on Days 1 and 15, and at predose only on Day 8.

Deviation from study protocol: Based on the analyses of dosing solutions, actual doses delivered were up to 14.4% below target. Accordingly, the actual doses would be as low as:

0.09mg/kg	(0.5mg/m <sup>2</sup> ),
0.17mg/kg	(1.0mg/m <sup>2</sup> )
0.21mg/kg	(1.3mg/m <sup>2</sup> )

## Observations and Results

### Mortality

Morbidity, requiring early euthanasia, was observed in one 0.25 mg/kg group male on Day 7 and two 0.20 mg/kg group males on Days 14 and 21, likely due to bacterial infections secondary to bone marrow toxicity.

Findings at both doses were similar and included:

- Necrotic changes, occasionally accompanied by the presence of bacterial colonies in various organs including adrenals, liver and spleen, and focal purulent pneumonia were observed, and were regarded as secondary to hypocellularity of the bone marrow and resulting immunocompromise. Related mild to severe myeloid hyperplasia of bone marrow was noted in all three rats, and extramedullary hematopoiesis in the spleen was observed in some animals.
- Minimal fiber degeneration of the sciatic nerve was observed in one male.
- Mild to moderate lymphoid atrophy of the spleen.
- Lymphoid atrophy of the Peyer's patches of the intestine in two animals.
- Findings noted in the testes, epididymis, prostate, seminal vesicles and thymus were similar to findings seen in animals sacrificed on Day 18 or 29.

### Clinical Observations-

Partially closed eyes, decreased activity, thin condition, skin pallor, tremors, hunched back and skin turgor were reported in above moribund animals. No other clinical signs were noted in remaining animals.

### Body Weights -

Body weights were recorded for all animals prior to dosing on Days 1, 3, 8, 15 and on Days 18, 21 and 27, as well as terminally prior to necropsy. Group mean body weight

losses, or reduced body weight gain, were noted at doses  $\geq 0.20$  mg/kg, in both sexes, throughout the treatment period. Evidence of some compensatory weight gain was apparent by Day 21 or 27 in affected groups.

#### Food Consumption-

No remarkable effects on food consumption (excluding moribund animals listed above).

#### Ophthalmoscopy –

Conducted at pretreatment, Day 18 (on day of terminal sacrifice), and Day 25 for recovery animals. There were no treatment-related ophthalmological changes.

#### ECG-

Not conducted.

#### Hematology

In all test article-treated groups on Day 18, dose-related reductions in white blood cells (ranging from 54 to 78% of controls; neutrophil reduction predominant), and red blood cells, hematocrit and hemoglobin concentration (ranging from 77 to 90% of controls) were apparent in males and females. These effects were associated with dose-dependent bone marrow hypocellularity. At  $\geq 0.20$  mg/kg in males, platelets were elevated (124% of controls for both groups). Coagulation parameters were unaffected.

Recovery: On Day 29, reductions in total red blood cell count, and increases in mean corpuscular volume and mean corpuscular hemoglobin persisted at doses  $\geq 0.20$  mg/kg.

#### Clinical Chemistry

Dose-related increases in aspartate aminotransferase (122 to 159% of controls), glucose (110 to 123% of controls) and cholesterol levels (128 to 143% of controls, males only), and decreases in phosphorus (86 to 93%) were noted in males and females of the 0.20 and 0.25 mg/kg groups, respectively.

Recovery: On Day 29 there were no remarkable differences between control and treated groups.

#### Urinalysis

No remarkable findings.

#### Gross Pathology

Changes related to test article:

Terminal Sacrifice – Day 18:

Dose-dependent, decreased size of the epididymides and testes, and small thymus was observed at doses  $\geq 0.20$  mg/kg. Decreased size of the prostate and seminal vesicles, and pale discoloration of the kidneys were observed at 0.25mg/kg.

Terminal Sacrifice – Day 29:

Dose-dependent decreased size of the epididymides and testes was observed in all 0.20 mg/kg and 0.25 mg/kg animals. Small prostate and seminal vesicles were found in one surviving rat at 0.25 mg/kg.

Organ Weights

Changes related to test article:

On Day 18 dose-related reduction (43 to 69% of controls) in absolute and relative testes weights was observed in  $\geq 0.20$ mg/kg males.

Recovery: On Day 29 dose-related reduction in absolute and relative testes weights was apparent in all treated groups (29 to 77% of controls).

Histopathology

Adequate Battery: yes (below Table excerpted from sponsor’s report).

ORGANS/TISSUES	Retain (•)	Weigh (√)	Examine (√)	ORGANS/TISSUES	Retain (•)	Weigh (√)	Examine (√)
Adrenals	•	√	√	Small intestine, duodenum	•		√
Animal identification	•			Small intestine, jejunum	•		√
Aorta (thoracic)	•		√	Small intestine, ileum	•		√
Blood	•		√	Spinal cord (cervical)	•		√
Bone marrow smears (3)	•			Spleen	•	√	√
Brain	•	√	√	Sternum + marrow	•		√
Cecum	•		√	Stomach	•		√
Colon	•		√	Testes	•a	√	√
Epididymides	•a		√	Thymus	•		√
Esophagus	•		√	Thyroid lobes + parathyroids	•		√
Eyes	•a		√	Tongue	•		√
Femur + marrow	•		√	Trachea	•		√
Heart	•	√	√	Urinary bladder	•		√
Kidneys	•	√	√	Uterus	•		√
Liver (2 lobes)	•	√	√	Vagina	•		√
Lungs + bronchi ( 2 Lobes)	•b		√				
Lymph node, mandibular	•		√	Abnormal findings	•		√
Lymph node, mesenteric	•		√				
Mammary gland (inguinal)	•		√	<b>Additional Tissues presented below</b>			
Optic nerves	•a		√	Harderian glands	•		√
Ovaries	•	√	√	Injection sites (tail vein)	•		√
Pancreas	•		√				
Pituitary	•		√				
Prostate	•		√				
Rectum	•		√				
Salivary gland (mandibular, subsublingual)	•						
Sciatic nerve	•		√				
Seminal vesicles	•		√				
Skeletal muscle	•						
Skin + subcutis (inguinal)	•		√				

Peer Reviewed – No

Histological Findings:

- Testicular hypocellularity of the seminiferous epithelium with epididymal hypospermia/aspermia in the epididymis, and decreased secretions in the prostate and seminal vesicles were observed at ≥0.20 mg/kg animals sacrificed on Day 18.

- Dose-dependent thymic atrophy was also noted in all treated groups, in both sexes.
- Fiber degeneration of the sciatic nerve was observed in two 0.25 mg/kg males.

Recovery: In animals sacrificed on Day 29, testicular hypocellularity persisted in all treated groups, and single fiber degeneration of the sciatic nerve was observed at doses  $\geq 0.20$  mg/kg in females, and at a dose of 0.25 mg/kg males.

Changes related to test article:

Test article-related changes were observed in the bone marrow, testes, epididymis, thymus, spleen and sciatic nerve, as described below:

Terminal Sacrifice – Day 18:

- Minimal/mild bone marrow hypocellularity was observed in 7/10 animals from 0.10 mg/kg, and moderate/severe hypocellularity in all 0.20 and 0.25 mg/kg animals.
- In the testes, dose-dependent minimal/moderate hypocellularity of the seminiferous epithelium, characterized by epithelial degeneration was observed in all 0.20 and 0.25 mg/kg animals. Hypocellularity of the seminiferous epithelium resulted in hypospermia/aspermia in the epididymis. Decrease of secretions in the prostate and seminal vesicles was observed in a subset of these animals, and was considered to be a secondary to the primary effects on the testes.
- Dose dependent thymic atrophy was noted as minimal in 4/10, and mild/moderate in 10/10 and 10/10 rats from the 0.10, 0.20 and 0.25 mg/kg respectively, and was characterized by necrosis/apoptosis of lymphoid tissue.
- Minimal fiber degeneration of the sciatic nerve in two 0.25 mg/kg males was reported.

Terminal Sacrifice – Day 29:

- Hypocellularity of seminiferous epithelium occasionally with focal calcification was minimal/mild in 3/5, moderate to severe in 3/3 and severe in 4/4 males from 0.10 mg/kg, 0.20 mg/kg and 0.25 mg/kg dose groups, respectively. Epithelial changes were characterized by atrophy of seminiferous epithelium. Hypospermia/aspermia in epididymis with increased luminal cell debris was also reported and regarded as a secondary change related to the primary effects on the testes.
- Dose-dependent increased incidence and severity of extramedullary hematopoiesis in the spleen was observed, with minimal severity reported in 3/10 males in the control group, and minimal/moderate severity in all treatment groups, indicating compensatory recovery from bone marrow hypocellularity.
- Minimal fiber degeneration of the sciatic nerve was observed in 1/8 and 3/9 animals from 0.20 and 0.25 mg/kg, respectively.

Special Evaluation - none

## Toxicokinetics (as excerpted from sponsor's report)

Dose (mg/kg)	0.10 <sup>a</sup>	0.20 <sup>a</sup>	0.25 <sup>a</sup>
	Male		
AUC <sub>0-24, Day 1</sub> (ng.hr/mL)	49.199 ±7.280	97.005 ±9.108	143.221 ±23.044
AUC <sub>0-24, Day 1/Dose</sub>	491.993 ±72.802 <sup>b</sup>	485.025 ±45.542 <sup>b</sup>	572.882 ±92.178 <sup>b</sup>
MRT <sub>last, Day 1</sub> (hr)	1.0 ±.2	5.0 ±1.0 <sup>c</sup>	4.1 ±0.1 <sup>c</sup>
AUC <sub>0-24, Day 15</sub> (ng.hr/mL)	35.077 ±9.891	59.321 ±14.186	77.401 ±4.269
AUC <sub>0-24, Day 15 /Dose</sub>	350.765 ±98.911 <sup>b,d</sup>	296.603 ±70.932 <sup>b,d</sup>	309.604 ±17.076 <sup>b,d</sup>
MRT <sub>last, Day 15</sub> (hr)	2.6 ±2.2	4.4 ±0.4 <sup>c</sup>	4.4 ±0.1 <sup>c</sup>
	Female		
AUC <sub>0-24, Day 1</sub> (ng.hr/mL)	37.658 ±6.033	82.152 ±9.504	100.266 ±8.312
AUC <sub>0-24, Day 1/Dose</sub>	376.580 ±60.326 <sup>b</sup>	410.759 ±47.521 <sup>b,c</sup>	401.062 ±33.249 <sup>b,c</sup>
MRT <sub>last, Day 1</sub> (hr)	1.2 ±0.1	5.2 ±0.5 <sup>c</sup>	5.0 ±0.3 <sup>c</sup>
AUC <sub>0-24, Day 15</sub> (ng.hr/mL)	16.515 ±1.779	49.923 ±1.535	61.401 ±5.362
AUC <sub>0-24, Day 15 /Dose</sub>	165.153 ±17.789 <sup>b,d</sup>	249.616 ±7.677 <sup>b,c,d</sup>	245.604 ±21.449 <sup>b,c,d</sup>
MRT <sub>last, Day 15</sub> (hr)	0.3 ±0.2	5.1 ±0.6 <sup>c</sup>	3.8 ±1.9 <sup>c</sup>

a Data expressed in mean ± standard deviation (n=4)

b Significant difference (p<0.05) compared to opposite sex

c Significant difference (p<0.05) compared to 0.1 mg/kg group

d Significant difference (p<0.05) compared to Day 1

MRT – mean residence time

**Summary:** Exposure generally increased with increasing dose. Day 1 values were consistently higher than Day 15 values. Gender-related differences were reported, with exposure in males shown to be higher than females.

#### Stability and Test Article Dosing Formulation Analysis:

- E7389 was stable in dosing vehicle at -20°C for at least five days and one freeze/thaw cycle.
- Results of analysis of two dosing solution samples were 1.6% and 14.4% below nominal (nominal = 0.1 mg/mL).

**Comment:** A 14.4% reduction is outside the normal acceptable criteria of ±10%. As such, actual doses received were reduced up to 14.4% from nominal doses.

### 6.2.2 Repeat-Dose Toxicity

Study title: E7389: A Chronic Intravenous Toxicity Study in Fischer 344 Rats

Study no.: 7640, 7640-DS  
 Study report location: eCTD - module 4.2.3.2.1  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: February 1, 2005  
 GLP compliance: yes  
 QA statement: yes  
 Drug, lot #, and % purity: E7389, Lot# BLDR001, 94.3% purity

### Key Study Findings

E7389 at 0.015, 0.05 and 0.15 mg/kg/day was administered by intravenous injection once weekly for 3 consecutive weeks, followed by a 14-day recovery period. This regimen was repeated for 6 cycles (18 doses). Mortality (20%-40%) occurred in all treatment groups, and as such a non-lethal dose was not identified in this study. Hypocellularity was observed in the epididymides, testis and bone marrow at 0.05 and 0.15 mg/kg. Enlarged spleen was reported at dose levels  $\geq 0.05$  mg/kg, and multifocal necrosis of the liver was reported at all dose levels. Hematological changes related to pharmacological effect on bone marrow were noted at 0.15 and 0.05 mg/kg/day. It should be noted that *all* animals were euthanized and assessed for macro- and microscopic pathology 14 days after the last dose, but not at the end of the dosing period. Clinical pathology, hematology and urinalysis sampling were also conducted at termination, 14 days after the last dose. As such, acute and/or transient pathology that occurred directly post dose was not assessed in this study.

### Methods

Doses: 0, 0.015, 0.05 or 0.15 mg/kg (0.9 mg/m<sup>2</sup>)  
 Frequency of dosing: once weekly for 3 consecutive weeks followed by a 14-day recovery period, repeated for a total of six times (a total of 18 doses)  
 Route of administration: intravenous injection  
 Dose volume: 4.29, 0.43, 1.43, 4.29 mL/Kg (for 1. 0.15, 0.05 and 0.15 doses, respectively)  
 Formulation/Vehicle: 5% ethanol in 0.9% NaCl  
 Species/Strain: Fischer 344  
 Number/Sex/Group: 15 rats/sex/group  
 Age: 10 to 12 weeks  
 Weight: 140 to 205 g for males; 137 to 165 g for females  
 Satellite groups: TK - 4 rats/sex/group (excluding control) were sampled at 5min, 1h, 2h, 4h, 8h, and 24h; on Days 1 and 141.  
 Unique study design: All animals were euthanized upon completion of the 6th cycle, after the final 2-week recovery period. No main study animals were euthanized

and assessed macro-/microscopically directly after the last dose, and as such acute (end-of-treatment) effects were not assessed.

Deviation from study protocol: Nasal cavities were retained for histopathological analysis, given macroscopic findings (respiratory distress, esophagectasis)

## Observations and Results

### Mortality

A number of deaths were reported in females (1 at 0mg/kg, 4 at 0.015mg/kg, 2 at 0.05mg/kg and 3 at 0.15mg/kg) on Days 105-164. These deaths were considered by the sponsor to be incidental, and were attributed to esophagectasis (animal choking on food), a syndrome specific to Fischer F344 rats. At necropsy stomach ingesta was found in esophagus and upper respiratory tract, and associated alveolar edema and histiocytosis were reported in histological analysis.

*Comment:* Although the number of deaths/group did not appear to increase with increasing dose, mortality in the treatment groups was significantly higher than the control group, indicating that they may be drug-related. Additionally, esophagectasis was not observed in any of the other repeat-dose toxicology studies using Fischer 344 rats.

### Clinical Signs:

No test article-related clinical signs were reported.

### Body Weights and Feed Consumption

Body weights were recorded on each day of dosing, and weekly thereafter.

There was a significant decrease in body weight gain or body weight loss, and associated decreased food consumption among animals at 0.15mg/kg, with some recovery apparent between treatments.

### Ophthalmoscopy

Conducted at pretreatment and week 24.

No test article-related changes were reported.

ECG – no studies conducted

**Laboratory investigations (hematology, coagulation, clinical chemistry and urinalysis):** were performed on all animals at termination (14 days post last dose).

*Comment:* Given that these assays were conducted 14 days post last dose, data regarding acute and transient drug effects were not obtained.

#### Hematology

Decreased RBC (as low as -11.6% vs. control) and related red blood cell parameters were noted and correlated with increases in reticulocytes (i.e. as high as 103.4% vs. control) at doses  $\geq 0.05$  mg/kg/day. There was no effect of E7389 on prothrombin time (PT) and activated partial thromboplastin time (APTT).

#### Clinical Chemistry

In high dose males and females increases in ALT (136.7% and 93.6% vs. control, respectively), AST (77.5% and 54.5% vs. control, respectively) and cholesterol (33.0% and 36.6% vs. control) were observed.

#### Urinalysis

No test article-related changes were reported.

#### Gross Pathology

Bilateral decrease in size of testes and epididymides in all rats at 0.15mg/kg, and in one rat at 0.015mg/kg.

#### Organ Weights

- A reduction in the weight of testes was reported at doses  $\geq 0.05$  mg/kg/day with histopathological correlates of the testicular hypocellularity of seminiferous epithelium and associated hypospermia/aspermia of the epididymides.
- Hypocellularity of bone marrow was also observed at doses  $\geq 0.05$  mg/kg/day, and was associated with compensatory increased extramedullary hematopoiesis and with increases of absolute and relative spleen weights.
- An increase in liver weights was reported in both sexes.

## Histopathology

## Adequate Battery –

All control and high-dose tissues were examined histologically. Target organs were then identified based on findings in the high dose group (testes, epididymis, liver, spleen, femur and sternum), and analyzed in low and mid-dose groups, as well.

Peer Review - yes

## Histological Findings:

Test article changes were observed in the testis, spleen, bone marrow and liver:

- At 0.15 mg/kg/day severe testicular hypocellularity of seminiferous epithelium and associated hypospermia/aspermia of the epididymides was reported. Minimal/mild effects were seen in epididymides at 0.05mg/kg.
- Hypocellularity of bone marrow was observed in males at doses  $\geq 0.05$ mg/kg.
- Extramedullary hematopoiesis was observed in the spleen at doses  $\geq 0.05$ mg/kg in both sexes; however the effect was more severe in males.
- Dose-related multifocal necrosis of the liver was observed at all doses, and was characterized by coagulative necrosis and inflammatory cell infiltrate.

Special Evaluation- **none**

Toxicokinetics (below Table excerpted from sponsor's report):

Dose <sup>a</sup> (mg/kg/day)	Day	Male		Female	
		C <sub>5min</sub> (ng/mL)	AUC <sub>0-t</sub> <sup>b</sup> (hr•ng/mL)	C <sub>5min</sub> (ng/mL)	AUC <sub>0-t</sub> <sup>b</sup> (hr•ng/mL)
Low Dose (0.015)	1	4.528 ± 0.290	NC	6.694 ± 2.256	NC
	141	12.320 ± 3.240	4.368 ~ 6.165	9.241 ± 0.978	4.701 <sup>c</sup>
Mid Dose (0.05)	1	17.675 ± 4.270	5.202 ~ 8.202	27.611 ± 4.964	12.271 ~ 17.970
	141	35.096 ± 5.820	16.868 ~ 19.230	37.170 ± 4.714	18.635 ~ 27.736
High Dose (0.15)	1	64.101 ± 16.789	42.633 ~ 64.005	67.841 ± 10.856	35.437 ~ 60.657
	141	113.022 ± 9.373	69.087 ~ 85.455	97.009 ± 22.315 <sup>d</sup>	60.368 ~ 89.261 <sup>d</sup>

<sup>a</sup>: Dose of E7389 expresses in equivalents of the bismesylate salt (1 mg of E7389 is equivalent to (b) (4)mg of the free base)

<sup>b</sup>: Results expressed as ranges

<sup>c</sup>: Results from one animal

<sup>d</sup>: Results from three animals

NC: Not calculated due to insufficient data

### Summary:

Samples were collected on Day 1 (1st dose of cycle 1) and Day 141 (1st dose of Cycle 6). Exposure increased with increasing dose. Exposure levels were higher on Day 141 as compared to Day 1 (AUC up to 1.7X), indicating some accumulation. No gender differences were apparent.

### Stability and Test Article Dosing Formulation Analysis –

The concentrations of the dosing solutions were analyzed, and the deviations from the intended concentrations were acceptable at <10%.

### 6.2.3 Repeat-Dose Toxicity

Study title: E7389: A Repeat Dose Intravenous Infusion Toxicity and Toxicokinetic Study in Beagle Dogs Followed by an up to 14-Day Observation Period

Study no.: 6288, 6288-TK  
 Study report location: eCTD - module 4.2.3.2.1  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: June 16, 2004  
 GLP compliance: yes  
 QA statement: yes  
 Drug, lot #, and % purity: E7389, Lot No. BLDR001

#### Key Study Findings-

E7389 at nominal doses of 0.02, 0.04, and 0.05mg/kg administered by 1-hour intravenous infusion to male and female Beagle dogs on Days 1, 8 and 15 resulted in reversible leukocytopenia, associated with a compensatory splenic extramedullary hematopoiesis. The maximum tolerated dose (MTD) was considered to be >0.05mg/kg nominal dose (actual dose is 0.034 mg/kg, or 0.68mg/m<sup>2</sup>).

It should be noted that all animals received 27-31% less than nominal dose. Additionally, all animals were euthanized/assessed macro- and microscopically 14 days post the last dose (Day 29). Accordingly, acute (end-of-treatment) or transient treatment-related effects occurring directly post dose were not assessed, and the toxicity profile for this study may be underestimated.

#### Methods:

Doses: 0.0, 0.02, 0.04, and 0.05 mg/kg (actual doses were 0.014, 0.028 and 0.034mg/kg<sup>1</sup>)  
 Frequency of dosing: Days 1, 8, and 15 (weekly for 3 weeks)  
 Route of administration: 1-hr. intravenous (IV) infusion

*Comment:* The infusion time in this dog study (1-hr) is not consistent with that proposed in the clinic (2-5min).

Dose volume: 1.5 mL/kg/hour for all groups  
 Formulation/Vehicle: 5% ethanol in 0.9% NaCl  
 Species/Strain: Dogs/Beagle  
 Number/Sex/Group: 3/sex/group  
 Age: 5 to 7 months  
 Weight: 5.8 to 7.8 kg for males; 5.0 to 7.7 kg for females  
 Satellite groups: None  
 Unique study design: None of the animals were assessed directly after

the dosing period (Day 15); rather all animals were euthanized 14 days post last dose on Day 29.

TK samples were collected from main study animals at pre-dose, 15 minutes, 1hr. , 1.1hr, 1.5 2, 4, 8 and 24 hours following the start of infusion. On Day 8, samples were collected pre-dose only.

Deviation from study protocol: <sup>1</sup>Nominal concentrations were 0.013, 0.027 & 0.033 mg/mL. Actual concentrations were reduced by a mean of -27.9%, -29.7% and -31.2%, respectively due to formulation problems.

#### Observations and Results

Mortality – no test article-related effects reported.

Clinical Signs - no test article-related effects reported.

Body Weights - no test article-related effects reported.

Feed Consumption - no test article-related effects reported.

Ophthalmoscopy - no test article-related effects reported.

ECG – not conducted

Laboratory investigations (hematology, coagulation, clinical chemistry and urinalysis): performed on all animals on Day 18 and Day 29.

Hematology – On Day 18, 0.05 mg/kg males and females, and 0.04 mg/kg females exhibited slight to moderate reductions in total white cell count (57 to 63%) and neutrophil count (42 to 56%), which were reversible by Day 29.

Clinical Chemistry - no test article-related effects reported.

Urinalysis - A statistically elevated value for specific gravity in 0.05 mg/kg females was reported on Day 29.

Gross Pathology - All dogs were euthanized on Day 29 (at the end of the recovery period). No test article-related effects were reported.

*Comment* – All dogs were euthanized at the end of recovery, and as such treatment-related effects were not assessed directly after completion of dosing.

**Organ Weights** –Dose-related increases in absolute and/or relative spleen weights were noted in the males and females at all dose levels.



Histological Findings - Splenic extramedullary hematopoiesis was reported in 1/6, 1/6 and 6/6 dogs at nominal doses 0.02, 0.04 and 0.05 mg/kg, respectively. This was associated with dose-related increases in group mean spleen weights.

Special Evaluation - none

Toxicokinetics (as excerpted from sponsor's report)

Dose (mg/kg)	0.02 <sup>a</sup>	0.04 <sup>a</sup>	0.05 <sup>a</sup>
	Male		
AUC <sub>0-24, Day 1</sub> (ng·hr/mL)	6.484 ± 1.832	25.592 ± 5.789	22.359 ± 3.080
AUC <sub>0-24, Day 1</sub> /Dose	324.200 ± 91.587	639.792 ± 144.718	447.187 ± 61.594
MRT <sub>last, Day 1</sub> (hr)	1.2 ± 0.4	3.7 ± 0.4 <sup>b</sup>	3.2 ± 0.6 <sup>b</sup>
AUC <sub>0-24, Day 15</sub> (ng·hr/mL)	8.887 ± 3.152	22.978 ± 5.316	27.950 ± 2.413
AUC <sub>0-24, Day 15</sub> /Dose	444.333 ± 157.598	574.442 ± 132.910	559.000 ± 48.264
MRT <sub>last, Day 15</sub> (hr)	2.1 ± 1.8	3.7 ± 0.3 <sup>b</sup>	3.6 ± 0.1 <sup>b</sup>
	Female		
AUC <sub>0-24, Day 1</sub> (ng·hr/mL)	6.807 ± 2.128	19.073 ± 4.020	29.196 ± 7.600
AUC <sub>0-24, Day 1</sub> /Dose	340.333 ± 106.401	476.833 ± 100.512	583.920 ± 152.005 <sup>b</sup>
MRT <sub>last, Day 1</sub> (hr)	2.1 ± 2.0	2.8 ± 1.4	3.6 ± 0.3 <sup>b</sup>
AUC <sub>0-24, Day 15</sub> (ng·hr/mL)	5.518 ± 0.659	14.495 ± 2.111	26.627 ± 2.292
AUC <sub>0-24, Day 15</sub> /Dose	275.900 ± 32.926	362.375 ± 52.775	532.533 ± 45.836 <sup>b</sup>
MRT <sub>last, Day 15</sub> (hr)	0.9 ± 0.1	2.0 ± 1.9	3.7 ± 0.4 <sup>b</sup>

<sup>a</sup> Data expressed in mean ± standard deviation (n = 3)

<sup>b</sup> Significant difference (p < 0.05) compared to 0.02 mg/kg group

Summary: Exposure to E7389 in dogs increased with an increase in dose, and accumulation of E7389 in dogs was not observed. No gender effects were apparent.

Stability and Test Article Dosing Formulation Analysis –

Dosing solutions were stable for six hours at room temperature. Analysis of the dose formulation samples revealed significant deviations from the nominal concentrations, ranging from -31.6 to -23.1% for 0.013 mg/mL nominal, -43.8 to -17.6% for 0.027 mg/mL, and -41.1 to -20.2% for 0.034 mg/mL nominal concentrations. These results were not in acceptable range, and intended doses were not achieved. Dosing solutions were found to be stable for six hours at room temperature.

*Comment:* The mean deviation from nominal concentrations were -27.85, -29.7 and -31.2% for 0.013, 0.027 and 0.034 mg/mL, respectively. Therefore calculated actual concentrations were 0.009, 0.019 and 0.023 mg/mL, and resultant actual doses were 0.014, 0.028 and 0.034mg/kg. Referenced doses throughout the sponsor's report are identified by using the nominal dose designations, rather than actual.

### 6.2.4 Repeat-Dose Toxicity

Study title: E7389: A Chronic Intravenous Toxicity Study in the Beagle Dog

Study no.: 6528  
 Study report location: eCTD - 4.2.3.2.1  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: February 1, 2005  
 GLP compliance: yes  
 QA statement: yes  
 Drug, lot #, and % purity: E7389 (b) (4) mesylate salt), Lot No. BLDR001, 94.3% purity

#### Key Study Findings

No mortality was observed in this study. Test article-related changes were observed in the testes (degeneration of seminiferous epithelium), epididymides (hypospermia), bone marrow (hypercellularity), thymic atrophy and lymphoid tissue (depletion) at 0.045 mg/kg. Hematological changes were noted at doses  $\geq 0.015$  mg/kg/day. The NOAEL was 0.015 mg/kg/day based on changes in the testis, bone marrow and lymphoid tissue.

It should be noted that all animals were necropsied 14 days post the last dose. Accordingly, acute or transient treatment-related effects occurring directly post dose were not assessed, and the toxicity profile for this study may be underestimated.

#### Methods

Doses: 0, 0.0045, 0.015 and 0.045 mg/kg  
 Frequency of dosing: once weekly for 3 consecutive weeks, followed by a 14-day recovery period. This regimen was repeated for a total of six times for a total of 18 doses.  
 Route of administration: 1-hour intravenous infusion  
*Comment: differs from clinical infusion which will be delivered over 2-5 minutes.*  
 Dose volume: 1.29, 0.13, 0.43 and 1.29 mL/kg  
 Formulation/Vehicle: 5% ethanol in 0.9% NaCl  
 Species/Strain: Beagle Dog  
 Number/Sex/Group: 4 dogs/sex/group  
 Age: Not reported  
 Weight: 4.9 to 7.3 kg for males; 4.6 to 6.1 kg for females  
 Satellite groups: On Days 1 and 141, blood samples were collected as following: pre-dose, 15 minutes, 1, 1.083 (1 hour and 5 min), 1.5, 2, 4, 8 and 24 hours post the start of infusion

Unique study design: All dogs were euthanized upon completion of the 6th cycle (i.e. after the final 2-week recovery period). As such, acute or transient effects were not assessed.

## Observations and Results

Mortality – no deaths reported.

Clinical Signs – a dose-dependent increase in the incidence of fecal changes and emesis was observed in all treatment groups, compared to the control.

Body Weights– no test article-related effects reported.

Feed Consumption – no test article-related effects reported.

Ophthalmoscopy — no test article-related effects reported.

ECG – no test article-related effects reported.

**Laboratory investigations (Hematology, Coagulation, Clinical chemistry and Urinalysis):** performed on all animals prior to start of treatment and on Days 18, 158 and 169 (7 days after last dose).

### Hematology

Decreases in white blood cell counts, hemoglobin, and reticulocytes were observed in animals treated at 0.045 mg/kg/day. At 0.015mg/kg there was a reduction in reticulocytes in males.

- A decrease in white blood cell count (WBC) was noted on Day 18 and Day 158 for males (- 36.4% and 41% vs. controls, respectively) and Day 158 for females (-28.1% vs. controls).
  - This change corresponded to a decrease primarily in neutrophils (as low as -41.5% vs. controls) and lymphocytes (as low as -43.9% vs. controls).
- A decrease in hemoglobin on Day 18 and 158 in males and females (as low as - 6.5% on Day 18, and -15.8% on Day 158, as compared to controls).
- A reduction in the reticulocytes on Days 18 and 158 (as low as -80.0% or -83.9% respectively vs. controls) was noted at 0.045 mg/kg/day, in both sexes. A slight

decrease (as low as -40.3% vs. controls) in reticulocytes was also noted in male dogs at 0.015 mg/kg.

- On Day 169 (7 days after the last dose), recovery from the above changes was reported, with a compensatory increase in WBC (as high as 24.6% vs. controls).
- No test article-related effects on coagulation parameters were reported.

Clinical Chemistry– no test article-related effects reported.

Urinalysis– no test article-related effects reported.

Gross Pathology – Increased incidence of small thymus was found in 1/4 male and 2/4 females treated at 0.045 mg/kg/day.

Organ Weights –

A significant decrease (up to -36.2%) in testes weight (relative to body weight) was reported at doses  $\geq 0.045$  mg/kg/day, as compared to controls.

Histopathology-

Adequate Battery (below Table excerpted from sponsor’s protocol):

ORGANS/TISSUES	Retain (*)	Weigh (√)	Examine (€)
Adrenals	•	√	€
Animal identification	•		
Aorta (thoracic)	•		€
Blood			
Bone marrow smears (3)	•		
Brain	•	√	€
Cecum	•		€
Colon	•		€
Epididymides	*a		€
Esophagus	•		€
Eyes	*a		€
Femur + marrow	•		€
Gallbladder	•		€
Heart	•	√	€
Kidneys	•	√	€
Liver (2 lobes)	•	√	€
Lungs + bronchi ( 2 Lobes)	*b		€
Lymph node, bronchial	*c		€
Lymph node, mandibular	•		€
Lymph node, mesenteric	•		€
Mammary gland (inguinal)	•		€
Optic nerves	*a		€
Ovaries	•	√	€
Pancreas	•		€
Pituitary	•		€
Prostate	•		€
Rectum	•		€

ORGANS/TISSUES	Retain (*)	Weigh (√)	Examine (€)
Salivary gland (mandibular)	•		€
Sciatic nerve	•		€
Skeletal muscle	•		€
Skin + subcutis (inguinal)	•		€
Small intestine, duodenum	•		€
Small intestine, jejunum	•		€
Small intestine, ileum	•		€
Spinal cord (cervical)	•		€
Spleen	•	√	€
Sternum + marrow	•		€
Stomach	•		€
Testes	*a	√	€
Thymus	•		€
Thyroid lobes + parathyroids	•		€
Tongue	•		€
Trachea	•		€
Urinary bladder	•		€
Uterus	•		€
Vagina	•		€
Abnormal findings	•		€
<b>Additional Tissues presented below</b>			
Lacrimal glands	•		€
infusion sites	•		€

Peer Review - no

Histological Findings –

- Minimal to moderate hypocellularity of the seminiferous epithelium with cell degeneration and accompanying regeneration was observed in all males at 0.045 mg/kg/day. Dose-dependent hypospermia/aspermia of the epididymides with increased luminal cell debris was noted at all dose levels. These changes were consistent with the effects noted in the absolute and relative (to body weight) testes weights.
- Minimal to mild hypercellularity of the bone marrow was observed 1/4 males and 3/4 females treated at 0.045 mg/kg/day.
- Minimal to mild thymic atrophy was found in 2/4 males at 0.045 mg/kg/day, and lymphoid depletion of the mesenteric lymph nodes was reported in all males and 2/4 females at 0.045 mg/kg/day. Increased frequency of lymphoid depletion in the iliac Peyer’s patches was observed in all males at 0.045 mg/kg/day.

- In the spleen, increased extramedullary hematopoiesis was found in 3/4 males and 4/4 females at 0.045 mg/kg/day.
- In the liver, increased extramedullary hematopoiesis was found in 1/4 males and 3/4 females at 0.045 mg/kg/day.

Special Evaluation - **none**

Toxicokinetics (as excerpted from sponsor's report)

Dose <sup>a</sup> (mg/kg)	Day	Male		Female	
		C <sub>1hr</sub> (ng/mL)	AUC <sub>0-t</sub> <sup>b</sup> (hr•ng/mL)	C <sub>1hr</sub> (ng/mL)	AUC <sub>0-t</sub> <sup>b</sup> (hr•ng/mL)
Low Dose (0.0045)	1	1.049 ± 0.174	0.756 ~ 1.224	1.967 ± 1.114	1.179 ~ 2.262
	141	2.066 ± 0.370	1.459 ~ 9.046	2.005 ± 0.463	1.662 ~ 2.401
Mid Dose (0.015)	1	4.040 ± 1.341	5.017 ~ 6.799	4.318 ± 0.671	3.930 ~ 6.124
	141	6.174 ± 0.629	6.268 ~ 13.079	5.485 ± 1.218	4.531 ~ 10.447
High Dose (0.045)	1	11.393 ± 3.413	19.743 ~ 25.017	14.575 ± 3.972	17.077 ~ 29.088
	141	18.300 ± 3.290	27.391 ~ 30.663	15.766 ± 2.450	16.339 ~ 24.078

<sup>a</sup>: Dose of E7389 expresses in equivalents of the bismesylate salt (1 mg of E7389 is equivalent to (b) (4) mg of the free base)

<sup>b</sup>: Results expressed as ranges

Summary: Exposure increased with increasing dose. Exposure on Day 141 was generally higher than that noted on Day 1 in both sexes, indicating some accumulation (AUC, up to 1.4X). There was no gender difference in exposure between males and females on Day 1, but the values were slightly higher for males on Day 141.

Stability and Test Article Dosing Formulation Analysis –

E7389 was stable in dosing vehicle at -20°C for at least five days, and for one freeze/thaw cycle. Analysis of dosing formulation samples revealed deviations that were in acceptable range (-6.3 to + 9.5% of nominal concentrations).

### 6.2.5 Repeat-Dose Toxicity

Study title: E7389: A 4-Week Intermittent Intravenous Toxicity Study of Impurity-containing Drug Substance in Rats

Study no.: S09043  
 Study report location: eCTD - 4.2.3.2.1  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: June 9, 2009  
 GLP compliance: yes  
 QA statement: yes  
 Drug, lot #, and % purity: E7389, Lot # 194P1404, Purity: 92.4%

### Key Study Findings

E7389 test article with high impurity content (Lot No. 194P1404) was administered intravenously at doses of 0.10 or 0.20 mg/kg on Days 1, 8, and 15 to male and female F-344 rats. The dosing regimen was the same as the previous 4-week intermittent intravenous toxicity study using the batch with less impurity content (Batch No. BLDR001, Purity: 96.2%). The primary findings observed in this study were comparable with those in the previous study, including bone marrow hypocellularity, thymic atrophy and testicular hypocellularity. The cytopenia observed at 0.20 mg/kg was severe and considered the dose-limiting toxicity. In conclusion, the presence of high impurity contents did not affect the toxicological profile of E7389 under the present study conditions.

### Methods

Doses: 0, 0.10 or 0.20 mg/kg (0.6 or 1.2 mg/m<sup>2</sup>, respectively).  
 Frequency of dosing: Days 1, 8, and 15  
 Route of administration: IV  
 Dose volume: 2.0, 1.0 and 2.0 mL/kg, respectively  
 Formulation/Vehicle: E7389 was dissolved in 5% final volume of (b) (4) ethanol, and then the solution was added to 0.9% Sodium Chloride for Injection  
 Species/Strain: Fischer 344 (F-344) rats  
 Number/Sex/Group: 10 rats/sex/group  
 Age: 6 weeks  
 Weight: 140 to 166g in males, 117 to 128g in females  
 Satellite groups: 5 of the 10 rats/sex/group were necropsied on either Day 18 (3 days post last dose), or Day 29 (14 days post last dose).  
 Unique study design: The purpose of the present study was to assess the toxicity of E7389 test article with high impurity content.  
 Deviation from study protocol: None deviations reported that impacted the study

## Observations and Results

Mortality - none

Clinical Signs – no test-article effects reported

### Body Weights-

Decrease in body weight at 0.20 mg/kg was observed in males and females on Day 21 (up to -8.4%). No body weight effects were observed on Day 29.

Feed Consumption– no test-article effects reported

Ophthalmoscopy– no test-article effects reported

ECG – not conducted

### Hematology

#### *Changes related to test article:*

Test article-related hematological changes were reported in both sexes at doses  $\geq 0.10$  mg/kg on Day 18, and included reduction in white blood cells (up to -51%), red blood cells (up to -25%), hemoglobin concentration (up to -23%), and reticulocytes (up to -97%). The leukopenia observed in this study was primarily due to reductions in neutrophil and lymphocyte counts. These changes were associated with dose-dependent bone marrow hypocellularity.

Recovery: Following the 14-day observation period, reduction in red blood cells and hemoglobin, and a compensatory increase in reticulocytes and splenic extramedullary hematopoiesis were reported.

**Text Table 2 Test article-related hematological changes (Sacrificed on Day 29)<sup>§</sup>**

Sex	Parameter	Dose (mg/kg)	Male			Female		
			0	0.10	0.20	0	0.10	0.20
	RBC counts (10 <sup>6</sup> /μL)		8.30	-3.0	-6.1**	8.27	-3.9*	-7.4**
	Hemoglobin (g/dL)		14.2	-0.7	-2.1	14.5	-3.4*	-4.1*
	Reticulocyte counts (10 <sup>9</sup> /L)		170.6	31.4*	75.6**	146.5	34.7**	69.8**
	WBC counts (10 <sup>3</sup> /μL)		4.64	-19.0	-16.4	2.75	-7.6	-6.2

<sup>§</sup> For controls, group means. For treated groups, percent difference from controls.

Significantly different from control (0 mg/kg) group: \* p<0.05, \*\* p<0.01 (Dunnett's test)

(As excerpted from the sponsor's report)

### Clinical Chemistry

A statistically significant increase in aspartate aminotransferase (AST) was observed in males at 0.10mg/kg (+24%) and 0.20 mg/kg (+65%).

### Gross Pathology

On Day 18, test article-related changes included small testes which were observed in one male at 0.20 mg/kg, and soft testes in three males at 0.20mg/kg. Enlarged spleen was observed in one male at 0.20 mg/kg.

Recovery: Small testes and epididymides were observed in all males at 0.20 mg/kg on Day 29.

### Organ Weights

The liver, kidneys, heart, spleen, brain, testes, ovaries, adrenals were weighed at necropsy. Test article-related changes in testes weight (absolute) were reported at 0.1mg/kg (-10%), and at 0.20mg/kg (-32%), as compared to controls. Increased spleen weight (64%) was observe in 1/5 males at 0.20mg/kg.

Statistically significant changes were noted in absolute adrenal, heart and liver weights and relative kidney and liver weights in males and/or females at 0.10 and/or 0.20 mg/kg; however, no histopathological correlates were found.

Recovery: A 12% and 49% decrease in testes weight was reported at 0.10 and 0.20mg/kg, respectively, as compared to controls.

## Histopathology

## Adequate Battery -yes

All organs in the control and high-dose groups were assessed. Only bone marrow, thymus, spleen, testes, and epididymides, and organs with macroscopic findings were assessed in the 0.10 mg/kg group.

## Peer Review - No

## Histological Findings:

Primary test article-related changes were limited to bone marrow, thymus, and testes.

- Dose-dependent bone marrow hypocellularity was observed in all animals at doses  $\geq 0.10$  mg/kg.
- Increased splenic extramedullary hematopoiesis observed in one male at 0.20 mg/kg, and was considered secondary to a compensatory response to bone marrow hypocellularity.
- Dose-dependent thymic atrophy was observed in all treatment animals.
- Dose-dependent hypocellularity (epithelial degeneration) of the testes seminiferous epithelium was observed in 3 males at 0.10 mg/kg, and all males at 0.20 mg/kg. Associated intratubular cellular debris and/or hypospermia/aspermia in the epididymides were noted in these animals.

Recovery: Testicular effects persisted in all males. Compensatory splenic extramedullary hematopoiesis was reported at doses  $\geq 0.10$  mg/kg. No bone marrow effects were noted on Day 29.

Special Evaluation –

Impurity levels in Lot 194P1404 (as excerpted from Sponsor’s report):

Tests	Results
Appearance	White powder
Identification (IR)	Conformed to standard
HPLC Impurities (wt%)	
(b) (4)	
Unspecified Impurities	(b) (4)
Total Impurities	
(b) (4)	
Specific Rotation	(b) (4)
(b) (4)	
Assay (HPLC)	

Toxicokinetics – none evaluated in this study.

## Stability and Test Article Dosing Formulation Analysis –

The stability of the dosing solutions at room temperature for 4 hours was confirmed. Analysis of dosing solutions confirmed that concentrations were within acceptable range.

## 7 Genetic Toxicology

*Overall Summary:* E7389 was found to be positive in two independently conducted 5178Y/TK Mouse Lymphoma Mutagenesis assays. It was also strongly positive in the *in vivo* rat micronucleus assay, indicating its potential for induction of chromosome damage. The generally large-sized micronuclei induced by E7389 in this assay indicated that the effect was due primarily to chromosome segregation interference rather than chromosome breakage. This is consistent with the mode of action for this drug class (microtubule inhibitor). Although E7389 is genotoxic, no long-term carcinogenicity studies will be required given the proposed clinical patient population.

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

Study title: **E7389: Bacterial Mutation Test**

Study no.:	Project No. 960905
Study report location:	eCTD - 4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 14, 2005
GLP compliance:	Yes, with following exceptions: The bulk test article was not characterized under GLP conditions. The concentration and stability of stock solution was conducted at Eisai (Wilmington, MA) under GLP conditions.
QA statement:	yes
Drug, lot #, and % purity:	BLDR001

Key Study Findings:

E7389 did not show any evidence of genotoxic activity in this *in vitro* mutagenicity assay when tested in accordance with regulatory guidelines.

## Methods

Strains: TA1535, TA1537, TA98, TA100, WP2 *trp*  
*uvrA*

Concentrations in definitive study: 78, 156, 312, 625, 1250, 2500,  
5000 $\mu$ g/plate (standard limit dose)

Basis of concentration selection: Based on initial test results

Negative control: Vehicle

Positive control: In absence of S9: Sodium azide, 2-Nitrofluorene (2NF), 4-Nitroquinoline N-oxide (NQO)  
In presence of S9: 2-Aminoanthracene (2AA), Benzo[a]pyrene (BaP)

Formulation/Vehicle: Dimethyl sulfoxide (DMSO)

Incubation & sampling time: 48 to 72 h, immediately post incubation

## Study Validity

- Analysis of the stock solution (50 mg/mL) and lowest dose level (0.391 mg/mL) formulations in the first test and third tests (Note: 2<sup>nd</sup> test did not meet criteria and was repeated) confirmed that nominal concentrations were effectively achieved (analyzed concentrations were within  $\pm 10\%$  of the nominal values).
- Visible thinning of the background lawn of non-revertant bacteria was obtained following exposure to E7389 in the pre-incubation version of the test, indicating that the test article was toxic towards several bacterial strains at the highest dose level(s) tested.
- The mean revertant colony counts for the vehicle controls were close to or within the laboratory historical control range. Appropriate positive control compounds (with S9 mix where required) induced increases in revertant colony numbers to at least twice the concurrent vehicle control levels with the appropriate bacterial strain (1.5 $\times$  for strain TA100), confirming sensitivity of the test system and activity of the S9 mix.

## Results:

E7389 did not cause any substantial increase in the revertant colony counts of any strain using either the plate-incorporation or pre-incubation versions of the bacterial mutation test in the absence or presence of S9 mix.

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

Study title: **E7389: Reverse Mutation Assay of Impurity-containing Drug Substance in Bacteria**

Study no.: S09044

Study report location: eCTD - 4.2.3.3.1

Conducting laboratory and location: (b) (4)

Date of study initiation: July 1, 2009

GLP compliance: Stated to be GLP, but no QA statement

QA statement: No

Drug, lot #, and % purity: E7389, Lot # 194P1404, Purity: 92.4%

### Key Study Findings

E7389 containing high impurity content, incubated either in the presence or absence of S9 mix, exhibited no mutagenic effects on *Salmonella typhimurium* TA100, TA1535, TA98 and TA1537, or *Escherichia coli* WP2 *uvrA*(pKM101) strains. These negative responses were the same as those of the previous test with E7389 containing less impurities (Lot Number: BLDR001, Purity: 96.2%).

## Methods

Strains: TA100, TA1535, TA98, TA1537 and WP2 *uvrA*(pKM101)

Concentrations in definitive study: 0 (control), 78, 156, 313, 625, 1250, 2500 and 5000 µg/plate

Basis of concentration selection: Based on previous test conducted by (b) (4) (Project No. 960905) in which doses used were 78 to 5000 µg/plate, at a common ratio of 1/2.

Negative control: Dimethyl sulfoxide (DMSO)

Positive control: For TA100, TA98 and WP2 *uvrA*(pKM101) in the absence of S9 mix: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (AF2).  
For TA1535 in the absence of S9 mix: Sodium azide (SA).  
For TA1537 in the absence of S9 mix: 9-Aminoacridine hydrochloride (9AA).  
For all strains in the presence of S9 mix: 2-Aminoanthracene (2AA).

Formulation/Vehicle: AF2 at 0.05, 0.1 and 1 µg/mL, 9AA at 800 µg/mL, and 2AA at 5, 10 and 20 µg/mL were prepared by dissolution in DMSO. SA was dissolved in water for injection at 5 µg/mL.

Incubation & sampling time: 48 hours

## Study Validity

- In the absence of S9 mix, cytotoxicity (a reduction in the number of revertants and clearing or diminution of the background lawn) was observed at 1250 µg/plate or more in TA100, TA1535, and TA1537 strains, and 5000 µg/plate in WP2 *uvrA*(pKM101) strain, but was not observed at up to 5000 µg/plate in TA98 strain.
- Precipitate was not observed at up to 5000 µg/plate in all of bacterial strains.
- The numbers of revertants for the negative and positive controls were similar to the historical data. No bacterial contamination was observed in any of the plates.

## Results:

There was no increase in the number of revertants generated following exposure to E7389 containing high impurity content in any bacterial strains tested with or without S9 mix. This negative response was the same as that of the previous test conducted by (b) (4) (Project No. 960905)1), in which Lot Number BLDR001 was used. Therefore, the level of high impurity content contained in the current lot (Lot Number: 194P1404) did not affect the potential of E7389 to induce gene mutations in all of bacterial strains.

### 7.3 *In Vitro* Chromosomal Aberration Assays in Mammalian Cells

**Study title: 5178Y/TK Mouse Lymphoma Mutagenesis Assay:**

Study no.: AA37RJ.703.BTL  
 Study report location: eCTD - 4.2.3.3.1  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: December 19, 2000  
 GLP compliance: Yes, with the following exception:  
 Characterization, stability analysis or concentration analysis of test or control mixtures of test article were not performed  
 QA statement: Yes  
 Drug, lot #, and % purity: NSC D707389-K/D4, Lot 570-285D (NCI contracted study)

**Key Study Findings:**

NCI drug NCS D707389-K/D4 was found to be weakly positive in this test. Increased frequency of small colonies (as well as medium and large) was observed indicating gene mutation/chromosomal damage and associated functional loss.

**Methods**

Cell line: L5178Y cells, clone 3.7.2C  
 Concentrations in definitive study: 0.025, 0.05, 0.075, 0.1, 0.15 and 0.2µg/mL (w/o activation); 0.3, 0.4, 0.5, 0.6 and 0.7µg/mL (with activation)  
 Basis of concentration selection: Based on preliminary toxicity assay (toxicity measured as suspension growth relative to (b) (4) control), using a 4-hour exposure of test article concentration ranging from 0.005 to 50µg/mL.  
 Negative control: Vehicle  
 Positive controls: MMS (10 and 20µg/mL) and 7,12-DMBA (1.0 and 1.5µg/mL)  
 Formulation/Vehicle: Dissolved in ethanol at maximum concentration of 5mg/mL, and subsequent dilutions were prepared in sterile distilled water.  
 Incubation & sampling time: 4-hour incubation/exposure time

**Study Validity:**

Criteria for validation of mutagenesis assay are as follows:

- Negative Controls: The spontaneous mutant frequency of the (b) (4) control cultures must be within 20 to 100 TFT-resistant mutants for 10 surviving cells. The cloning efficiency of the (b) (4) control group must be greater than 50% and less than 130%.
- Positive Controls: At least one concentration of each positive control must exhibit mutant frequencies greater than twice the background level.
- Test Article-Treated Cultures: There must be at least four analyzable concentrations with cloning efficiency between 10% and 130%, and total growth greater than 1%.

#### Results:

Two non-activated ( $\geq 0.15 \mu\text{g/mL}$ ) and two S9-activated cloned cultures ( $\geq 0.4 \mu\text{g/mL}$ ) exhibited mutant frequencies that were at least twice that of the (b) (4) control. The data on colony size distributions showed an increase in the frequency of small, medium and large colonies, as compared to (b) (4) control cultures. A dose-response trend was not observed. Small colony frequency is consistent with chromosomal damage associated with functional loss of the TK locus. Thus the test article was considered weakly positive.

### 7.4 *In Vitro* Chromosomal Aberration Assays in Mammalian Cells

#### Study title: **E7389: Mouse Lymphoma TK Assay of Impurity-containing Drug Substance**

Study no.:	Study # S09045
Study report location:	eCTD - 4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 9, 2009
GLP compliance:	Stated to be conducted GLP, but no QA statement was provided
QA statement:	No
Drug, lot #, and % purity:	E7389, Lot 194P1404, 92.4% purity

#### Key Study Findings:

E7389 containing high impurity content showed gene mutations and/or clastogenic effects when incubated in the presence or absence of S9 mix in this mouse lymphoma TK assay.

## Methods

Cell line:	L5178Y TK+/- cells derived from mouse lymphoma
Concentrations in definitive study:	- With activation (short)=0.6, 1.2, 2.4, 2.7, 3, 3.3, 3.6, 3.9, 4.2, 4.5µg/mL - Without activation = 0.3, 0.6, 0.9, 1.2, 1.5, 1.8, 2.1µg/mL - Without activation (continuous) = 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08µg/mL
Basis of concentration selection:	Preliminary test conducted by Eisai using concentration of 0.002 to 2.0 µg/mL
Negative control:	Dehydrated ethanol, water for injection
Positive control:	With S9 Activation: Cyclophosphamide monohydrate (CP 2µg/mL). Without S9 activation: Methyl methanesulfonate (MMS, 5 or 20µg/mL). Both MMS and CP dissolved in DMSO.
Formulation/Vehicle:	E7389 dilutions from 0.4 to 450 µg/mL in 5% ethanol/ Vehicle is 5% ethanol
Incubation & sampling time:	3 hours for short, 24 hours for continuous

## Study Validity

### Validity:

- In the range-finding cytotoxicity test, a marked decrease (approximately 80%) in relative survival was observed at concentrations of 2 µg/mL in the absence of S9 mix- short treatment method; and 0.06 µg/mL in the absence of S9 mix - continuous treatment method, but was not observed at up to 2 µg/mL in the presence of S9 mix- short treatment method.
- In the mutagenicity test, the negative and positive control mutant frequencies were similar to those in the historical data.
- E7389 was assayed up to concentrations which produced a marked decrease (more than 80%) in relative total growth in both the short treatment method in the presence or absence of S9 mix; and the continuous treatment method in the absence of S9 mix.

### Results:

Statistically significant increases in mutant frequency were observed at concentrations of 3.3 to 3.9 µg/mL in the presence of S9 mix, and 1.2 µg/mL in the absence of S9 mix in the short treatment method; and 0.06 µg/mL in the continuous treatment method in the absence of S9 mix. These values reached levels from 1.9- to 2.6-fold, compared to

the concurrent negative control. A concentration-dependent increase was also reported in all treatment methods. The percentage of small colony was similar in all treatment methods, and therefore, the genotoxic potential was characterized as gene mutations and/or clastogenic effects.

## 7.5 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: **E7389: Rat Micronucleus Test**

Study no.: 960994

Study report location: eCTD - 4.2.3.3.1

Conducting laboratory and location:

(b) (4)

Date of study initiation: February 21, 2006

GLP compliance: Yes,

QA statement: Yes

Drug, lot #, and % purity: E7389 (Lot No.: BLDR001)

### Key Study Findings:

Fischer male rats were administered E7389 at 0, 0.05, 0.13, 0.5 mg/kg (or 0, 0.3, 0.78 and 3mg/m<sup>2</sup>), and animals were euthanized either 24 or 48 hours after treatment. Examination of bone marrow smears showed strong evidence of genotoxic activity in this *in vivo* test for induction of chromosome damage. The generally large-sized micronuclei induced by E7389 indicated that the effect was due primarily to chromosome segregation interference rather than chromosome breakage, consistent with the mode of action for this drug class (anti-tubule).

### Methods

Doses in definitive study: 0, 0.05, 0.13, 0.5 mg/kg (or 0, 0.3, 0.78 and 3mg/m<sup>2</sup>)

Frequency of dosing: single administration (animals were euthanized 24 or 48 hours after treatment and bone marrow smear obtained)

Route of administration: IV (slow bolus over 1-2 min)

Dose volume: 5, 0.5, 1.3 and 5 mL/kg

Formulation/Vehicle: Test article concentration was 0.1 mg/mL prepared in vehicle of 5% EtOH/0.9% NaCl

Species/Strain: Fischer F-344 male rats

Number/Sex/Group: 10 males/group, 5/sampling time (24 or 48 hours)

Satellite groups: none

Basis of dose selection: Based on the results of a multiple dose range-finding study (Study No. G465520A) in which the MTD of a

single intravenous dose was determined to be 0.5 mg/kg  
Negative control: Vehicle  
Positive control: Concurrent positive control group were treated with Cyclophosphamide (CP, 20mg/mL) orally by intragastric gavage at 10mL/kg; animals euthanized at 24 hours.

### Study Validity

- The dose formulation of test article used in this study met the acceptance criteria of being within  $\pm 10\%$  of nominal concentration. Test article was not detected in the vehicle control sample.
- The positive control (CP) caused large, highly significant increases ( $p \leq 0.001$ ) in the frequency of MN-PCEs, confirming sensitivity of the system.
- Animals treated with E7389 did not show any substantial increases in the incidence of MN-NCEs at either sampling time. The incidence of MN-NCEs for all groups was uniformly low, confirming the absence of micronucleus-like artifacts.

### Results:

- Animals treated with E7389 showed highly statistically significant increases in the number of micronucleated polychromatic erythrocytes (MN-PCEs) at either sampling time ( $p \leq 0.001$ ). Individual and group mean values for animals treated with the test article generally fell out of the historical range for control animals.
- Animals treated with E7389 showed highly significant decreases in the proportion of PCEs at either sampling time ( $p \leq 0.001$ ), which may indicate some degree of bone marrow depression.
- Micronucleus sizes for animals treated with E7389 were generally large compared with those induced by the clastogenic agent Cyclophosphamide.
- A slight decrease in body weight gain was noted at the highest dose.

## 8 Carcinogenicity – No studies conducted.

## 9 Reproductive and Developmental Toxicology

### 9.1 Embryonic Fetal Development

Study title: FINAL PILOT REPORT:  
E7389: An Intravenous Embryo-Fetal Development Study in Rats by Intermittent Injection during the Mid-Organogenesis Period

Study no.: LFA00033

Study report location: eCTD - 4.2.3.5.2.1

Conducting laboratory and location:

(b) (4)

Date of study initiation: 5 March 2006

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: E7389, BLDR001, 94.3%  
(impurities at release were (b) (4), retested  
at (b) (4) (specification = <(b) (4)).

## Key Study Findings

- *Maternal:* Early delivery, adverse clinical signs and/or enlarged spleen were observed in dams at 0.15 mg/kg. Decreased body weight and food consumption were observed in maternal animals given 0.10 and 0.15 mg/kg.
- *Fetal:* Fetal external and soft tissue malformations occurred at 0.15 mg/kg (0.9mg/m<sup>2</sup>) indicating E7389 has teratogenic potential. At 0.10 (0.6mg/m<sup>2</sup>) and 0.15 mg/kg (0.9mg/m<sup>2</sup>), embryoletality occurred and fetal body weights were reduced.
- A no-observed-adverse-effect-level (NOAEL) of E7389 is concluded to be 0.03 mg/kg (0.18mg/m<sup>2</sup>) for general maternal toxicity and embryo-fetal development in this study.

Thus, the teratogenic potential of E7389 was confirmed in the rat at doses ( $\geq 0.6\text{mg/m}^2$ ) considerably lower (0.42X) than the proposed dose in humans (1.4mg/m<sup>2</sup>). E7389 is a tubulin-binding anticancer agent that inhibits microtubule polymerization. The positive teratogenic potential observed in this study was an expected finding for this class of compound.

## Methods

Doses: 0.0, 0.01, 0.03, 0.10 or 0.15 mg/kg  
 Frequency of dosing: once daily on Days 8, 10 and 12 of presumed gestation  
 Dose volume: 1.5 , 0.1, 0.3, 1.0 and 1.5 mL/kg (for 0.0, 0.01, 0.03, 0.10 or 0.15 mg/kg dosage groups, respectively)  
 Route of administration: Intravenously (IV) injection at ~1.5-2.0 mL/min  
 Formulation/Vehicle: 5.0% (v/v) ethanol in 0.9% sodium chloride, USP  
 Species/Strain: Rat/Sprague-Dawley  
 Number/Sex/Group: Female (8/group)  
 Satellite groups: None  
 Study design: The following maternal parameters were evaluated:  
 Mortality  
 Clinical signs  
 Body weight  
 Food consumption  
 Pregnancy status  
 Maternal necropsy

All surviving rats were euthanized and Caesarean sections were performed, and the following data collected on GD 21.  
 Corpora lutea numbers  
 Implantation sites

Resorption numbers  
Fetal weights  
Fetal Sex  
External fetal exams  
Staples exam and free hand razor sectioning of heads of ½ of the fetuses.  
Skeletal exams on remaining ½ of the fetuses.

Deviation from study protocol: One high dose female delivered early, and the dam and pups were necropsied on GD 21.

## Observations and Results

### *Maternal Observations:*

In the 0.15 mg/kg group:

- One dam (#7885) in the 0.15 mg/kg group delivered and was sacrificed on GD 21. This early delivery may have been related to the test article because it occurred at the highest dosage tested, and 69% of the litter was resorbed.
- In another dam in the 0.15 mg/kg dosage group, pale extremities, scant feces, dehydration, red or brown perivaginal substance and hyperreactivity were observed in clinical observations, and this rat had a litter consisting of 100% early resorptions.
- Two rats in the 0.15 mg/kg group had enlarged spleens at necropsy.

In the 0.10 and 0.15 mg/kg groups:

- Decreases in body weight gains with reductions in food consumption were observed during the entire dosing and post dosing periods.

### *Fetal Observations:*

In the 0.15 mg/kg group:

- In the 0.15mg/kg group, all conceptuses in three litters in the 0.15 mg/kg group were completely resorbed. External and/or soft tissue anomalies were noted in two fetuses in the 0.15 mg/kg group. One fetus had agnathia, a small oral opening and an absent tongue, and the other fetus had an absent stomach and spleen.

In the 0.10 and 0.15 mg/kg groups:

- In the 0.10 and 0.15 mg/kg groups, the number of early resorptions was increased, and fetal body weight was reduced in the 0.10 and 0.15 mg/kg groups.
- There were no test article-related changes in fetal parameters at doses of 0.03 mg/kg or less.

## Mortality

No maternal lethality reported.

### Clinical Signs

One dam in the 0.15 mg/kg dosage group had pale extremities, scant feces, dehydration, red or brown perivaginal substance and hyperreactivity (full litter found resorbed at C-section).

### Maternal Body Weight

Rats at doses  $\geq 0.03$  mg/kg group lost weight or had reduced body weight gain as compared to the vehicle control group on the day of each injection of the test article (GD 8 to 9, 10 to 11 and 12 to 13).

Reduced body weight gain during the dosing and postdosing period (GD 8 to 21) occurred at doses  $\geq 0.10$  mg/kg, likely reflecting the litter resorption that occurred in these groups.

### Feed Consumption

Absolute and relative food consumption values were reduced in a dose-dependent manner in the 0.03, 0.10 and 0.15 mg/kg groups during the dosage period (GD 8 to 13), and continued to be reduced at doses  $\geq 0.10$  mg/kg during the postdosage period (GD 13 to 21).

### Toxicokinetics – Not conducted

### Stability and Homogeneity

E7389 was stable in dosing vehicle at  $-20^{\circ}\text{C}$  for at least five days, and for one freeze/thaw cycle. The concentrations of the dosing solutions deviated from the intended concentration by 0.5% and 3.5% for samples collected on 03/14/06 and 03/17/06, respectively. These deviations were within the acceptable limit for dosing solution analysis.

### Necropsy

Two dams in the 0.15 mg/kg group had enlarged spleens at necropsy, a finding that was considered to be a secondary change as a compensatory reaction to bone marrow toxicity of E7389. Another dam in this group had bilateral dilation of the renal pelves.

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

The average numbers of early resorptions were significantly increased in a dosage-dependent manner in the 0.10 and 0.15 mg/kg groups. The resultant number of live

fetuses in these dosage groups was therefore reduced (39%-86%). Three litters (3/6) in the 0.15 mg/kg group were completely resorbed.

Offspring (Malformations, Variations, etc.)

- 1.) Two fetuses (2 fetuses/3 litters) in the 0.15 mg/kg group had serious malformations.
  - One fetus (7888-1) in the 0.15 mg/kg group had agnathia and a small oral opening at gross external examination. This fetus had an absent tongue at soft tissue examination.
  - At soft tissue examination, one fetus (7886-8) in the 0.15 mg/kg group had an absent stomach and spleen.
- 2.) Fetal body weights were reduced in the 0.10 and 0.15 mg/kg groups; values were 73.2% and 74.1% of the vehicle control group value, respectively.
- 3.) Other fetal alterations observed in single litters were considered variations and not dose-dependent.
  - At soft tissue examination, 1-2 fetuses from single litters in the control, 0.03 and 0.10 mg/kg groups had the umbilical artery descend to the left of the urinary bladder.
  - One fetus in the 0.10 mg/kg group had a non-ossified sternal centrum.
  - One fetus in the 0.03 mg/kg group had cervical ribs.
  - One fetus in the 0.01 mg/kg group had dilation of the renal pelvis.
  - One fetus in each of the 0.01 and 0.15 mg/kg groups had a bifid centrum of a thoracic vertebra.

## 10 Special Toxicology Studies-

Myelotoxicity Studies (eCTD - 4.2.3.7.7):

Hipple Cancer Research Center developed *in vitro* technologies for quantitatively estimating myelotoxicity of investigational drugs to the progenitors of human, dog and murine hematopoietic myeloid lineage (CFU-GM). Three non-GLP studies were conducted by Hipple Cancer Research Center (contracted by NCI) to provide an evaluation of the myelotoxicity of NCS-707389 (E7389) on granulocyte-macrophage precursors in canine, murine and human bone marrow as determined by *in vitro* colony forming assays (CFU-GM assay). AZT (azidodeoxythymidine) was used a positive control in these studies.

Results: Inhibition of CFU-GM was obtained at five increasing concentrations of the test article (0.01 to 100nM). The IC<sub>50</sub> for first and second order regression analysis for the dog are 0.44 and 0.41, respectively; in the mouse are 1.04 and 2.2, respectively; and in the human are 0.53 and 0.60, respectively.

In a separate non-GLP study conducted by Hemogenix (HG-Eisai-01) the IC<sub>50</sub> of E7389 in bone marrow mononuclear cells was compared to Paclitaxel and Vinblastine. In the human, the IC<sub>50</sub> for E7389 was found to be similar to Vinblastine and 3-fold lower than Paclitaxel. As such ranked compound sensitivity to multipotential stem cells is E7389>Vinblastine>Paclitaxel, as assessed in these studies.

## 11 Integrated Summary and Safety Evaluation

Repeat-dose Toxicology Studies

Species	STD <sub>10</sub> (mg/kg)	Safety Margin
Rat	0.10 (0.6 mg/m <sup>2</sup> )	0.43X proposed clinical dose <sup>1</sup>
Rat – high impurity	0.20 (1.2 mg/m <sup>2</sup> )	0.86X proposed clinical dose <sup>1</sup>
Rat- Chronic	< 0.015 (0.09 mg/m <sup>2</sup> )	<0.06X proposed clinical dose <sup>1</sup>
Dog	> 0.034 (0.7 mg/m <sup>2</sup> )	>0.48X proposed clinical dose <sup>1</sup>
Dog - Chronic	> 0.045 (0.9 mg/m <sup>2</sup> )	>0.64X proposed clinical dose <sup>1</sup>

<sup>1</sup> dose in mg/m<sup>2</sup>

Eribulin mesylate is genotoxic, teratogenic and lethal in rats at doses lower than the proposed clinical dose. Additionally, acute (end-of-treatment) toxicities were not assessed in the chronic rat and dog studies, or the 29-day dog study. As such, the toxicity profiles for these studies are likely underestimated. Accordingly, a careful assessment of submitted clinical data will be necessary to fully estimate the safety of eribulin mesylate.

## 12 Appendix/Attachments - none

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/s/

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LORI E KOTCH  
09/20/2010

ANNE M PILARO  
09/20/2010

## PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

<b>NDA/BLA Number:</b>	<b>Applicant:</b>	<b>Stamp Date:</b>
201,532	Eisai Inc 300 Tice Blvd Woodcliff Lake, NJ 07677	3-30-2010
<b>Drug Name:</b>	<b>NDA/BLA Type:</b> original NDA (new molecular entity)	
Eribulin Mesylate		

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).			Formulation appears similar.
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 <u>submitted</u> a rationale to justify the alternative route?	x		
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	x		Toxicology studies have been conducted GLP (not pharmacology)

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR  
NDA/BLA or Supplement**

	<b>Content Parameter</b>	<b>Yes</b>	<b>No</b>	<b>Comment</b>
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	x		The types of studies discussed at the pre-submission meeting were submitted. A persistent request throughout multiple reviews, however, addressed increasing the numbers of animals used in nonclinical studies to enable adequate interpretation. Determination regarding adherence to this request will be a review issue.
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		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	x		(b) (4) reported in the drug substance are not listed in ICH Q3C. Only the most recent 4 batches have these (b) (4) listed in the drug substance specification, and their levels being tested. The earlier 12 batches (include tox batches had no specification for these (b) (4) and their levels were not tested). Qualification of these (b) (4) will be a review issue.
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.

The specifications for (b) (4) reported in the most recent 4 batches of drug substance were not reported for earlier batches,

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR  
NDA/BLA or Supplement**

including the toxicology lots. Please provide specifications for these (b) (4) in the toxicology lots.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-201532	ORIG-1	EISAI INC	eribulin mesylate

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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.**

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/s/

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LORI E KOTCH  
05/03/2010

ANNE M PILARO  
05/03/2010

I concur with the reviewer's decisions regarding the acceptability of this application for filing. RPM please NOTE: one comment included for communication to the sponsor in the 74-day deficiency letter.