CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

207793Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Date: October 8, 2015
From: Whitney S. Helms, Ph.D.
Supervisory Pharmacologist
Division of Hematology Oncology Toxicology for Division of Oncology Products 2
To: File for NDA #207793
ONIVYDE (irinotecan liposome)
Re: Approvability of Pharmacology and Toxicology

Merrimack submitted NDA 207793 as a 505(b)(2) application for ONIVYDE in combination with 5-fluorouracil (5-FU) and leucovorin (LV) for the treatment of patients with metastatic adenocarcinoma of the pancreas who have received a prior gemcitabine-containing regimen. Clinical trials were conducted with ONIVYDE at a dose of 80 mg/m^2 administered as an intravenous infusion once every 2 weeks, however, due to changes in the FDA salt policy a decision was made late in the review cycle, and following the completion of Dr. Brower's review of the nonclinical data, to base the recommended dose on the free base rather than the salt form. Thus the final recommended dose for patients without UGT1A1*28 was changed from 80 mg/m^2 to 70 mg/m^2 and corresponding changes to other dose levels were made throughout the label. This NDA is a 505(b)(2) application relying on data from Camptosar, NDA 20571. Because the exposure comparisons for nonclinical and clinical data for ONIVYDE were based on Camptosar, which describes the dose in terms of the irinotecan hydrochloride salt form that does not exist in ONIVYDE and because the comparisons are approximations based on limited data from the innovator application, these changes do not represent significant changes for the nonclinical comparisons. A new review of the pharmacology/toxicology data or the dose comparisons would not contribute significantly to patient safety or represent real changes in the data, thus, while Dr. Brower's review describes comparisons to the original 80 mg/m^2 dose, the actual amount of irinotecan recommended in the irinotecan liposome product label is unaffected and this change does not affect the nonclinical review of ONIVYDE.

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/s/

WHITNEY S HELMS
10/08/2015

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	NDA 207,793
Supporting document/s:	000
Applicant's letter date:	February 2015
CDER stamp date:	February 2015
Product:	ONIVYDE (Irinotecan liposome injection)
Indication:	Metastatic pancreatic adenocarcinoma in combination with 5-FU and leucovorin in patients previously treated with gemcitabine
Applicant:	Merrimack Pharmaceuticals, Inc.
Review Division:	DHOT/DOP2
Reviewer:	Margaret E. Brower, PhD
Supervisor/Team Leader:	Whitney S. Helms, PhD
Division Director:	John K. Leighton, PhD, DABT (DHOT)/ Patricia Keegan, MD (DOP2)
Project Manager:	Deanne Varney

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1 Executive Summary

1.1 Introduction

Merrimack Pharmaceuticals has submitted New Drug Application (NDA) 207,793 for Onivyde (irinotecan liposome injection) in combination with 5-fluorouracil (5-FU) and leucovorin (LV) for the treatment of patients with metastatic adenocarcinoma of the pancreas who have received a prior gemcitabine-containing regimen. The recommended clinical dose of Onivyde is 80 mg/m^2 administered as an intravenous infusion once every 2 weeks, followed by LV 400 mg/m^2 and 5-FU 2400 mg/m^2. This NDA is a 505(b)(2) application relying on data from Camptosar, NDA 20571.

Nonclinical pharmacology, pharmacokinetic, and toxicology studies have been submitted to support the approval of liposomal irinotecan for the proposed indication. The 6-cycle (18 week) repeat-dose rat and dog toxicology studies were previously reviewed under IND 102,799 by Dr. Whitney S. Helms. Relevant portions of that review are appended to the current review as Appendix 1.

1.2 Brief Discussion of Nonclinical Findings

Irinotecan liposome is a liposomal encapsulation of irinotecan hydrochloride, the active ingredient. The encapsulated irinotecan is present in a precipitated state as a sucrosofate salt. Irinotecan is a water-soluble semi-synthetic derivative of camptothecin, a topoisomerase I inhibitor. Irinotecan and its active metabolite SN-38 bind reversibly to the topoisomerase 1 – DNA complex and induce single-strand breaks. The presence of SN-38 or irinotecan complexes with topoisomerase 1 appears to prevent re-ligation of these single strand breaks leading to double-strand breaks and subsequent cell death. The liposomal formulation of irinotecan resulted in longer exposure of both irinotecan and SN-38 compared to free irinotecan in all species tested. The activity of irinotecan liposome was evaluated in several xenograft tumor models, including pancreatic tumor models. Irinotecan liposome exhibited improved tumor growth inhibition compared to equivalent doses of free irinotecan. The presence of irinotecan liposome also resulted in improved anti-tumor activity when used in combination with 5-fluorouracil (5-FU) compared to the activity of the single agents alone.

Irinotecan liposome was evaluated in 4-week and 6-cycle studies in rats and dogs. In general, toxicities were similar following dosing of irinotecan liposome or free irinotecan. Following 4 weeks of dosing, irinotecan liposome was lethal at 260 mg/kg in rats, with toxicities including bone marrow hypocellularity, renal tubular hypertrophy and thymic atrophy, as well as histiocytosis of multiple organs. Additional findings following 6 cycles of dosing with 30, 75, or 190 mg/kg irinotecan liposome in rats, included hepatic necrosis and hematopoiesis of the spleen. Since histiocytosis was observed in control animals, the finding was considered by the Applicant to possibly be a vehicle related finding. Since a saline control was not included in either study, verification of this conclusion was not possible; however, similar findings of increased histiocytosis have occurred following treatment of animals with other liposomal drug formulations. Infusion

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site toxicities included inflammation, necrosis, and thrombosis. Body weights were consistently depressed in the 6-cycle study at irinotecan liposome doses of 75 and 190 mg/kg. The incidence of dental loss/damage and neurological findings (tremors, uncoordinated gait/walking on toes) was reduced with irinotecan liposome compared to irinotecan hydrochloride, although a comparative increase in aggressiveness was observed with the liposome.

Weekly administration of irinotecan lipoosome for 4 weeks was lethal at 16 mg/kg in dogs. Primary target sites in dogs administered 8 and 16 mg/kg irinotecan liposome included the gastrointestinal tract, bone marrow, Peyer's patch, and spleen. No cardiovascular changes were observed in the 4-week study and there were no cardiovascular effects observed in dogs administered single doses of up to 21 mg/kg irinotecan liposome in a safety pharmacology study.

Irinotecan liposome doses of 36 mg/kg every 3 weeks were lethal to dogs administered the drug in a 6-cycle repeat dose study. The primary target organs of animals administered irinotecan liposome at doses of 15 and 21 mg/kg during this study were the gastrointestinal tract, lymphatic and hematopoietic tissues (lymphoid atrophy/epithelial necrosis of GI, lymphoid atrophy of spleen, histiocytosis of spleen and liver and uterine atrophy). In general, histopathological findings were similar for irinotecan liposome and irinotecan hydrochloride with the exception of gastrointestinal epithelial necrosis which was not observed with irinotecan hydrochloride.

A greater degree of platelet and WBC depletion was observed following administration of irinotecan liposome compared to irinotecan hydrochloride. Increased exposure and prolonged half-life of irinotecan and SN-38 occurred following irinotecan liposome administration compared to irinotecan HCl in both rats and dogs, with increased clearance observed with irinotecan HCl. Irinotecan liposome exhibited an exposure up to 70-fold higher by AUC when compared to irinotecan HCl.

Reproductive toxicity studies with irinotecan liposome were not conducted. Instead, the Applicant relied on the Agency's previous findings for the effects of irinotecan hydrochloride, the reference drug for this 505(b)(2) application, on reproductive and developmental toxicity to support the application for irinotecan liposome. Intravenous administration of irinotecan HCl at a dose of 6 mg/kg/day to rats and rabbits during the period of organogenesis resulted in increased post-implantation loss and decreased numbers of live fetuses. In separate studies in rats, this dose resulted in an irinotecan exposure of approximately 0.002 times the exposure of irinotecan based on AUC in patients administered ONIVYDE at the 80 mg/m^2 dose. Structural abnormalities and growth delays were observed in rats at doses greater than 1.2 mg/kg/day (approximately 0.0002 times the clinical exposure to irinotecan from 80 mg/m^2 ONIVYDE based on the clinical AUC of 1364 µg•h/mL) and in rabbits at 72 mg/m^2/day. Teratogenic effects included external, visceral, and skeletal abnormalities. Irinotecan HCl administered to rat dams for the period following organogenesis through weaning at doses of 6 mg/kg/day caused decreased learning ability and decreased female body weights in the offspring. Irinotecan HCl crosses the placenta of rats following

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intravenous administration at 10 mg/kg. Finally, atrophy of male and female reproductive organs was observed in dogs administered ≥9 mg/kg liposomal irinotecan once every 3 weeks for 6 cycles, suggesting potential effects on fertility. A warning for embryofetal risk is warranted in the label along with warnings for females and males to use effective contraception during treatment with irinotecan liposome and for 1 and 4 months, respectively, following the final dose.

No studies have been performed to assess the potential of liposomal irinotecan for genetic toxicity. Irinotecan HCl, the reference drug, was clastogenic both in vitro (chromosome aberrations in Chinese hamster ovary cells) and in vivo (micronucleus test in mice). Consistent with these genotoxic effects, when irinotecan HCl was administered to rats once weekly for 13 weeks followed by a 91 week recovery period, a significant linear trend between irinotecan HCl dosage and the incidence of combined uterine horn endometrial stromal polyps and endometrial stromal sarcomas was observed. Neither irinotecan HCl nor its active metabolite, SN-38, was mutagenic in the *in vitro* Ames assay.

1.3 Recommendations

1.3.1 Approvability

From the nonclinical perspective, liposomal irinotecan is approvable in combination with 5-fluorouracil and leucovorin for the treatment of patients with metastatic adenocarcinoma of the pancreas.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

A separate labeling review will be completed as necessary.

2 Drug Information

2.1 Drug

CAS Registry Number
136572-09-3

Generic Name
Nanoliposomal irinotecan, Irinotecan hydrochloride liposome

Code Name
MM-398, PEP02, HBI-202
PEP02
Chemical Name
(*S*)-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo1*H*-pyrano[3',4':6,7]-indolizino[1.2-b]quinolin-9-yl-[1,4'bipiperidine]-1'-carboxylate

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Molecular Formula/Molecular Weight
Formula = $C_{33}H_{38}N_4O_6HCl.3H2O$; Molecular weight = 677.18

Structure/ Biochemical Description

Figure 1 Schematic view of MM-398/PEP02

(b) (4)

Pharmacologic Class
Topoisomerase 1 Inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND (b) (4), IND 102799, NDA 20571

2.3 Drug Formulation

The drug product liposome is a unilamellar lipid bilayer vesicle, ~110 nm in diameter, which encapsulates an aqueous space containing irinotecan in a gelated or precipitated state as sucrosofate salt. The lipid membrane is composed of 1, 2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol, and N-(carbonyl-methyoxypolyethylene glycol-2000)-1, 2-distearoly-sn-glycero-3 phosphoethanolamine. Similar liposomal formulations have been used for other oncology drugs in an effort to prolong exposure and decrease toxicity.

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Table 1: Drug Formulation

Component	Quality Standard	Function	Amount per Batch
Irinotecan hydrochloride	USP	Drug substance	(b) (4)
(b) (4)			

(Excerpted from Applicant's submission)

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

None

2.6 Proposed Clinical Population and Dosing Regimen

- Indication: Treatment of patients with metastatic adenocarcinoma of the pancreas
- Dosing Regimen: 80 mg/m^2 administered as an intravenous infusion once daily every 2 weeks in combination with 5-fluorouracil and leucovorin

2.7 Regulatory Background

- October, 2008: PharmaEngine submitted IND 102,799 for PEP02 [PEP02 licensed to PharmaEngine for clinical development by Hermes Biosciences, Inc].
- June 2011: PEP02 acquired by Merrimack Pharmaceuticals, Inc.
- July, 2013: Orphan drug designation granted for indication of pancreatic cancer

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3 Studies Submitted

3.1 Studies Reviewed

Pharmacology

- Antitumor activity of PEP02/MM-398 in orthotopic L3.6pl-T human pancreatic cancer xenografts in nude mice (Report #MM-398-NC-N-Ph-015)
- Antitumor activity of PEP02/MM-398 in orthotopic BxPC3-luc2 human pancreatic cancer xenografts in SCID mice (Report #MM-398-NC-N-Ph-016)
- Antitumor activity of MM-398 in orthotopic OCIP51 human pancreatic cancer xenografts in NOD-SCID mice (Report #MM-398-NC-N-Ph-023)
- Antitumor efficacy of MM-398 in an ectopic patient -derived pancreatic cancer xenograft model (#18254) in SCID mice (Report #MM-398-NC-N-Ph-024)
- Antitumor efficacy of MM-398 against ectopic pancreatic cancer xenograft models in NOD-SCID mice (Report #MM-398-NC-N-Ph-018)
- Evaluation of antitumor activity of PEP02 and 5-Flurouracil (5-FU) in the xenograft tumor model of human HT-29 colon tumor cells in SCID mice (Study #PEP02-NC-N-PH-010)
- A Cardiovascular and Respiratory Safety Pharmacology Assessment in Telemetered Beagle Dogs (Report #20036143)

Pharmacokinetics/Toxicokinetics

- Disposition of ^{14}C-CPT-11 in Male Long-Evans Rats Following a Single 90-Minute Intravenous Dose of Liposome Encapsulated (14C-PEP02) and Non-Encapsulated Formulation (^{14}C-CPT-11) (Report #007)
- Mass balance and excretion of radioactivity in Sprague Dawley rats following an intravenous infusion dose of liposome encapsulated [^{14}C] irinotecan (2 studies) (Report (125N-0502)
- A 4-week study of the toxicokinetics of PEP02 administered by intravenous infusion to Sprague Dawley rats (Report #7391**).**
- A 4-week study of the toxicokinetics of PEP02 administered by intravenous infusion to Sprague Dawley rats Amendment. (Report #7391a).

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General Toxicology/Toxicokinetics

- A 4-week toxicity and toxicokinetics study (with a 2-week recovery/observation period) of PEP02 administered by intravenous infusion to Sprague-Dawley rats (Study #PEP02-NC-G-Tx-006)

- A 4-week toxicity and toxicokinetics study (with a 2-week recovery/observation period) of PEP02 administered by intravenous infusion to Beagle dogs (Study #PEP02-NC-G-Tx-009)

- A 6-cycle (3 weeks per cycle) infusion toxicity study in Sprague Dawley rats followed by a 6-week recovery period (Study #1005-3071).

- A 6-cycle (3 weeks per cycle) toxicity and toxicokinetics study (with a 6-week recovery/observation period) of PEP02 administered by intravenous infusion to Beagle dogs (Study #1006-2162).

3.2 Studies Not Reviewed

Pharmacology

- The uptake of fluorescently-tagged low-PEG liposomes in select *in vitro* cell cultures (Report #MM-398-NC-N-Ph-017)

- Distribution of fluorescently-tagged liposomes in tumor cell populations of a xenograft tumor model of human HT-29 colon cancer in NOD-SCID mice without and with pretreatment of animals with an anti-CSF-1 antibody (Report #MM-398-NC-N-Ph-019)

- Distribution of fluorescently-tagged liposomes in tumor cell populations of a syngeneic tumor model of murine LLC lung cancer in C57Bl6 mice without and with pretreatment of animals with an anti-CSF-1 antibody (Report #MM-398-NC-N-Ph-020)

- Distribution of fluorescently-tagged liposomes in tumor cell populations of a syngeneic tumor model of murine MC-38 colon carcinoma cells in C57Bl/J6 mice without and with pretreatment of animals with an anti-CSF-1 antibody (Report #MM-398-NC-N-Ph-021)

- Longitudinal tumor hypoxia imaging with [$_{18}$F]FAZA-PET to assess treatment activity of MM-398 when dosed with equivalent tumor AUC_{SN-38} active drug exposure compared to free irinotecan (Efficacy) (Report #MM-398-NC-N-Ph-022)

- The release of drug payload from macrophage cell lines after uptake of MM-398 in select in vitro cell cultures (Report #MM-398-NC-N-Ph-025)

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- Conversion of free irinotecan to SN-38 in whole tumor tissue Lysates (Report #MM-398-NC-N-Ph-026)

- Antitumor efficacy of prototype CPT-11 liposomes (PEP02-A) against breast cancer xenografts in nude mice (Report #MM-398-NC-N-Ph-001s)

- Comparative evaluation of CAMPTOSAR® and Lipo-II, a liposomal irinotecan (CPT11), in human gastric MKN45 tumor xenograft in nude mice (Report #MM-398-NC-N-Ph-002)

- Antitumor efficacy of prototype CPT-11 liposomes against HT-29 human colon cancer xenografts in nude mice (Report #MM-398-NC-N-Ph-003s-1)

- Antitumor activity of CPT-11 and Lipo-CPT 11 in the xenograft tumor model of human HT-29 colon tumor cells in SCID mice (Report #MM-398-NC-N-Ph-005)

- Evaluate possible antitumor activity of PEP02 and CPT-11 in the xenograft tumor model of human HT-29 colon tumor cells in SCID mice (Report #MM-398-NC-N-Ph-006)
- Evaluate possible antitumor activity of PEP02 and CPT-11 in the xenograft tumor model of human HT-29 colon tumor cells in SCID mice [580100] (second repeat test). (Report #MM-398-NC-N-Ph-007)

- Evaluate possible antitumor activity of PEP02 and CPT-11 in the xenograft tumor model of human HT-29 colon tumor cells in SCID mice [580100] (third repeat test). (Report #MM-398-NC-N-Ph-008)

- Evaluate possible antitumor activity of PEP02 and Cisplatin in the Tumor Xenograft model of Cervix, SiHa Cell [580050] in SCID mice (Report #MM-398-NC-N-Ph-011)

- Evaluate possible antitumor activity of PEP02 and CPT-11 in the Tumor Xenograft model of Cervix, SiHa Cell [580050] in SCID mice (Report #MM-398-NC-N-Ph-012)

- Evaluate possible antitumor activity of PEP02 and Cisplatin in the Tumor Xenograft model of Cervix, SiHa Cell [580050] in SCID mice (Report #MM-398-NC-N-Ph-013)

Pharmacokinetics

- Validation of a High Performance Liquid Chromatography Method with Ultraviolet Detection for the Determination of MM-398 Concentration in Dose Formulations (Report # 20045567)

- CPT-11 HPLC analytical method validation for the assay pf CPT-11 in dosing formulations – Lipo-CPT-11 formulated in 5% dextrose (Report #012)

- LC/MS/MS assay validation of irinotecan and SN-38 in rat plasma- 3 studies (Report #128-0401)

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- ❖ LC/MS/MS assay validation of irinotecan and SN-38 in dog plasma- 2 studies (Report #128-0402)

- ❖ Plasma Concentration in the SD Rats after a Single Intravenous Administration of PEP02 (Pilot Study) (Report # 001)

- ❖ Tissue Distribution in the SD Rats after a Single Intravenous Administration of PEP02 (Pilot Study) (Report # 002)

- ❖ Plasma Concentration in the Beagle Dogs after a Single Intravenous Administration of PEP02 (Pilot Study) (Report #004)

- ❖ To measure the tissue and tumor distribution in the xenograft tumor model of human HT-29 colon tumor cells in SCID mice [580100] after single intravenous bolus administration of PEP02 and Irinotecan Hydrochloride (Report #005)

- ❖ Plasma PEP02 (Liposome encapsulated irinotecan) concentration in Beagle dogs after a single intravenous administration of PEP02 (Report# 006)

Toxicology

- ❖ Acute intravenous infusion toxicity study in mice – PEP02 (Report # 003)

- ❖ Lipo-CPT-11: A single intravenous infusion pharmacokinetics study in Sprague Dawley rats (Report # 7095)

- ❖ Acute intravenous infusion pilot study in male rats (Report # 004)

- ❖ A determination of the acute toxicity of PEP02 (LIPO-CPT 11) after single administration by intravenous infusion (followed by a 14-day recovery/observation period) to Sprague Dawley rats (Report #7082)

- ❖ PEP02: An acute intravenous infusion toxicity study in Beagle dogs (Report #6151)

- ❖ LIPO-CPT-11: A determination of the maximum tolerated dose following administration to Beagle dogs by intravenous infusion (Study #6160)

- ❖ A determination of the maximum tolerated dose following administration to Beagle dogs by intravenous infusion (Study #6171)

- ❖ In vitro evaluation of the influence of PEP02 on human whole blood hemolysis and plasma flocculation (Study #PEP02-NC-G-Tx-008)

3.3 Previous Reviews Referenced

IND 102,799 reviewed by Whitney Helms, Ph.D., November 14, 2008

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4 Pharmacology

4.1 Primary Pharmacology

Kalra et al. 2014[1]

The purpose of this paper was to examine parameters that maximize the intratumoral activity of liposomal irinotecan in a NOD/SCID mouse xenograft model of human colon carcinoma. Following single weekly intravenous administration of CPT-11 (50 mg/kg or 60 mg/kg) or liposomal irinotecan (10 mg/kg) for a total of 3 doses, similar SN-38 (active metabolite) exposures were observed in plasma and tumor. Administration of liposomal irinotecan resulted in similar intratumoral exposures of SN-38 at doses 5-fold lower than free irinotecan, but with 2- to 2.7-fold increases in tumor response. Calculated tumor growth inhibition rates were 32, 43 and 86% for 50 mg/kg CPT-11, 60 mg/kg CPT-11 and 10 mg/kg liposomal irinotecan, respectively.

Figure 2: Anti-tumor activity of liposomal vs free irinotecan

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(Excerpted from Kalra et al.)

In a separate study, following single weekly intravenous administration of liposomal irinotecan (ranging from 1.25 to 20 mg/kg) for a total of 3 doses, tumor response and pharmacokinetic modeling identified the duration for which concentrations of SN-38 persisted above an intratumoral threshold of 120 nmol/L as a determinant for antitumor activity. The tumor duration of prolonged SN-38 levels from free irinotecan was 50 hours compared to 168 hours for liposomal irinotecan. Tumors with longer SN-38 duration displayed greater growth inhibition compared with tumors with shorter SN-38 duration.

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Figure 3: Duration of SN-38 tumor exposure correlating with anti-tumor activity

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4.1.1 Antitumor activity of PEP02/MM-398 in orthotopic L3.6pl-T human pancreatic cancer xenografts in nude mice (Report #MM-398-NC-N-Ph-015)

The purpose of this study was to evaluate the effect of PEP02 in a nude mouse pancreatic xenograft model. In an orthotopic model established with human L3.6pl-T pancreatic tumor cells in nude mice, EGFR-targeted or untargeted liposomal or free irinotecan were administered intravenously at 10 or 20 mg/kg, q7d for a total of 3 doses. (Targeted liposomes were described as immunoliposomes incorporated with lipid-anchored anti-EGFR C225 Fab fragments into the outer lipid monolayer.) Comparative tumor volume, mouse body weights, and drug levels were measured, and the metastatic tumor burden of select organs was determined. The effects of the two liposomal irinotecans (targeted or untargeted) were similar during treatment; a more robust response was observed with the EGFR-targeted liposomal irinotecan at D63-105. In contrast, response to free irinotecan (CPT-11) was similar to the vehicle control (see figure below). L3.6pl tumors, harvested 24 h following dosing of 20 mg/kg free CPT 11 contained 0.043 µg/g CPT-11 and 0.019 µg/g SN-38. In contrast, following dosing of liposomal irinotecan, tumors contained 6.1 µg/g CPT-11 and 0.17 µg/g SN-38, 142- and 9-fold higher, respectively, than levels observed with free drug. Tumors from mice treated with 10 mg/kg PEP02 were ~5 - 29x smaller in size than tumors from mice treated with free irinotecan.

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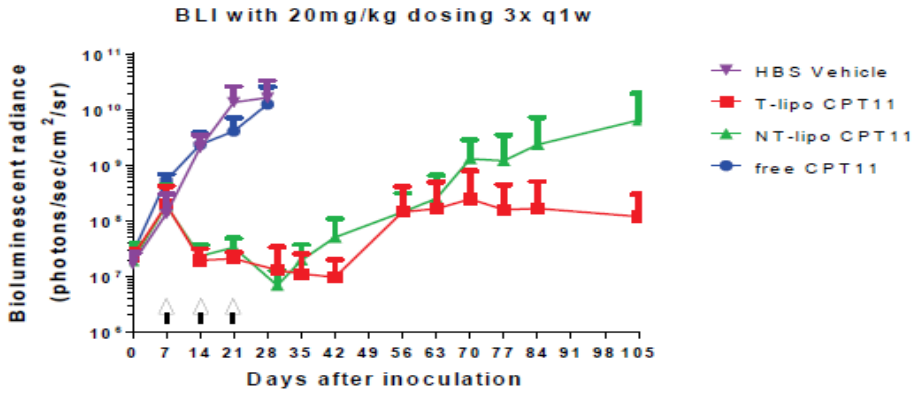
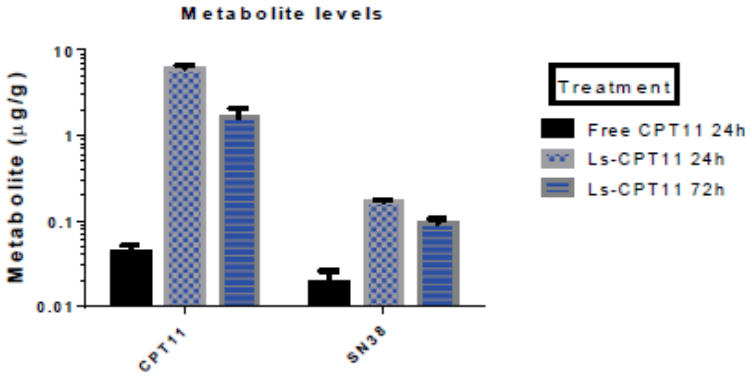
Figure 4: Mean and SD of bioluminescent signal for L3.6pl tumors after treatment with 20 mg/kg 3xqw



(Excerpted from Applicant's submission)

Figure 5: Metabolite levels after dosing with free or liposomal irinotecan (20 mg/kg)

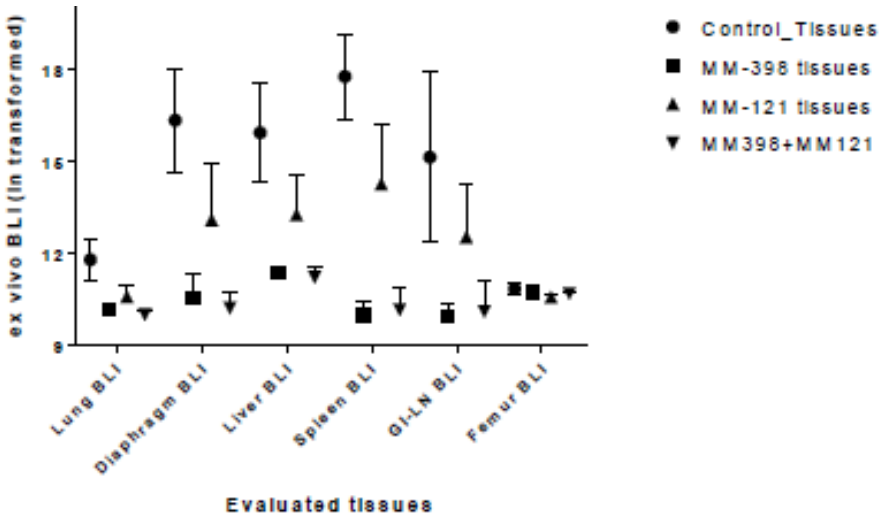


(Excerpted from the Applicant's submission)

4.1.2 Antitumor activity of PEP02/MM-398 in orthotopic BxPC3-luc2 human pancreatic cancer xenografts in SCID mice (Report #MM-398-NC-N-Ph-016)

In an orthotopic model, human BxPC3-luc2 pancreatic tumor cells in SCID mice, liposomal irinotecan either alone or in combination with abraxane (MM-121) was administered intravenously at 10 mg/kg q7d for a total of 5 doses. Comparative tumor volume, mouse body weights, and drug levels were measured, and the metastatic tumor burden of select organs was determined. Metastatic tumor load was significantly lower in the spleen, GI-lymph node, liver and lung ($p<0.0001$), and diaphragm ($p<0.05$) of animals treated with MM-398 compared to vehicle controls.

Figure 6: BxPC3-luc2 tumor burden

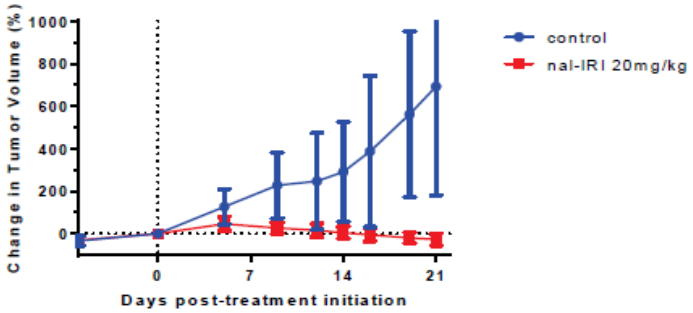


(Excerpted from the Applicant's submission)

4.1.3 Antitumor activity of MM-398 in orthotopic OCIP51 human pancreatic cancer xenografts in NOD-SCID mice (Report #MM-398-NC-N-Ph-023)

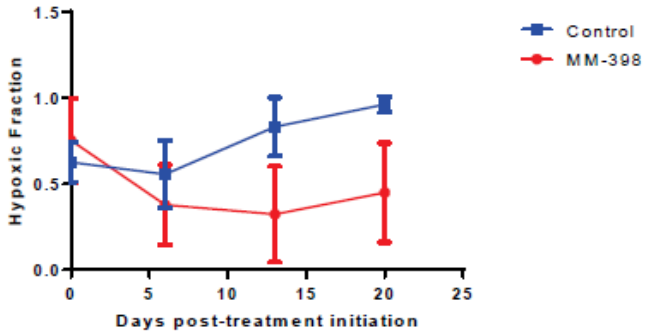
The purpose of this study was to evaluate the effects of MM-398 in a high hypoxia xenograft mouse tumor model of pancreatic cancer. Tumor hypoxia has been linked to aggressive disease progression and resistance to therapy. An orthotopic tumor model was established with human patient-derived OCIP51 pancreatic tumors in NOD-SCID mice. Twenty mg/kg liposomal irinotecan was administered intravenously q7d for a total of 4 doses. Tumor volume, mouse body weights, and hypoxia status of tumors were measured. Tumor hypoxia decreased by 30% following treatment 1 cycle of MM-398 administration and tumors grew to only 35% of the volume of tumors in control-treated animal by D5. From D5, tumors regressed to study termination with a 50% decrease relative to maximal size. Control tumors increased 127% by D5. Tumor weights were ~4x less in MM-398 treated mice at study termination compared to control mice.

Figure 7: Relative tumor volume



(Excerpted from Applicant's submission)

Figure 8: Hypoxic Fraction

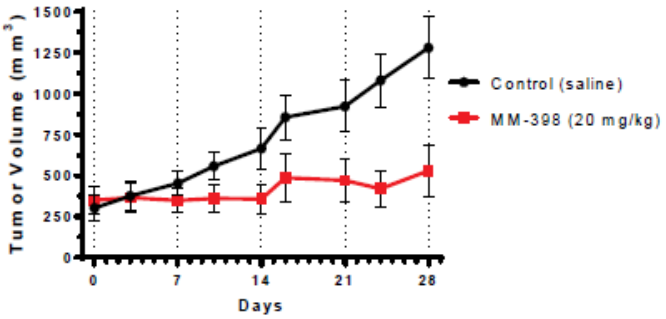


(Excerpted from the Applicant's submission)

4.1.4 Antitumor efficacy of MM-398 in an ectopic patient -derived pancreatic cancer xenograft model (#18254) in SCID mice (Report #MM-398-NC-N-Ph-024)

In an ectopic tumor model of patient-derived pancreatic tumor tissue propagated in SCID mice, 20 mg/kg liposomal irinotecan was administered intravenously q7d for a total of 4 doses. [The Applicant defined the #18254 model as tumor tissue fragments propagated in mouse strains to maintain tissue architecture]. Tumor volume and mouse body weights were recorded for 28 days. At study termination (D28), tumors treated with MM-398 were ~58% smaller compared to control tumors.

Figure 9: Decreased tumor volume in tumor fragment implant model



(Excerpted from the Applicant's submission)

4.1.5 Antitumor efficacy of MM-398 against ectopic pancreatic cancer xenograft models in NOD-SCID mice (Report #MM-398-NC-N-Ph-018)

In ectopic tumor models of pancreatic tumor cells (PNAC-1, MiaPaCa-2, BxPC3, and AsPC-1) in NOD-SCID mice, liposomal irinotecan at 20 mg/kg ws administered iv q7d for a total of 2-5 doses. All cell lines were derived from patient biopsies. Gemcitabine was included as a comparator. Tumor volume and mouse body weights were recorded for a total of 10-33 days. Significant tumor growth inhibition was observed with the four pancreatic tumor models (~91% smaller compared to control tumors: Figures 10-13).

Reference ID: 3813066

Figure 10: MM398 Treatment of Panc-1 xenografts

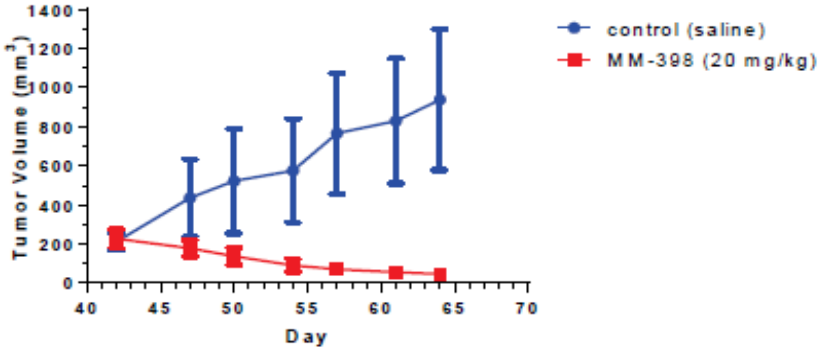


Figure 11: MM398 Treatment of MiaPaCa2 xenografts

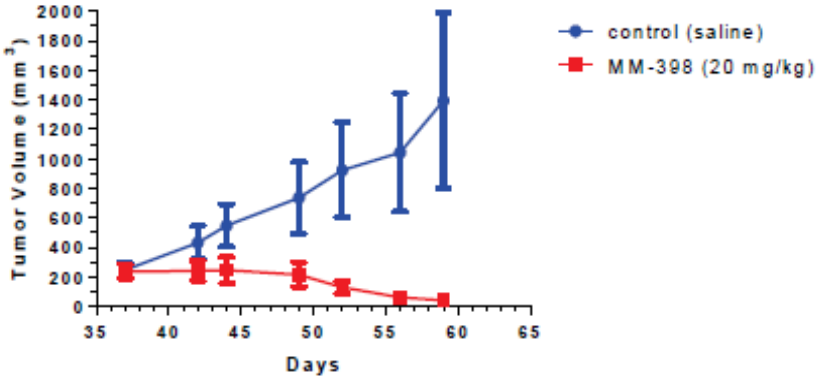


Figure 12: MM398 Treatment of BxPC3 xenografts

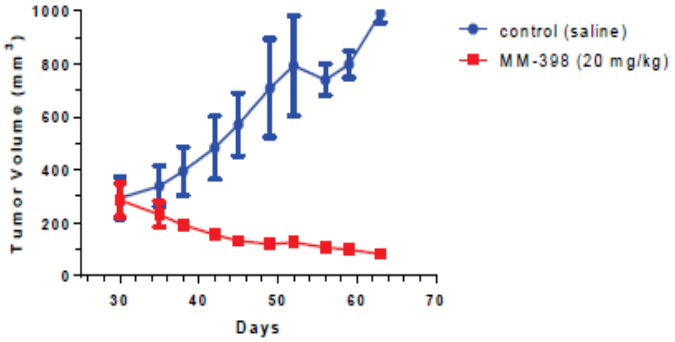
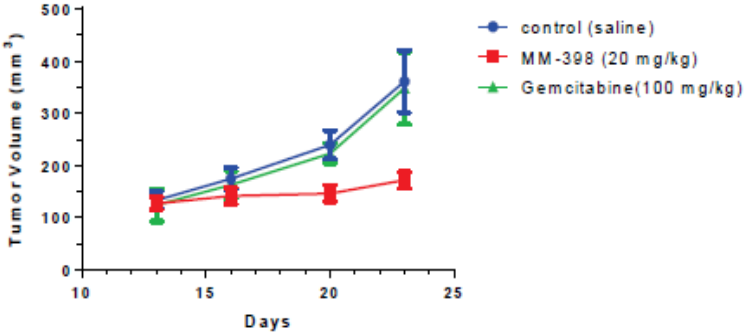


Figure 13: MM398 Treatment of AsPC1 xenografts

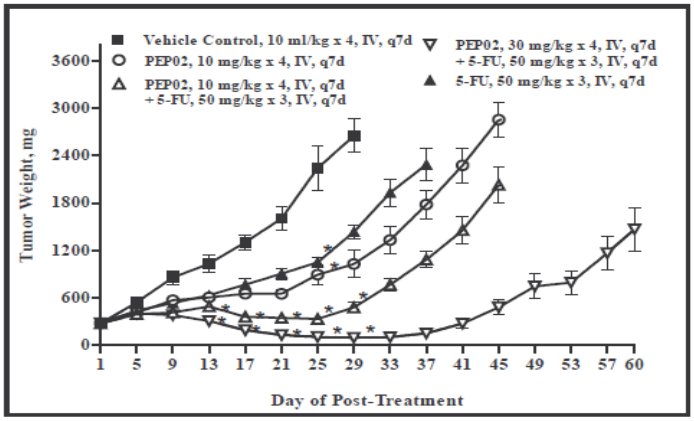


(figures Excerpted from the Applicant's submission)

4.1.6 Evaluation of antitumor activity of PEP02 and 5-Flurouracil (5-FU) in the xenograft tumor model of human HT-29 colon tumor cells in SCID mice (Study #PEP02-NC-N-PH-010)

The objective of this study was to evaluate the activity of PEP02 and 5-FU, both as single agents and in combination, in a tumor xenograft model of human HT-29 colon tumor cells in SCID mice. Treatment with 50 mg/kg 5-FU in combination with 10 mg/kg PEP02, resulted in greater decrease in tumor volumes and extended delay of tumor growth compared to either drug alone (Figure 14).

Figure 14: Activity of PEP02 and 5-FU in the HT-29 colon tumor model



(Excerpted from Applicant's submission)

4.2 Secondary Pharmacology

Not conducted

4.3 Safety Pharmacology

4.3.1 Study title: A Cardiovascular and Respiratory Safety Pharmacology Assessment in Telemetered Beagle Dogs (Report # 20036143)

Study no.:	20036143
Study report location:	M4.2.1.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 24, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	MM-398, Batch #QB2713-B09; 99.5% pure

Key Study Findings

● No drug-related changes in cardiovascular hemodynamic, electrocardiographic, or respiratory parameters or body temperature following a single infusion of 9-21 mg/kg of liposomal irinotecan.

Group No.	No. of Animals[a]		Test Material	Dose Level (mg/kg)	Concentration (mg/mL)	Dose Volume (mL/kg)
	Male	Female				
1	3	3	Control Article	0	0	15
2	3	3	MM-398	9	0.6	
3	3	3		15	1.0	
4	3	3		21	1.4	

[a] Animals were dosed via a 90 minute intravenous infusion at a rate of 10 mL/kg/hr.

(Excerpted from Applicant's submission)

In a single dose safety pharmacology study, electrocardiograms (ECG) were evaluated at 4 and 0.5 hours prior to dosing and at 0.5, 2.5, 4, and 24 hours after the start of dosing with MM-398, which included the time points around Cmax of irinotecan and SN-38. There were no MM-398-related changes in cardiovascular hemodynamic or electrocardiographic parameters, including heart rate, rhythm, and conduction, arterial blood pressure, or RR, QRS, PR, QT, and QTca intervals following administration at single dose levels up to 21 mg/kg MM-398.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Disposition of ^{14}C-CPT-11 in Male Long-Evans Rats Following a Single 90-Minute Intravenous Dose of Liposome Encapsulated (^{14}C-PEP02) and Non-Encapsulated Formulation (^{14}C-CPT-11) (Report #125N-0402)

Drug, lot #, and radiochemical purity:	MM-398, Batch #QB2713-B09; 99.5% pure
Dose, ROA, dose volume:	10 mg/kg, 15.3 and16.6 µCi/g for

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	[^{14}C]PEP02 and [^{14}C]CPT-11, dose volume not provided
Formulation/Vehicle:	5% dextrose
Species/Strain:	Rat/Long Evans
Number/Sex/Group:	6 males/group
Sample collection:	[^{14}C]PEP02: post infusion, 8h, 24h, D2, 3, and 7; [^{14}C]CPT-11: post infusion, 8h, 24h, D2 and 3

The Applicant compared radioactivity distribution into tissues, organs, and body fluids following administration of a single 10 mg/kg intravenous dose of liposome encapsulated and non-encapsulated [^{14}C]CPT-11 to male Long-Evans rats.

In general, tissues from animals administered liposomal encapsulated [^{14}C]PEP02 (Group 1 in table below) exhibited higher CPT-11 concentrations that remained elevated for extended times compared to non-encapsulated irinotecan. The highest concentrations were observed immediately following infusion and the highest [^{14}C]PEP02 distribution occurred to the liver, kidney, bile duct, lung, heart, spleen, bone marrow, trachea, brown adipose, and thyroid. Ocular uveal tract radioactivity distribution was associated with both liposomal and free iriniotecan suggesting drug binding with ocular melanin; levels remained high 7 days post-dose. CNS tissues had moderate concentrations of [^{14}C]PEP02 through 8h post-dose; CNS levels decreased to below quantifiable levels (BQL) within 8h post-dose. CNS levels of free irinotecan were not measurable except immediately post-infusion when it was minimally detected in the cerebellum.

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Table 2: Comparative concentrations of radioactivity in tissues of Long-Evans rats following [^{14}C]PEP02 and [^{14}C]CPT-11 infusion

		Mean - µg equiv/g tissue											
		GROUP 1						GROUP 2					
		Rat #1	Rat #2	Rat #3	Rat #4	Rat #5	Rat #6	Rat #7	Rat #8	Rat #9	Rat #10	Rat #11	Rat #12
Tissue Type	Tissue	End of Infusion	8 h	24 h	2 Days	3 Days	7 Days	End of Infusion	4 h	8 h	24 h	2 Days	3 Days
Adipose	Adipose (brown)	17.437	13.504	3.117	1.789	0.343	0.282	14.320	2.921	0.715	BQL	BQL	BQL
	Adipose (white)	0.946	1.606	BQL	BQL	BQL	BQL	0.539	BQL	BQL	BQL	BQL	BQL
Alimentary Canal	Cecum	5.716	9.613	18.109	9.486	1.244	BQL	11.424	5.333	48.912	NI	BQL	NI
	Cecum (contents)	3.282	22.883	31.844	14.322	6.264	0.852	43.972	322.085	394.893	NI	1.103	0.288
	Esophagus	5.482	5.673	0.864	1.056	0.310	BQL	6.401	1.283	1.611	NI	NI	NI
	Large Intestine	4.792	2.857	4.403	1.460	1.735	BQL	5.258	3.273	2.730	0.302	BQL	BQL
	Large Intestine (contents)	BQL	25.699	49.954	35.985	12.575	0.851	11.739	524.450	26.975	29.670	1.470	0.759
	Small Intestine	6.271	9.622	21.095	22.673	3.538	BQL	11.255	13.442	25.535	0.293	NI	BQL
	Small Intestine (contents)	7.275	6.219	26.863	8.478	5.460	0.317	250.623	107.696	177.518	1.266	NI	BQL
	Stomach (contents)	0.333	4.461	8.889	BQL	2.354	0.248	26.340	3.275	2.762	1.415	BQL	BQL
	Stomach (gastric mucosa)	6.864	3.486	4.771	2.345	0.577	BQL	27.409	5.711	1.148	0.259	BQL	NI
Central Nervous System	Brain (cerebellum)	3.370	1.370	0.779	0.342	BQL	BQL	0.188	BQL	BQL	NI	BQL	BQL
	Brain (cerebrum)	1.622	0.846	0.582	0.221	BQL	BQL	BQL	BQL	BQL	NI	BQL	BQL
	Spinal Cord	2.161	1.073	0.597	0.258	BQL	NI	BQL	BQL	BQL	NI	NI	NI
Dermal	Skin (pigmented)	2.799	5.352	3.536	1.933	1.089	0.225	5.074	1.418	1.502	0.748	0.834	0.511
	Skin (non-pigmented)	2.056	2.626	4.400	2.670	2.096	0.266	4.812	1.137	1.195	0.870	BQL	0.353
Endocrine	Adrenal Gland	45.614	22.127	15.674	11.722	2.338	0.361	30.148	6.254	3.566	0.661	0.433	0.391
	Pituitary Gland	14.546	2.187	5.564	3.695	0.940	0.679	32.897	12.506	14.089	1.059	0.271	1.693
	Thyroid	15.173	9.718	30.455	15.293	6.164	0.330	32.080	10.503	2.038	2.355	0.199	NI
Excretory/ Metabolic	Bile (in duct)	128.105	198.577	58.171	43.288	41.328	NI	95.132	27.902	5.479	NI	NI	NI
	Renal Cortex	41.056	26.644	18.136	9.661	2.712	1.049	35.332	5.022	2.269	0.409	0.251	0.287
	Renal Medulla	50.635	31.319	20.240	7.502	0.969	BQL	20.996	3.041	1.215	BQL	BQL	BQL
	Liver	49.940	23.405	19.568	11.231	3.104	0.908	31.142	5.809	3.043	0.295	0.201	0.251
	Urinary Bladder	4.897	3.899	1.088	1.263	3.171	NI	81.447	3.360	0.685	1.785	NI	NI
	Urinary Bladder (contents)	1.767	21.492	0.717	1.197	0.877	NI	165.456	56.448	13.686	0.440	BQL	NI

		Mean - µg equiv/g tissue											
		GROUP 1						GROUP 2					
		Rat #1	Rat #2	Rat #3	Rat #4	Rat #5	Rat #6	Rat #7	Rat #8	Rat #9	Rat #10	Rat #11	Rat #12
Tissue Type	Tissue	End of Infusion	8 h	24 h	2 Days	3 Days	7 Days	End of Infusion	4 h	8 h	24 h	2 Days	3 Days
Ocular	Eye (lens)	BQL	BQL	BQL	BQL	BQL	BQL	0.204	BQL	BQL	BQL	BQL	BQL
	Eye (uveal tract)	2.980	3.671	4.567	5.526	7.672	9.584	25.586	19.137	25.855	20.153	14.161	20.277
Reproductive	Epididymis	2.318	4.335	7.206	7.871	1.274	0.854	2.761	2.621	1.253	BQL	NI	BQL
	Prostate Gland	3.323	5.386	2.447	0.721	0.286	NI	11.384	7.930	2.349	4.629	NI	NI
	Seminal Vesicles	0.651	1.585	0.464	0.362	0.211	NI	2.812	2.255	2.472	0.749	0.223	NI
	Testis	1.638	1.234	1.658	1.253	0.650	0.427	0.837	0.549	0.543	0.195	BQL	BQL
Respiratory Tract	Lung	68.836	36.539	26.677	10.320	1.848	0.455	15.299	1.950	0.564	BQL	BQL	BQL
	Trachea	19.967	6.374	3.152	1.615	2.401	NI	6.570	1.629	0.538	NI	NI	NI
Secretory	Harderian Gland	2.746	2.068	2.222	1.367	0.255	BQL	15.932	10.935	9.728	0.484	NI	BQL
	Pancreas	3.816	27.813	10.690	5.976	2.133	0.186	43.169	17.219	10.047	0.830	BQL	NI
	Salivary Gland	5.986	4.662	2.972	1.232	0.349	BQL	39.049	11.878	8.569	BQL	NI	BQL
Skeletal/ Muscular	Bone	0.798	0.657	0.446	0.273	0.255	NI	1.143	0.196	0.405	NI	NI	NI
	Heart	25.416	17.304	18.252	10.944	3.803	0.604	8.668	1.064	0.425	BQL	NI	NI
	Skeletal Muscle	0.891	1.441	0.761	0.322	BQL	BQL	5.466	0.973	0.355	NI	NI	BQL
Vascular/ Lymphatic	Blood (by QWBA)	142.038	70.180	37.122	14.529	1.387	BQL	2.643	0.592	0.267	NI	NI	NI
	Blood (by LSC)	129.795	85.160	40.069	13.585	1.598	BQL	2.633	0.458	BQL	BQL	BQL	BQL
	Plasma (by LSC)	264.840	149.886	69.977	23.174	2.968	BQL	3.434	0.344	BQL	BQL	BQL	BQL
	Bone Marrow	21.229	32.833	14.059	9.524	1.681	NI	23.819	5.832	1.770	NI	NI	NI
	Lymph Node	10.797	6.503	8.202	3.821	1.630	BQL	12.361	4.449	1.840	NI	NI	NI
	Spleen	33.096	66.967	97.421	134.896	60.255	15.404	34.146	6.464	2.722	0.196	BQL	BQL
	Thymus	4.317	5.175	2.524	1.637	0.457	BQL	5.926	3.543	3.234	NI	NI	NI

NI = Not identified on autoradioluminograph.
BQL = Value is below the LLOQ
* = AQL = Above Upper Limit of Quantitation (ULOQ)
QWBA LLOQ = 0.181 µg equivalent / g tissue
QWBA ULOQ = 1839.498 µg equivalent / g tissue
LSC LLOQ = 0.013 µg equivalent / g tissue

(Excerpted from Applicant's submission)

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5.1.2 Mass balance and excretion of radioactivity in Sprague Dawley rats following an intravenous infusion dose of liposome encapsulated [^{14}C] irinotecan (2 studies) (Report (125N-0502)

The purpose of the study was to determine the cumulative percentage and time course of excretion of encapsulated [^{14}C] irinotecan following a single intravenous infusion of 5 mg/kg, as well as evaluating gender differences. Fecal excretion was the major route of elimination in male and female rats accounting for 78 and 83%, respectively, of the administered dose. Excretion of radioactivity occurred primarily within the first 48h, averaging 63% of the dose. Urinary excretion accounted for 23 and 17% of the administered dose in males and females, respectively. There were no major gender differences in excretion.

Table 3: Excretion of MM-398 in Rats

	Percent of Dose Recovered					
	Group 1 Males (0-168 h)			Group 1 Females (0-168 h)		
Feces	78.264	±	2.842	83.436	±	2.260
Bile		NA			NA	
Urine	22.930	±	0.783	16.527	±	1.597
Cage Rinse	1.881	±	0.379	3.435	±	1.079
Cage Wash	0.152	±	0.105	0.150	±	0.063
Cage Wipe	0.088	±	0.085	0.107	±	0.029
Carcass		ND			ND	
Total	103.315	±	2.566	103.654	±	2.843

Note: Values are the mean ± standard deviation (n=3)
NA: Not Applicable
ND: Not Determined, average recovery >90%

(Excerpted from Applicant's submission)

5.2 Toxicokinetics

5.2.1 A 4-week study of the toxicokinetics of PEP02 administered by intravenous infusion to Sprague Dawley rats (Report #7391).

Sponsored by PharmaEngine Inc, Taiwan, Republic of China, August, 2005.

5.2.2 A 4-week study of the toxicokinetics of PEP02 administered by intravenous infusion to Sprague Dawley rats Amendment. (Report #7391a).

October 2008.

Sprague-Dawley rats were administered 65, 130, and 260 mg/kg PEP02 by intravenous infusion once weekly for a 28-day period, followed by a 14-day recovery period. CPT-11, the reference drug was administered at 130 mg/kg. Plasma samples were collected at 10 time-points for toxicokinetic analyses.

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Irinotecan exposure following administration of PEP02 was significantly increased compared to CPT-11 at D1 and D22. In addition, the half-lives of irinotecan liposome and the active metabolite, SN-38 were prolonged in the PEP02-treated group, indicating an increase in systemic residence time with the liposomal formulation. Dose dependent irinotecan drug accumulation ranged from 0.8 to 1.6 at D22.

The amended study report (#7391a) documented minor changes in exposure at D22 and accumulation factors for PEP02 and SN-38. Amended tables have been excerpted from the Applicant's submission below.

Table 4: Summary of toxicokinetic parameters of irinotecan

Day 1, Irinotecan

Group	Sex	Treatment	Dose Level (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	$AUC_{(0-168h)}$ or $AUC_{(0-48h)}$ (ng·h/mL)	$AUC_{(0-\infty)}$ (ng·h/mL)
1	M	PEP02	65	1,068,840	1.5	17.2	29,530,261	27,994,368
2	M	PEP02	130	1,752,860	4.0	14.8	56,700,830	56,732,550
3	M	PEP02	260	2,971,558	1.5	16.5	131,716,900	131,906,725
4	M	CPT-11	130	17,728	1.5	2.1	80,341[a]	80,459
1	F	PEP02	65	1,177,427	1.5	13.2	22,459,065	21,764,798
2	F	PEP02	130	1,883,692	1.5	9.3	51,260,635	51,261,427
3	F	PEP02	260	2,376,723	4.0	20.5	90,063,717	90,437,820
4	F	CPT-11	130	17,754	1.5	2.2	84,371[a]	84,587

Day 22, Irinotecan

Group	Sex	Treatment	Dose Level (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	$AUC_{(0-168h)}$ or $AUC_{(0-48h)}$ (ng·h/mL)	AF[b]
1	M	PEP02	65	2,362,126	1.5	11.7	41,905,975	1.4
2	M	PEP02	130	1,434,680	4.0	20.2	56,287,349	1.0
3	M	PEP02	260	2,047,713	4.0	20.4	108,249,296	0.8
4	M	CPT-11	130	18,006	1.5	2.2	82,409[a]	1.0
1	F	PEP02	65	970,249	4.0	9.9	36,035,932	1.6
2	F	PEP02	130	1,792,738	1.5	13.4	42,360,874	0.8
3	F	PEP02	260	2,954,585	4.0	16.4	102,197,690	1.1
4	F	CPT-11	130	19,989	1.0	2.0	83,286[a]	1.0

[a] $AUC_{(0-48h)}$ after administration of CPT-11.

[b] AF (Accumulation Factor) = Day 22 $AUC_{(0-168h)}$ or Day 22 $AUC_{(0-48h)}$/Day 1 $AUC_{(0-168h)}$ or Day 1 $AUC_{(0-48h)}$

(Excerpted from Applicant's submission)

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Table 5: Summary of toxickinetic parameters of SN-38

Day 1, SN-38

Group	Sex	Treatment	Dose Level (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	$AUC_{(0-168h)}$ or $AUC_{(0-48h)}$ (ng·h/mL)	$AUC_{(0-\infty)}$ (ng·h/mL)
1	M	PEP02	65	1,394	1.5	51.1	57,353	65,780
2	M	PEP02	130	1,486	1.0	46.3	50,318	53,552
3	M	PEP02	260	501	1.0	ND	26,627	ND
4	M	CPT-11	130	95.0	0.5	4.2	598[a]	587
1	F	PEP02	65	1,345	1.5	35.4	39,640	40,473
2	F	PEP02	130	1,230	0.5	20.1	45,895	45,985
3	F	PEP02	260	507	4.0	ND	47,532	ND
4	F	CPT-11	130	70.7	1.5	3.8	443[a]	464

Day 22, SN-38

Group	Sex	Treatment	Dose Level (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	$AUC_{(0-168h)}$ or $AUC_{(0-48h)}$ (ng·h/mL)	AF[b]
1	M	PEP02	65	4,264	1.5	16.2	152,311	2.7
2	M	PEP02	130	539	48.0	45.7	43,439	0.9
3	M	PEP02	260	1,246	1.5	34.7	82,238	3.1
4	M	CPT-11	130	59.8	1.0	4.8	403[a]	0.7
1	F	PEP02	65	4,161	1.5	37.9	132,528	3.3
2	F	PEP02	130	366	0.5	16.0	27,815	0.6
3	F	PEP02	260	1,391	1.5	37.7	46,164	1.0
4	F	CPT-11	130	85.1	1.5	3.9	470[a]	1.1

ND: Not Determined. Insufficient data to determine PK parameters.
NC: Not Calculated.
[a] $AUC_{(0-48h)}$ after administration of CPT-11.
[b] AF (Accumulation Factor) = Day 22 $AUC_{(0-168h)}$ or Day 22 $AUC_{(0-48h)}$/Day 1 $AUC_{(0-168h)}$ or Day 1 $AUC_{(0-48h)}$

(Excerpted from Applicant's submission)

6 General Toxicology

6.2 Repeat-Dose Toxicity

6.2.1 A 4-week toxicity and toxicokinetics study (with a 2-week recovery/observation period) of PEP02 administered by intravenous infusion to Sprague-Dawley rats (Study #PEP02-NC-G-Tx-006)

Sprague-Dawley rats were administered 65, 130, and 260 mg/kg PEP02 by intravenous infusion once weekly for a 28-day period, followed by a 14-day recovery period. CPT-

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11, the reference drug was administered at 130 mg/kg. One mortality occurred at 260 mg/kg PEP02 on D13; 2 deaths occurred in rats treated with CPT-11 on Days 7 and 22. Body weights were reduced in a dose related manner in both genders. High-dose PEP02-treated (260 mg/kg) rats exhibited bone marrow hypocellularity, renal tubular hypertrophy and thymic atrophy. Cardiac histiocytosis was limited to the high dose, although histiocytosis was observed in the spleen, liver, and kidney in vehicle controls as well as all treatment groups. Infusion site toxicities included inflammation, necrosis, and thrombosis. Bone marrow and renal toxicities continued to be observed following the recovery period in HD PEP02-treated animals.

Due to a procedural error that compromised the D1 plasma samples, the toxicokinetic phase of the study was repeated independently under (b) (4) Reports #7391 and #7391a. These data are reviewed under Toxicokinetics.

6.2.2 A 4-week toxicity and toxicokinetics study (with a 2-week recovery/observation period) of PEP02 administered by intravenous infusion to Beagle dogs (Study #PEP02-NC-G-Tx-009)

In a 4-week repeat toxicity dose study), 4, 8, and 16 mg/kg MM-398 was given as a 90 minute intravenous infusion to beagle dogs once weekly for 28 days, with a 14-day recovery period; CPT-11 was administered at 16 mg/kg. Two HD PEP02-treated dogs died following 2 doses with findings of GI hemorrhage and necrosis and severe bone marrow hypocellularity observed macroscopically and microscopically. Peyer's patch atrophy and splenic hematopoiesis were observed in PEP02-treated dogs administered 8 and 16 mg/kg of the drug.

Heart rate, rhythm, and conduction, and electrocardiograms (limb leads I, II and III, and augmented leads aVR, aVL and aVF) were obtained for each dog prior to dosing on Days 1 and 22, and at approximately 1 and 6 hours following the conclusion of the 90 minute infusion period on each dosing day. No cardoiovascular changes were observed.

As observed with rodent toxicokinetics, the irinotecan liposome formulation resulted in extended drug exposure at D1 and 22 due to longer half-life and increased C_{max} and AUC, compared to CPT-11 alone. The irinotecan liposome half-live was prolonged up to 8-fold. Exposure and half-life of SN-38 were also increased relative to CPT-11. See tables below excerpted from Applicant's submission.

Table 6: Toxicokinetic parameters in Beagle dogs on Day 1 and Day 22 following weekly infusion of PEP02

Irinotecan, Day 1

Group	Sex	Treatment	Dose Level (mg/kg)		C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	r^2 (λ determination)	$AUC_{(0-t)}$ (ng·h/mL)	$AUC_{(0-\infty)}$ (ng·h/mL)	$MRT_{(0-\infty)}$ (h)
2	M	PEP02	4	Mean	76181	1.5	31.1	0.982	720746	721836	6.4
				SD	7872	0.0	7.3	0.012	106728	106662	0.9
3	M	PEP02	8	Mean	105478	1.5	17.5	0.920	1297080	1238846	7.7
				SD	2353	0.0	NC	NC	125288	NC	NC
4	M	PEP02	16	Mean	283830	1.5	32.6	0.938	3568966	3570079	9.1
				SD	5220	0.0	3.6	0.025	254022	254081	0.3
5	M	CPT-11	16	Mean	7908	1.5	3.8	0.969	36022	37038	4.0
				SD	1741	0.0	0.7	0.036	10314	9724	0.5
2	F	PEP02	4	Mean	65981	1.5	17.6	0.998	652140	737960	7.5
				SD	11590	0.0	NC	NC	151933	NC	NC
3	F	PEP02	8	Mean	84496	1.5	25.9	0.995	803233	804305	6.4
				SD	12664	0.0	4.6	0.004	115293	115599	1.0
4	F	PEP02	16	Mean	271538	1.5	32.7	0.914	3942032	2928449	9.1
				SD	15868	0.0	NC	NC	991849	NC	NC
5	F	CPT-11	16	Mean	10573	1.3	4.0	0.979	44722	45237	3.3
				SD	1684	0.3	1.1	0.023	5925	6213	0.8

Irinotecan, Day 22

Group	Sex	Treatment	Dose Level (mg/kg)		C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	r^2 (λ determination)	$AUC_{(0-t)}$ (ng·h/mL)	$MRT_{(0-\infty)}$ (h)	D22/D1 AUC Ratio*
2	M	PEP02	4	Mean	71601	1.5	9.1	0.833	647401	7.6	0.9
				SD	13680	0.0	NC	NC	195710	NC	0.2
3	M	PEP02	8	Mean	97172	1.5	19.7	0.869	1411822	10.1	1.1
				SD	8568	0.0	NC	NC	337473	NC	0.3
4	M	PEP02	16	Mean	301724	1.5	18.0	0.820	5613457	17.4	1.5
				SD	NC	NC	NC	NC	NC	NC	NC
5	M	CPT-11	16	Mean	8892	1.5	3.9	0.962	37990	3.3	1.1
				SD	2333	0.0	NC	NC	12170	NC	0.3
2	F	PEP02	4	Mean	54697	1.5	38.8	0.899	676355	11.7	1.0
				SD	2797	0.0	NC	NC	386937	NC	0.5
3	F	PEP02	8	Mean	80812	1.5	8.9	0.842	734539	7.7	0.9
				SD	16558	0.0	NC	NC	213652	NC	0.2
4	F	PEP02	16	Mean	208396	1.5	23.2	0.889	3936777	14.4	1.0
				SD	37029	0.0	12.6	0.029	822144	4.2	0.1
5	F	CPT-11	16	Mean	10513	1.3	4.5	0.910	38932	3.0	0.9
				SD	1759	0.3	0.8	0.084	4939	0.4	0.2

NC: Not Calculated. SD was not calculated due to n<3.

Note: Time is expressed relative to the start of IV infusion.

* D22/D1 AUC Ratio = mean of individual [Day 22 $AUC_{(0-t)}$/Day 1 $AUC_{(0-t)}$]

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Table 7: Toxicokinetic parameter of SN-38 in Beagle dogs on Days1 and 22 following weekly infusion of PEP02

SN-38, Day 1

Group	Sex	Treatment	Dose Level (mg/kg)		C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	r^2 (λ determination)	$AUC_{(0-t)}$ (ng·h/mL)	$AUC_{(0-\infty)}$ (ng·h/mL)	$MRT_{(0-\infty)}$ (h)
2	M	PEP02	4	Mean	5.32	1.5	10.4	0.993	48.2	73.4	15.4
				SD	1.08	0.0	7.3	0.010	11.9	26.4	10.2
3	M	PEP02	8	Mean	11.2	1.5	7.5	0.973	138	157	11.3
				SD	3.5	0.0	NC	NC	32	NC	NC
4	M	PEP02	16	Mean	16.8	1.5	8.3	0.942	187	232	12.4
				SD	2.0	0.0	NC	NC	22	NC	NC
5	M	CPT-11	16	Mean	14.3	1.5	3.1	0.998	59.6	67.0	4.6
				SD	4.1	0.0	1.1	0.003	18.0	19.6	1.3
2	F	PEP02	4	Mean	4.72	1.3	10.4	0.961	38.2	78.2	15.3
				SD	1.15	0.3	NC	NC	8.3	NC	NC
3	F	PEP02	8	Mean	7.93	1.3	11.2	0.939	68.5	137	16.5
				SD	1.06	0.3	6.0	0.080	10.1	62	9.1
4	F	PEP02	16	Mean	17.1	1.5	6.9	0.965	191	209	10.2
				SD	2.7	0.0	0.7	0.008	5	10	0.5
5	F	CPT-11	16	Mean	16.1	1.3	2.4	0.965	62.2	70.8	3.8
				SD	2.6	0.3	0.8	0.030	19.1	18.7	0.8

SN-38, Day 22

Group	Sex	Treatment	Dose Level (mg/kg)		C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	r^2 (λ determination)	$AUC_{(0-t)}$ (ng·h/mL)	$MRT_{(0-\infty)}$ (h)	D22/D1 AUC Ratio*
2	M	PEP02	4	Mean	2.71	1.5	15.0	0.819	13.0	21.8	0.2
				SD	0.76	0.0	NC	NC	11.0	NC	0.2
3	M	PEP02	8	Mean	7.17	2.8	11.2	0.951	98.3	16.1	0.7
				SD	0.23	2.8	1.5	0.082	23.5	1.5	0.1
4	M	PEP02	16	Mean	14.5	1.5	13.5	0.968	174	18.0	1.0
				SD	NC	NC	NC	NC	NC	NC	NC
5	M	CPT-11	16	Mean	15.2	1.3	1.7	0.992	42.6	2.9	0.7
				SD	4.5	0.3	NC	NC	16.1	NC	0.1
2	F	PEP02	4	Mean	3.79	1.5	13.8	0.985	20.1	20.5	0.5
				SD	1.92	0.0	NC	NC	7.4	NC	0.2
3	F	PEP02	8	Mean	7.51	1.5	11.3	0.909	56.8	17.0	0.8
				SD	2.71	0.0	NC	NC	15.0	NC	0.2
4	F	PEP02	16	Mean	15.9	1.5	7.5	0.984	193	11.3	1.0
				SD	2.5	0.0	NC	NC	37	NC	0.2
5	F	CPT-11	16	Mean	13.4	1.2	2.4	0.941	45.8	3.8	0.8
				SD	1.0	0.3	1.1	0.052	9.8	1.2	0.3

NC: Not Calculated. SD was not calculated due to n<3.
Note: Time is expressed relative to the start of IV infusion.
* D22/D1 AUC Ratio = mean of individual [Day 22 $AUC_{(0-t)}$/Day 1 $AUC_{(0-t)}$]

(Excerpted from Applicant's submission)

6.2.3 A 6-cycle (3 weeks per cycle) infusion toxicity study in Sprague Dawley rats followed by a 6-week recovery period (Study #1005-3071).

This study was previously reviewed under IND 102,799 by Dr. Whitney S. Helms. Relevant portions of that review are appended to the current review as Appendix 1. The study is summarized for this review.

Sprague-Dawley rats were administered a single intravenous infusion of 30, 75, and 190 mg/kg PEP02 on days 1, 22, 43, 64, 85, and 106 (6 cycles), followed by a 6-week recovery period. The administered dose of CPT-11 was 75 mg/kg.mg/kg.

Key Study findings:

❖ Single drug-related lethality at the LD and HD; bone marrow hypocellularity and histiocyte accumulation of multiple organs in HD decedent.

❖ Accumulation of foamy histiocytes in multiple organs of control and HD animals following dosing and recovery; possibly vehicle related. Not exhibited with CPT-11.

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❖ Comparative reduction in incidence of dental loss or damage, tremors, and uncoordinated gait/walking on toes with PEP02, increased aggressiveness in PEP02-treated animals.
❖ Bone marrow hypocellularity, splenic hematopoiesis, and hepatic necrosis in HD PEP02 animals; hepatic necrosis observed following recovery
❖ Mid-dose and high-dose body weights were significantly reduced
❖ Increased exposure and prolonged half-life with irinotecan liposome and SN-38 compared to CPT-11

Note: Accumulation of foamy histiocytes also observed in 4-week rat study

Table 8: Toxicokinetics of rats administered PEP02 for 6 cycles

Daily Dose (mg/kg)		0 (Control)		30 (Low Dose)		75 (Mid Dose)		190 (High Dose)		75 (Reference)	
Number of Animals		M: 15	F: 15	M: 10	F: 10	M: 10	F: 10	M: 15	F: 15	M: 15	F: 15
Toxicokinetics of Irinotecan											
Day 1	AUC_{0-168h} (ng•h/mL)	-	-	19414731	14137698	51302953	45859924	116672588	114271250	38544	44308
	C_{max} (ng/mL)	-	-	6800687	575923	17260286	1549373	4217100	3981660	10698	9716
	T_{max} (h)	-	-	1.5	4	1.5	1.5	4	1.5	1.5	1.5
	$t_{½}$ (h)	-	-	15.84	15.72	20.52	21.07	23.34	21.11	9.79	9.93
Day 106	AUC_{0-168h} (ng•h/mL)	-	-	37688133	24292002	90277680	80220620	251655061	248874586	82610	82898
	C_{max} (ng/mL)	-	-	1003821	827747	2170757	2160715	5733162	5402740	21181	18529
	T_{max} (h)	-	-	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
	$t_{½}$ (h)	-	-	28.4	17.98	35.12	30.02	34.57	31.3	4.79	5.02
	AF[a]	-	-	1.92	1.72	1.76	1.53	2.16	1.94	2.14	1.87
SN-38:											
Day 1	AUC_{0-168h} (ng•h/mL)	-	-	289357	207619	1137255	488596	1558211	791771	14722	5274
	C_{max} (ng/mL)	-	-	3525	3184	5855	3935	3804	2991	2199	1211
	T_{max} (h)	-	-	0.5	12	1.5	48	24	48	4	1
	$t_{½}$ (h)	-	-	45.5	19.14	184.54	59.83	263.74	155.01	10.4	9.36
Day 106	AUC_{0-168h} (ng•h/mL)	-	-	292359	168939	292338	310389	334862	319691	10488	4623
	C_{max} (ng/mL)	-	-	3568	3041	4864	4435	5074	4102	1520	1251
	T_{max} (h)	-	-	1.5	0.5	4	48	1	72	1	1
	$t_{½}$ (h)	-	-	347.05	NC	278.55	366.5	NC	NC	7.68	4.5
	AF[a]	-	-	1.37	0.87	0.86	1.06	1.06	1.27	0.71	0.88

[a]AF (accumulation factor) = Day 106 AUC $_{(0-168)}$/ Day 1 AUC $_{(0-168)}$; NC= not calculated

6.2.4 A 6-cycle (3 weeks per cycle) toxicity and toxicokinetics study (with a 6-week recovery/observation period) of PEP02 administered by intravenous infusion to Beagle dogs (Study #1006-2162).

This study was previously reviewed under IND 102,799 by Dr. Whitney S. Helms. Relevant portions of that review are appended to the current review as Appendix 1. The study is summarized for this review.

Beagle dogs were administered a single intravenous infusion of 9, 15, 36/21 mg/kg PEP02 on days 1, 22, 43, 64, 85, and 106 (6 cycles), followed by a 6-week recovery period. The administered dose of CPT-11 was 36/21 mg/kg. Following excessive mortality (8/12 PEP02 and 9/12 CPT-11 dogs found dead or sacrificed moribund) at 36 mg/kg, the HD dose for PEP02 as well as the CPT-11 dose was lowered to 21 mg/kg. Additional mortality included a single undetermined death at 15 mg/kg and a single

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death at 21 mg/kg PEP02. Histopathology of decedent animals indicated bone marrow, gastrointestinal and lymphoid tissue toxicities.

Key Study findings:

❖ 70% lethality at 36 mg/kg PEP02; single drug-related deaths at 15 and 21 mg/kg with bone marrow, gastrointestinal and lymphoid tissue organ toxicities
❖ The primary targets were the gastrointestinal tract, lymphatic and hematopoietic tissues (lymphoid atrophy/epithelial necrosis of GI, lymphoid atrophy of spleen, histiocytosis of spleen and liver, uterine atrophy).
❖ In general, histopathological findings similar for PEP01 and CPT-11 with exception of epithelial necrosis of GI not observed with CPT-11
❖ Greater degree of platelet and WBC depletion following PEP02 compared to CPT-11
❖ Increased exposure and prolonged half-life with irinotecan liposome and SN-38 compared to CPT-11; increased clearance with CPT-11

Table 9: Toxicokinetics of dogs administered PEP02 for 6 cycles

Daily Dose (mg/kg)		0 (Control)		9 (Low Dose)		15 (Mid Low Dose)		21 (Mid High Dose)		36 (High Dose)		21 (CPT11)		36 (CPT11)	
Number of Animals		M:4	F:4	M:4	F:4	M:4	F:4	M:4	F:4	M:4	F:4	M:4	F:4	M:4	F:4
Toxicokinetics:															
Irinotecan															
Day 1	AUC_{0-168h} (µg•h/mL)	-	-	2075	2080	4088	3446	5750	5394	10198	12663	47.7	54.5	141	98.4
	C_{max} (µg/mL)	-	-	147	150	308	227	332	317	550	527	13.6	13.8	21.9	19.8
	T_{max} (h)	-	-	1.5	1.5	1.51	1.51	1.5	1.5	1.51	1.5	1.5	1.5	1.38	1.5
	$t_{½}$ (h)	-	-	5.3	6.1	6.2	6.1	5.4	6.6	7.5	8	2.4	2.6	4.4	4.1
Day 106	AUC_{0-168h} (µg•h/mL)	-	-	2344	1748	4660	4127	9423	8775	-	-	57.3	61.8	-	-
	C_{max} (µg/mL)	-	-	142	136	226	207	340	320	-	-	14.6	16.6	-	-
	T_{max} (h)	-	-	1.5	1.5	1.51	1.51	2.63	1.5	-	-	1.38	1.5	-	-
	$t_{½}$ (h)	-	-	5.8	6.4	7.2	7.1	11.6	11.4	-	-	2.5	3	-	-
	AF*	-	-	1.13	0.84	1.14	1.2	1.64	1.63	-	-	1.2	1.09	-	-
SN-38:															
Day 1	AUC_{0-168h} (µg•h/mL)	-	-	135	126	287	250	339	316	527	589	131	143	294	172
	C_{max} (µg/mL)	-	-	11.2	10.1	21.6	20.2	27	25.7	47.4	45.1	27.6	26.5	43.9	31.3
	T_{max} (h)	-	-	1.5	1.38	1.51	1.38	1.5	1.5	1.51	1.5	1.5	1.63	1.51	1
	$t_{½}$ (h)	-	-	16.6	16.1	29.9	20.6	34.1	30.8	25.3	28.3	5.8	8.3	7.8	7.5
Day 106	AUC_{0-168h} (µg•h/mL)	-	-	135	119	267	269	456	382	-	-	154	211	-	-
	C_{max} (µg/mL)	-	-	10.3	11.1	17.1	18.2	23.2	21.1	-	-	34.8	42.9	-	-
	T_{max} (h)	-	-	1.5	1.38	1.38	1.51	1.5	1.5	-	-	1.13	1.38	-	-
	$t_{½}$ (h)	-	-	19.8	16.5	38.6	36	40.3	30.3	-	-	4.6	5.6	-	-
	AF*	-	-	1	0.94	0.93	1.08	1.35	1.21	-	-	1.18	1.48	-	-

*AF (accumulation factor) = Day 106 AUC ($_{0-168h}$)/ Day 1 AUC ($_{0-168h}$); NC= not calculated

7 Genetic Toxicology

No studies have been performed to assess the potential of liposomal irinotecan for genetic toxicity, and no studies are warranted for a 505(b)(2) relying on the previous findings from the listed drug.

Irinotecan HCl, the reference drug, was clastogenic both in vitro (chromosome aberrations in Chinese hamster ovary cells) and in vivo (micronucleus test in mice). Neither irinotecan HCl nor its active metabolite, SN-38, was mutagenic in the *in vitro* Ames assay.

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8 Carcinogenicity

No studies have been conducted to assess the potential of liposomal irinotecan for carcinogenicity and none are warranted for a product intended for the treatment of advanced cancer.

When irinotecan HCl, the reference drug, was administered to rats once weekly for 13 weeks followed by a 91 week recovery period, a significant linear trend between irinotecan HCl dosage and the incidence of combined uterine horn endometrial stromal polyps and endometrial stromal sarcomas was observed.

9 Reproductive and Developmental Toxicology

Reproductive toxicity studies, including embryofetal development studies, with liposomal irinotecan were not conducted. Instead, the Applicant used reproductive toxicity studies conducted for irinotecan HCl, the reference drug. In addition, atrophy of male and female reproductive organs was observed in dogs administered ≥9 mg/kg liposomal irinotecan once every 3 weeks for 6 cycles.

10 Special Toxicology Studies

None

11 Integrated Summary and Safety Evaluation

Pharmacology

Irinotecan liposome is a liposomal encapsulation of irinotecan hydrochloride, the active ingredient. Irinotecan is a water-soluble semi-synthetic derivative of camptothecin, a topoisomerase I inhibitor. Irinotecan and its active metabolite SN-38 bind reversibly to the topoisomerase 1 – DNA complex and induce single-strand breaks. The presence of SN-38 or irinotecan complexes with topoisomerase 1 appears to prevent re-ligation of these single strand breaks leading to double-strand breaks and subsequent cell death..The liposomal formulation of irinotecan resulted in longer exposure of both irinotecan and SN-38 compared to free irinotecan in all species tested. The effectiveness of irinoecan liposome was evaluated in several xenograft tumor models, including pancreatic tumor models, and exhibited improved tumor growth inhibition compared to equivalent doses of free irinotecan. The presence of irinotecan liposome also resulted in improved anti-tumor activity when used in combination with 5-FU compared to the activity of the single agents alone. There were no cardiovascular effects observed in dogs administered up to 21 mg/kg irinotecan liposome in a safety pharmacology study.

Pharmacokinetics

The Applicant evaluated the in vivo pharmacokinetics of irinotecan liposome following IV administration to rats and dogs. In general, the AUC and C_{max} of irinotecan and the active metabolite, SN-38, were significantly increased in animals treated with irinotecan

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liposome compared to irinotecan hydrochloride in both species. Overall plasma, blood, and tumor concentrations of the liposome were increased; irinotecan liposome has exhibited a 70-fold higher AUC when compared to irinotecan hydrochloride. In addition, the half-lives of irinotecan liposome and SN-38 were prolonged, indicating an increase in systemic residence time with the liposomal formulation. Dose dependent irinotecan liposome drug accumulation was minimal. A mass balance study using ^{14}C-labelled irinotecan liposome showed that after release from the liposome, encapsulated irinotecan follows the same pharmacokinetic pattern as the unencapsulated drug. The half-life of irinotecan following irinotecan liposome administration in rats ranged from 15-32h compared to 4-10h for irinotecan hydrochloride, while in dogs the irinotecan half-lives were lower at 5-7 and 2-3 hours for irinotecan liposome and irinotecan hydrochloride, respectively. This compares to an irinotecan half-life of ~26 hours following clinical administration of irinotecan liposome. Based on irinotecan AUC data from dogs administered 9 mg/kg [180 mg/m^2]), irinotecan exposures in the 6-cycle dog study using irinotecan liposome were ~ 1.5 times the clinical AUC of 1364 µg•h/mL when ONIVYDE was administered at a dose of 80 mg/m^2 as a monotherapy.

Fecal excretion was the major route of elimination of irinotecan liposome in male and female rats, accounting for 78 and 83%, respectively, of the administered dose. Excretion of radioactivity occurred primarily within the first 48 hours, averaging 63% of the dose. Urinary excretion accounted for 23 and 17% of the administered dose in males and females, respectively. While no study was conducted with iriinotecan liposome, radioactively labelled irinotecan hydrochloride was detected in rat milk shortly after infusion and concentrated up to 65-fold at 4 hours after administration.

In general, tissues from animals administered irinotecan liposome exhibited higher free irinotecan concentrations with tissue levels remaining elevated for extended times compared to non-encapsulated irinotecan. The highest irinotecan tissue concentrations occurred in the liver, kidney, bile duct, lung, heart, spleen, bone marrow, trachea, brown adipose and thyroid. Ocular uveal tract radioactivity distribution was associated with both liposomal and free irinotecan. CNS tissues had moderate concentrations of irinotecan through 8h following dosing with irinotecan liposome

General Toxicology

Irinotecan liposome was evaluated in 4-week and 6-cycle studies in rats and dogs. In general, toxicities were similar following dosing of irinotecan liposome or free irinotecan. Following 4 weeks of dosing in rats, toxicities in animals administered irinotecan liposome at the 260 mg/kg dose level included bone marrow hypocellularity, renal tubular hypertrophy and thymic atrophy, as well as histiocytosis of multiple organs. A lower incidence of thymic atrophy and histiocytosis was observed at irinotecan liposome doses of 65 and 130 mg/kg. Since histiocytosis was observed in control animals, the finding was considered to be a vehicle related finding, and, although this was not confirmed, it is consistent with findings from other liposomal drug formulations. Infusion site toxicities included inflammation, necrosis, and thrombosis. Irinotecan liposome was lethal at 16 mg/kg in dogs. Primary target sites in dogs administered irinotecan liposome

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at the 8 and 16 mg/kg dose levels included the gastrointestinal tract, bone marrow, Peyer's patch and spleen. No cardiovascular changes were observed. Gastrointestinal and hematological toxicities observed in rodents and nonrodents are consistent with findings observed in the clinic following treatment with Onivyde; four patient deaths were reported as a result of gastrointestinal toxicity, enterocolitis, and septic shock. Irinotecan and SN-38 toxicokinetics following dosing of both species indicated significantly higher systemic exposure and prolonged half-life in irinotecan liposome-treated animals compared to irinotecan hydrochloride.

Following 6 cycles of dosing with 30, 75 or 190 mg/kg irinotecan liposome in rats, findings of bone marrow hypocellularity, hepatic necrosis, hematopoiesis of the spleen, and accumulation of foamy histiocytes in multiple organs were observed. As in the 4-week study, histiocytes were also observed in multiple organs of control animals and were considered to be vehicle related. Body weights were consistently depressed at 75 and 190 mg/kg. The incidence of dental loss/damage and neurological findings (tremors, uncoordinated gait/walking on toes) was reduced with irinotecan liposome compared to irinotecan hydrochloride, although a comparative increase in aggressiveness was observed with the liposome.

Irinotecan liposome was lethal to dogs at the dose of 36 mg/kg administered in a 6-cycle repeat dose study. The primary target organs identified at irinotecan dose levels of 15 and 21 mg/kg during this study were the gastrointestinal tract and lymphatic and hematopoietic tissues (lymphoid atrophy/epithelial necrosis of GI, lymphoid atrophy of spleen, histiocytosis of spleen and liver and uterine atrophy). In general, histopathological findings were similar for irinotecan liposome and irinotecan hydrochloride with the exception of gastrointestinal epithelial necrosis which was not observed with free irinotecan. In addition, a greater degree of platelet and WBC depletion was observed following administration of irinotecan liposome compared to irinotecan hydrochloride. Increased exposure and prolonged half-life were exhibited for irinotecan and SN-38 following administration of irinotecan liposome compared to irinotecan hydrochloride in both rats and dogs, associated with faster clearance observed following administration of irinotecan hydrochloride.

Reproductive Toxicology

Atrophy of male and female reproductive organs was observed in dogs administered ≥9 mg/kg liposomal irinotecan once every 3 weeks for 6 cycles, a dose resulting in an exposure of approximately 1.5 times the clinical exposure of irinotecan following administration of ONIVYDE.

Intravenous administration of irinotecan HCl at a dose of 6 mg/kg/day to rats and rabbits during the period of organogenesis resulted in increased post-implantation loss and decreased numbers of live fetuses. In separate studies in rats, this dose resulted in an irinotecan exposure of approximately 0.002 times the exposure of irinotecan based on AUC in patients administered ONIVYDE at the 80 mg/m^2 dose. Administration of irinotecan HCl resulted in structural abnormalities and growth delays in rats at doses greater than 1.2 mg/kg/day (approximately 0.0002 times the clinical exposure to

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irinotecan in ONIVYDE based on AUC) and in rabbits at 72 mg/m^2/day. Teratogenic effects included a variety of external, visceral, and skeletal abnormalities. Irinotecan HCl administered to rat dams for the period following organogenesis through weaning at doses of 6 mg/kg/day caused decreased learning ability and decreased female body weights in the offspring.

References

Kalra AV, Kim J, Klinz SG, Paz N, Cain J, Drummond DD, Nielsen UB, Fitzgerald JB. 2014. Preclinical activity of nanoliposomal irinotecan is governed by tumor deposition and intratumor prodrug conversion. Cancer Res. **74(23**):7003-7013.

12 Appendix/Attachments

Excerpted from the original review of IND 102799

Study title: A 6-Cycle (3 weeks per cycle) Infusion Toxicity Study in Sprague Dawley Rats Followed by a 6-Week Recovery Period

Key study findings:

- There was increased exposure (Cmax and AUC) for PEP02 compared to test article alone (CPT-11).
- The major toxicity appeared to be an accumulation of foamy histiocytes/ macrophages in multiple organs including the adrenals and peripheral immune organs. This was partially resolved in the recovery animals. The vehicle could be contributing to this toxicity as it was seen in the control group animals as well as the PEP02 animals, particularly in the high dose group.
- There was a single mortality judged to be study related—a male in the high dose group (group 4) was euthanized early. Upon histological examination, hematopoietic hypocellularity in the femur was found.
- There appeared to be a difference between PEP02 and CPT-11on the dentition. PEP02 treated animals had a reduction in tooth loss and tremors.

Study no.: 1005-3071
Volume #, and page #: Vol. 4 p.723
Conducting laboratory and location: (b) (4)
Date of study initiation: January 9, 2006
GLP compliance: yes
QA report: yes ( ) no (x) Draft Report
Drug, lot #, and % purity: PEP02, LIIAS3001, to be supplied
PEP02, LIIA12, to be supplied
PEP02, LIIA14, to be supplied
CPT-11, 70495AA002,99.8%
CPT-11, 70495AA006, to be supplied

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PEP02 Placebo, LII12P, to be supplied
PEP02, Placebo, LII14P, to be supplied
PEP02 Placebo, LII14P-2, to be supllied

Methods

Treatment Group	Dose Level* (mg/kg)	Number of Animals					
		Main		Recovery		Toxicokinetics	
		M	F	M	F	M	F
1.Control Article	0	20	20	10	10	0	0
2. Low Dose	30	20	20	0	0	6	6
3. Mid Dose	75	20	20	0	0	6	6
4. High Dose	190	20	20	10	10	6	6
5. Ref. Article (CPT-11)	75	20	20	10	10	6	6

* expressed as dose of CPT-11

Doses: 0, 30, 75, or 190 mg/kg given on days 1, 22, 43, 64, 85, and 106.
Species/strain: Rats/Sprague Dawley
Number/sex/group or time point (main study): 20/sex/group

Route, formulation, volume, and infusion rate:

Treatment Group	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Conc. (mg/mL)	Infusion Rate (mL/kg/hr)
1. Control	0	15	0	90
2. Low	30	15	2	90
3. Mid	75	15	5	90
4. High	190	15	12.67	90
5. CPT-11	75	15	5	90

Control, PEP02, or CPT-11 were given by IV tail vein injection over a period of 90 minutes on days 1, 22, 43, 64, 85, and 106.

Satellite groups used for toxicokinetics or recovery: 6/group/sex except group 1 (control article).
Age: ~6 weeks
Weight: M: 268-358g F: 180-243g
Sampling times: During weeks 9 and 10 and at necropsy

Observation and Times:
Clinical signs: upon arrival, prior to randomization, and weekly starting 1 week prior to dosing, prior to each dose, the day before necropsy, and prior to necropsy
Body weights: upon arrival, prior to randomization, last week pre-treatment and then twice weekly until necropsy
Food consumption: last week pre-treatment and weekly until necropsy
Ophthalmoscopy: once during pre-treatment and once during week 18 (days 120-126)

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EKG: not performed
Hematology: once during week 9 and at necropsy
Clinical chemistry: once during week 9 and at necropsy
Urinalysis: once during week 9 and at necropsy
Gross pathology: necropsies conducted on all animals regardless of cause of death
Organ weights : see histopath table
Histopathology:
Adequate Battery: yes (x), no ( )
Peer review: yes ( ), no (x) not stated

Results
Mortality:

Animal #	Group	Day	Sex	Fate	Findings
1511E	1 (control)	34	F	euthanized	Fracture at distal right femur
1501A	1 (control)	67	F	euthanized	Broken right tibia
1026J	1 (control)	113	M	euthanized	Red discharge from genitals/evidence of pain follwing ab. palpation
2016G	2 (30mg/kg)	57	M	found dead	No cause of death determined; dark discoloration of the thymus and lymph node were found at necropsy along with enlargement of the lymph node
4023I	4 (190mg/kg)	117	M	euthanized	Conjunctivitis-yellow discharge from eyes, hematopoietic hypocelluarity in the femur/sternal bone and thymus, histiocyte/macrophage accumulation in adrenals, lymph nodes and spleen

Clinical signs:

	Group 1 (Vehicle)		Group 2 (30 mg/kg)		Group 3 (75 mg/kg)		Group 4 (190 mg/kg)		Group 5 CPT-11 (75 mg/kg)	
	M	F	M	F	M	F	M	F	M	F
Oral-dental/tooth loss or damage		2	7	9	1	3	7	6	29	30

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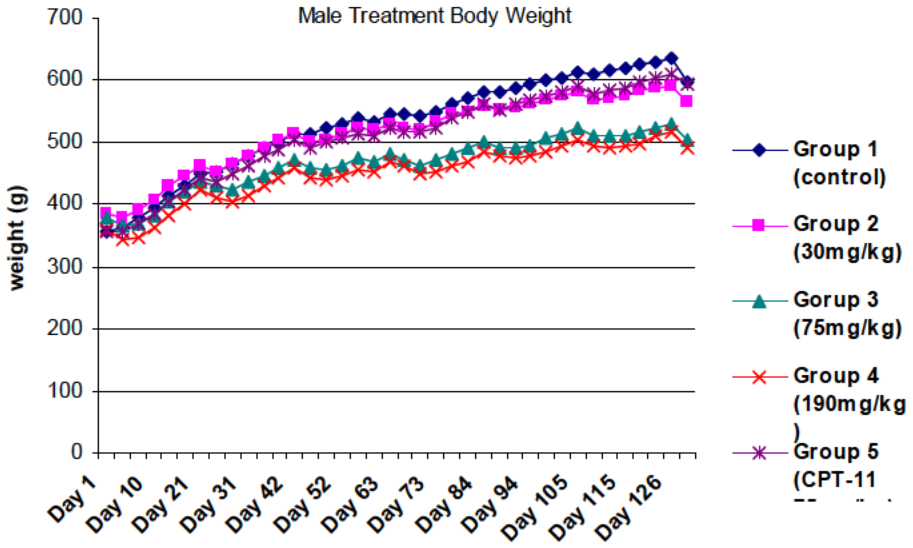
	Group 1 (Vehicle)		Group 2 (30 mg/kg)		Group 3 (75 mg/kg)		Group 4 (190 mg/kg)		Group 5 CPT-11 (75 mg/kg)	
	M	F	M	F	M	F	M	F	M	F
Increased aggressiveness			5	1	2		1		1	
Changed gait/loss of gait		2		1	1	1	1	1	3	3
Decreased activity/weaknes s							1		5	
Thin body condition							4			2
Walking on toes/posture						2	1		6	6
Tremors		2							29	30
Eyes partially closed		1							3	
Eating behavior		1		1					2	2

Body weights:

After Days 84 or 45, Groups 3 and 4 respectively showed a consistently lower body weight (greater than 10% change) than group 1 controls. This remained consistent in the Group 4 recovery mice, but did not increase to more than a 22% difference.



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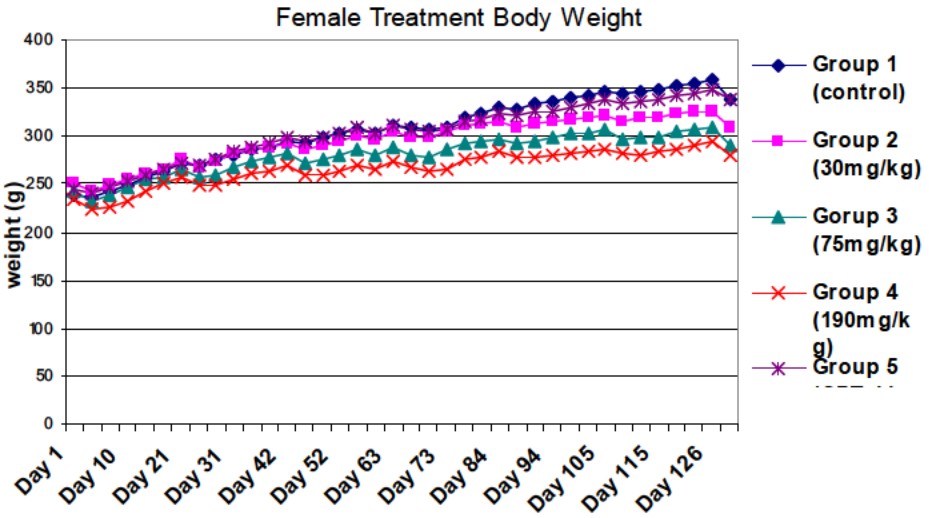

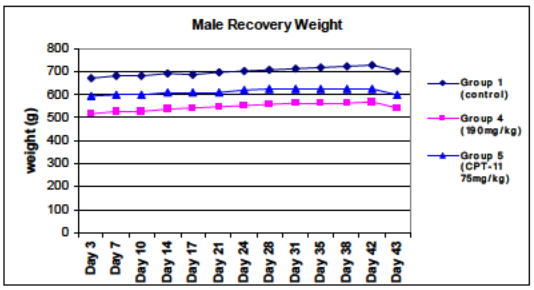

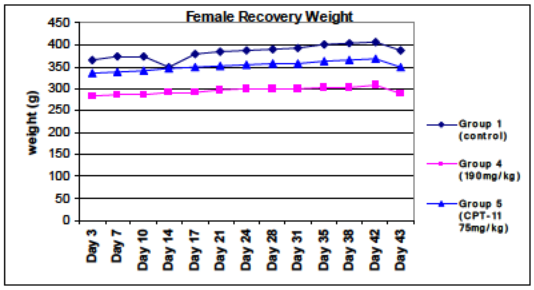

Food consumption:

There was a consistent drop in food consumption the week after treatment for all groups. Group 4 rats of both sexes consumed ≥10% less food than animals control starting at Week 4 (after day 22). Group 3 rats showed the same food consumption decrease except that decreases in female rats were not as consistent until Week 9. The decrease was dose dependent.

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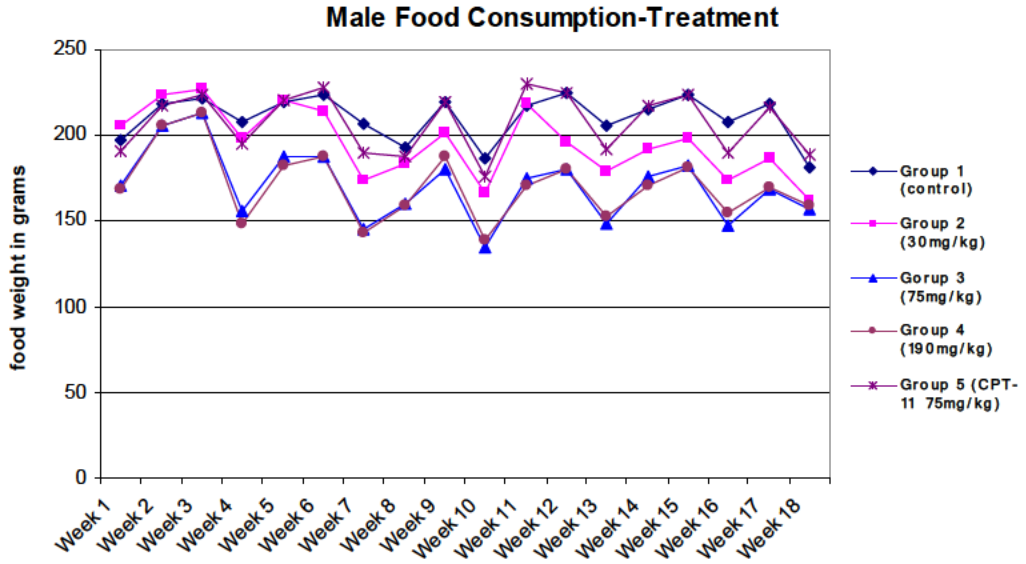

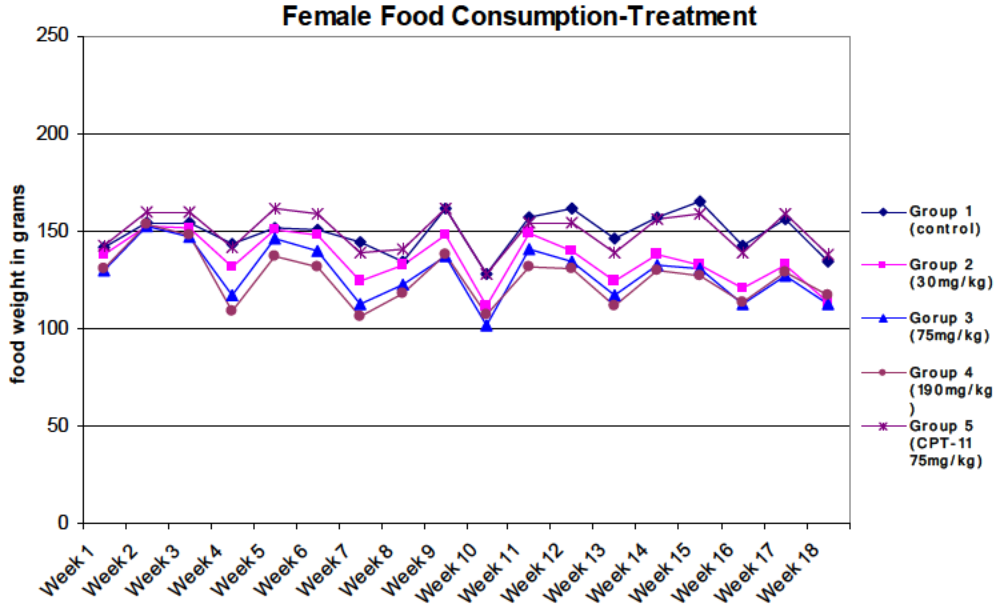

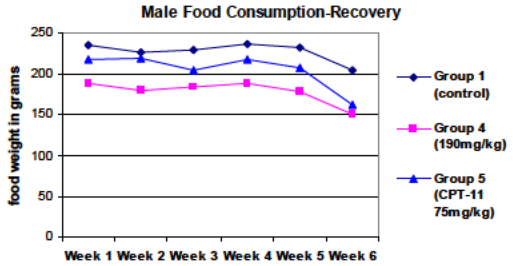

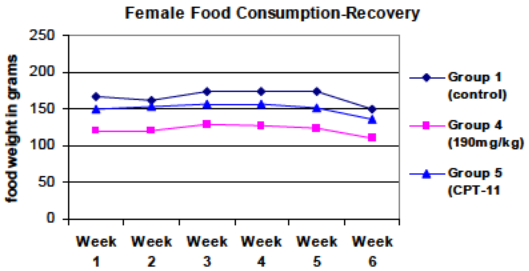

Ophthalmoscopy: no test article related findings

Hematology:

- Female WBC and individual WBC subtypes are consistently about half of what is seen in males in all groups—including vehicle control.
- Most changes, even when statistically significant were within acceptable ranges for rats. Changes in WBC and RBC appeared to be dose dependent with higher toxicities seen in the Group 4 animals.
- Neutrophils were not specifically depleted.
- Changes were mostly reversible in the recovery groups.

Percent Change from Vehicle Control

	Group 2 (30 mg/kg)		Group 3 (75 mg/kg)		Group 4 (190 mg/kg)		Group 5 CPT-11 (75 mg/kg)	
	M	F	M	F	M	F	M	F
RBC			↓5% ↓**8%**		↓21% ↓**21%** ↓*11%*	↓5% ↓**5%**		
HGB					↓14% ↓**10%**	↓4% ↓**3%** ↑*5%*		
HCT					↓13% ↓**%5**	↓4%		
MCV			↑3% ↑**7%**		↑10% ↑**20%** ↑*5%*			
MCH			↑2% ↑**6%**		↑7% ↑**14%**			
MCHC					↓2% ↓**4%**	↑*2%*		
RDW			↑17% ↑**12%**		↑40% ↑**30%**	↑7% ↑**8%** ↓*10%*	↑8%	↓*6%*
HDW			↑9% ↑**9%**		↑20% ↑**15%**	↑4% ↓*8%*		
PLT			↑**13%**	↓10%	↑24% ↑**37%**			
PCT		↓14%		↓14%	↑33% ↑**50%**			
WBC		↓**25%**	↓22% ↓**18%**	↓**16%**	↓37% ↓**32%** ↓*22%*	↓**26%**		
NEUT			↓22%		↓40%		↓**40%**	
LYMPH		↓**27%**	↓22% ↓**23%**	↓**23%**	↓36% ↓**38%**	↓**31%**		
MONO			↓12%		↓32%			
EOS			↓33%		↓62% ↓**38%** ↓*43%*			
BASO		↓**50%**	↓44%	↓**50%**	↓56% ↓**33%**	↓**50%**		
NEUT %					↓9% ↓**37%**		↓**38%**	

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	Group 2 (30 mg/kg)		Group 3 (75 mg/kg)		Group 4 (190 mg/kg)		Group 5 CPT-11 (75 mg/kg)	
	M	F	M	F	M	F	M	F
LYMPH%					↓**8%**		↓**8%**	
MONO%					↓**45%**	↑33%		
EOS%			↓14%		↓14%	↑**75%**		
BASO%		↓50%		↓33%	↓**33%**			↓33%
RETIC			↑45% ↑**48%**		↑96% ↑**101%**	↑44% ↑**30%**	↑27%	
RETIC%			↑62% ↑**67%**		↑151% ↑**174%**	↑54% ↑**37%**	↑33%	

Statistically Significant at Week 9/**Terminal**/*recovery*

Clinical chemistry: No remarkable changes.

Urinalysis: No remarkable changes

Gross pathology: Gross pathology indicates that there may be an effect on the kidney, but it is not directly related to the test article, as both the vehicle control and the drug only control show similar effects.

Macroscopic Observations		Group 1 (control)		Group 2 (30 mg/kg)		Group 3 (75 mg/kg)		Group 4 (190 mg/kg)		Group 5 CPT-11 (75 mg/kg)	
Sex		M	F	M	F	M	F	M	F	M	F
Kidneys	depressed area	5	2	3		3	2	5	4	6	3
	cyst	1	1		1				1		
	adhesion					1	1			2	1
	enlargement				1						
Thymus	dark focus	3		3				1		1	
	small							1	1		
Adrenals	dark/pale discoloration	1	1		2		2		3		
	rasied /depressed focus				1			1			
	soft		1								
	cyst				1						

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Macroscopic Observations		Group 1 (control)		Group 2 (30 mg/kg)		Group 3 (75 mg/kg)		Group 4 (190 mg/kg)		Group 5 CPT-11 (75 mg/kg)	
Sex		M	F	M	F	M	F	M	F	M	F
Lymph node (mandibular)	dark discoloration	1	1	1		1		3	1	1	1
Stomach	dark discoloration	1									
	depressed area							1			
Spleen	enlargement							3			
Liver	dark focus/rasied focus		1				1	2	5		
Testes	soft/small							1			
Lymph node (mesenteric)	dark discoloration							1			
Femur and marrow	pale discoloration							1			
Pancreas	pale discoloration							1			
Prostate	pale area									1	
Seminal vesicles	small									1	
Infusion site	dark discoloration									1	
Eyes	dark area									1	
Ears	thickening		1							1	
Pituitary	enlargement		2		1		1		1		1
	dark area										1
Thyroid/para-thyroid	dark focus		1								
Skin	mass						1		1		1

Organ weights :

Percent Change in Absolute Organ Weights Relative to Vehicle

	Group 1 (control)		Group 2 (30 mg/kg)		Group 3 (75 mg/kg)		Group 4 (190 mg/kg)		Group 5 CPT-11 (75 mg/kg)	
sex	1-M	1-F	2-M	2-F	3-M	3-F	4-M	4-F	5-M	5-F
Heart					↓11%					
Kidneys					↓13%		↓10%			

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Liver					↓19%		↓15%			
Pituitary			↑13%							
Spleen							↓7%	↓13%		
Ovaries								↓27%		

Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes ( ), no (x) not stated

The most obvious histopathology findings involved accumulation of foamy macrophages/histiocytes in various organs including the adrenals, spleen, liver, lymph nodes, ovaries and uterus. These findings were typically seen in both the control and group 4 animals indicating that the toxicity may be vehicle related rather than test article related. This accumulation did not fully resolve during the recovery period. There was also some infiltration in the heart specifically in group 4 females in the main study group. Finally there was hypocellularity seen in the femurs of some group 4 males.

Tables extracted from the Applicant submission

Main:

Tissues	Findings	1-F (20-Females)	1-M (20-Males)	2-F (20-Females)	2-M (20-Males)	3-F (20-Females)	3-M (20-Males)	4-F (20-Females)	4-M (20-Males)	5-F (20-Females)	5-M (20-Males)	Total
Abdominal Cavity	Nodule: inflammation: interstitial chronic, multifocal, fatty tissue	0	1	0	0	0	0	0	0	0	0	1
	Total	0	1	0	0	0	0	0	0	0	0	1
Adrenals	Accumulation: histiocyte/macrophage, foamy, sinusoidal	20	18	2	0	0	0	20	20	0	0	80
	Degeneration/necrosis: cortical	0	0	1	0	1	0	0	0	0	0	2
	Degeneration/necrosis: cortical, with RBC	0	0	1	0	0	0	0	0	0	0	1
	Degeneration: cortical	0	0	0	0	1	0	1	0	0	0	2
	Erythrocytosis/hemorrhage: sinusoidal	8	0	2	1	0	0	9	0	5	0	25
	Vacuolar change: cortical, with RBC	0	0	0	0	0	0	3	0	0	0	3
	Vacuolar change: cortical, with fibrin and RBC	2	0	0	0	0	0	1	0	0	0	3
	Total	30	18	6	1	2	0	34	20	5	0	116
Brain	Hemorrhage: interstitial, focal, forebrain	0	0	0	0	0	0	0	0	1	0	1
	Hemorrhage: interstitial, multifocal, hindbrain	0	0	0	0	0	0	0	0	1	0	1
	Total	0	0	0	0	0	0	0	0	2	0	2
Cecum	Hemorrhage: mucosal	1	0	0	0	0	0	0	1	0	0	2
	Total	1	0	0	0	0	0	0	1	0	0	2
Colon	Dilatation	0	0	0	0	0	0	2	0	0	0	2
	Total	0	0	0	0	0	0	2	0	0	0	2
Diaphragm	Cyst, single	0	0	0	0	1	0	0	0	0	0	1
	Total	0	0	0	0	1	0	0	0	0	0	1
Ears	Infiltration: interstitial, histocytic	0	0	0	0	0	0	0	0	0	1	1
	Total	0	0	0	0	0	0	0	0	0	1	1

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Epididymides	Aspermia	0	0	0	0	0	0	0	1	0	0	1
	Total	0	0	0	0	0	0	0	1	0	0	1
Femur & Marrow	Hypocellularity: hematopoietic	0	0	0	0	0	0	0	4	0	0	4
	Total	0	0	0	0	0	0	0	4	0	0	4
Fibula	Fracture/crack: bone	1	0	0	0	0	0	0	0	0	0	1
Heart	Accumulation: histiocyte/marcrophage, foamy, myocardial	0	0	0	0	0	0	6	0	0	0	6
	Degeneration/necrosis: myocardial, multifocal with hemorrhage and	0	1	0	0	0	0	0	0	0	0	1

Tissues	Findings	1-F (20-Females)	1-M (20-Males)	2-F (20-Females)	2-M (20-Males)	3-F (20-Females)	3-M (20-Males)	4-F (20-Females)	4-M (20-Males)	5-F (20-Females)	5-M (20-Males)	Total
Liver (2 lobes)	Adenocarcinoma: parenchymal	1	0	0	0	0	0	0	0	0	0	1
	Cyst, single	0	0	0	0	1	0	0	0	0	0	1
	Degeneration/necrosis: parenchymal, multifocal, with hemorrhage	0	0	0	0	0	0	0	1	0	0	1
Liver (2 lobes)	Degeneration/necrosis: parenchymal, with RBC	0	0	0	1	2	0	0	0	0	0	3
	Necrosis: parenchymal, with RBC	0	0	0	0	0	0	5	0	0	0	5
	Swollen sinusoidal lining cells, foamy	8	8	0	0	0	0	6	5	0	0	27
	Total	9	8	0	1	3	0	11	6	0	0	38
Lungs (with Bronchi)	Inflammation: bronchioloalveolar, chronic, diffuse, histiocytic	0	0	0	0	0	0	0	1	0	0	1
	Total	0	0	0	0	0	0	0	1	0	0	1
Lymph node, Mediastinal	Erythrocytosis/hemorrhage: sinusal	0	0	0	0	0	0	0	1	0	0	1
	Total	0	0	0	0	0	0	0	1	0	0	1
Lymph node, Mandibular	Accumulation: histiocyte/macrophage, sinusal	0	0	0	0	0	0	2	0	0	0	2
	Erythrocytosis/hemorrhage: sinusal	2	0	0	0	0	1	1	1	0	0	5
	Erythrocytosis/hemorrhage: sinusal, capsular	0	0	0	1	0	0	0	1	0	0	2
	Hyperplasia: lymphoid	1	0	0	0	0	0	0	0	1	0	2
	Total	3	0	0	1	0	1	3	2	1	0	11
Ovaries	Accumulation: histiocyte/macrophage, foamy, ovarian	20	0	0	0	0	0	19	0	0	0	39
	Total	20	0	0	0	0	0	19	0	0	0	39
	Erythrocytosis/hemorrhage: sinusal	0	0	0	0	0	0	0	1	0	0	1
	Total	4	0	0	0	0	0	17	12	0	0	33
Lymph node, Renal	Accumulation: histiocyte/macrophage, foamy, sinusal	0	0	0	0	0	0	1	0	0	0	1
	Erythrocytosis/hemorrhage: sinusal	0	0	0	1	0	0	1	1	0	0	3
	Total	0	0	0	1	0	0	2	1	0	0	4
Pancreas	Accumulation: histiocyte/macrophage, foamy, Islet of Langerhans	0	1	0	0	0	0	0	0	0	0	1
	Degeneration: acinar	0	1	0	0	0	0	0	1	0	0	2
	Hemorrhage, interstitial	0	0	0	0	0	0		0	0	1	1
	Pigment: intrahistiocytic, cytoplasmic, light brown/pale tan	0	1	0	0	0	0	0	0	0	0	1
	Total	0	3	0	0	0	0	0	1	0	1	5
Parathyroids	Atrophy: parenchymal	0	0	0	0	0	0	0	0	0	1	1
	Total	0	0	0	0	0	0	0	0	0	1	1
Pituitary	Congestion: sinusoidal	1	0	1	0	1	0	1	0	0	0	4
	Cyst, multiple	0	1	0	0	0	0	1	0	0	2	4
	Cyst	0	1	0	0	0	0	0	0	0	0	1
	Degeneration: parenchymal	0	0	0	0	0	0	0	0	0	1	1
	Erythrocytosis/hemorrhage: sinusoidal	0	0	0	0	0	0	1	0	1	0	2
	Total	1	2	1	0	1	0	3	0	1	3	12
Prostate	Inflammation: interstitial, chronic	0	0	0	0	0	0	0	0	0	1	1
	Total	0	0	0	0	0	0	0	0	0	1	1

Reference ID: 3813066

Tissues	Findings	1-F (20-Females)	1-M (20-Males)	2-F (20-Females)	2-M (20-Males)	3-F (20-Females)	3-M (20-Males)	4-F (20-Females)	4-M (20-Males)	5-F (20-Females)	5-M (20-Males)	Total
Spleen	Accumulation: histiocyte/macrophage, foamy, red pulp, with light brown/pale tan pigmentation	5	13	0	0	0	0	0	7	0	0	25
	Accumulation: histiocyte/macrophage, foamy, red and white pulps, with light light brown/pale tan pigmentation	11	3	0	0	0	0	16	7	0	0	37
	Accumulation: histiocyte/macrophage, foamy, red pulp, with brownish pigmentation	0	1	0	0	0	0	0	0	0	0	1
	Hematopoiesis: extramedullary, increased	0	0	1	1	1	3	5	11	1	0	23
	Accumulation: histiocyte/macrophage, foamy, sinusoidal, red and white pulps, with light brown/pale tan pigmentation	2	0	0	0	0	0	0	0	0	0	2
	Total	18	17	1	1	1	3	21	25	1	0	88
Sternum & Marrow	Hypocellularity: hematopoietic	0	0	0	0	0	0	0	3	0	0	3
	Total	0	0	0	0	0	0	0	3	0	0	3
Stomach	Hemorrhage: mucosal	0	2	0	0	0	0	0	1	0	1	4
	Hemorrhage: interstitial	0	1	0	0	0	0	0	0	0	0	1
	Total	0	3	0	0	0	0	0	1	0	1	5
Testes	Atrophy: seminiferous tubules, germinal epithelium	0	0	0	0	0	0	0	1	0	0	1
	Degeneration/necrosis: seminiferous tubules, germinal epithelium	0	0	0	0	0	0	0	1	0	0	1
	Mineralization: seminiferous tubules, germinal epithelium	0	0	0	0	0	0	0	1	0	0	1
	Total	0	0	0	0	0	0	0	3	0	0	3
Thymus	Hemorrhage: interstitial	3	14	0	3	0	1	11	11	8	12	63
	Hypocellularity/atrophy: lymphoid	0	0	0	0	0	1	1	0	0	0	2
	Total	3	14	0	3	0	2	12	11	8	12	65
Thyroids	Hypocellularity/atrophy: glandular	0	0	0	0	0	0	1	0	0	0	1
	Total	0	0	0	0	0	0	1	0	0	0	1
Tibia	Fracture/crack: bone	1	0	0	0	0	0	0	0	0	0	1
	Total	1	0	0	0	0	0	0	0	0	0	1
Urinary bladder	Accumulation: histiocytic/macrophage, foamy, mucosal	0	0	0	0	0	0	1	0	0	0	1
	Dilatation	0	0	0	0	0	0	1	0	0	0	1
	Hemorrhage: interstitial	0	1	0	0	0	0	0	0	0	0	1
	Total	0	1	0	0	0	0	2	0	0	0	3
Uterus	Accumulation: histiocyte/macrophage, foamy, endometrial	5	0	0	0	0	0	6	0	0	0	11
	Accumulation: histiocyte/macrophage, foamy, endometrial and myometrial	1	0	0	0	0	0	0	0	0	0	1
	Cyst, single	0	0	0	0	0	0	0	0	1	0	1
	Hemorrhage: interstitial	0	0	0	0	0	0	1	0	0	0	1
	Total	6	0	0	0	0	0	7	0	1	0	14

Reference ID: 3813066

Recovery:

Tissues	Findings	1-F (10-Females)	1-M (10-Males)	4-F (10-Females)	4-M (10-Males)	5-F (10-Females)	5-M (10-Males)	Total
Abdominal Cavity - nodule	Degeneration/necrosis: fatty tissue	0	0	0	0	1	0	1
	Total	0	0	0	0	1	0	1
Adrenals	Accumulation: histiocyte/macrophage, foamy, sinusoidal	10	10	9	9	0	0	38
	Accumulation: histiocyte/macrophage, sinusoidal	0	0	0	1	0	0	1
	Atrophy: cortical	0	1	0	0	0	0	1
	Atrophy: medullary	0	0	0	1	0	0	1
	Degeneration: cortical	0	1	0	0	0	0	1
	Erythrocytosis/hemorrhage: sinusoidal	8	0	4	0	5	0	17
	Hyperplasia: nodular	0	0	0	1	0	0	1
	Vacuolar change: cortical with RBC	6	0	2	0	3	0	11
	Vacuolar change: cortical, with fibrin and RBC	2	0	1	0	0	0	3
	Total	26	12	16	12	8	0	74
Ears	Inflammation: interstitial	2	0	0	0	0	0	2
	Total	2	0	0	0	0	0	2
Esophagus	Deposits: bacterial, mucosal surface	0	0	0	0	0	1	1
	Total	0	0	0	0	0	1	1
Eyes	Hemorrhage: ciliary	0	0	0	0	0	1	1
	Total	0	0	0	0	0	1	1
Femur & Marrow	Hypocellularity: hematopoietic	0	0	0	1	0	0	1
	Total	0	0	0	1	0	0	1
Heart	Accumulation: histiocyte/macrophage, foamy, myocardial	0	1	0	0	0	0	1
	Total	0	1	0	0	0	0	1
Infusion site (Tail)	Congestion: subcutis	0	0	0	0	0	1	1
	Vesiculation: epidermal, with cellular debris and eosinophilic keratin	0	0	0	0	0	1	1
	Total	0	0	0	0	0	2	2
Kidneys	Accumulation: histiocytic/macrophage, foamy, cortical	0	1	0	0	0	0	1
	Basophilia : tubular, cortical	1	2	0	3	0	2	8
	Cyst, single	0	0	0	1	0	0	1
	Cyst, single, cortical	0	1	0	0	0	0	1
	Deposit: proteinaceous	0	0	0	0	0	1	1
	Deposit: proteinaceous, tubular, medullary	1	0	0	0	0	0	1
	Deposits: proteinaceous, tubular, cortical	1	0	0	0	1	0	2
	Deposits: proteinaceous, tubular, cortical, and medullary	1	0	0	0	0	0	1
	Dilatation : pelvis	0	0	0	0	0	1	1
	Dilatation : tubular, cortical	0	0	0	1	0	0	1
	Infiltration, mixed cell, pelvis	0	0	0	0	1	1	2
	Inflammation: interstitial, subacute, multifocal, bilateral, pelvis	0	0	0	0	1	0	1
	Proliferation: capsular, fibrous tissue	0	0	0	0	0	1	1
	Proliferation: cortical, fibrous tissue	0	0	0	0	1	0	1
	Total	4	4	0	5	4	6	23
Liver (2 lobes)	Congestion: vascular	0	0	1	0	0	0	1
	Cyst	0	0	1	0	0	0	1
	Degeneration/necrosis: parenchymal	0	1	0	0	0	0	1
	Degeneration/necrosis: parenchymal, with RBC	0	1	2	2	0	0	5
	Total	0	2	4	2	0	0	8
Lymph node, Mandibular	Erythrocytosis/hemorrhage: sinusal	0	1	0	1	0	1	3
	Erythrocytosis/hemorrhage: sinusal, capsular	0	0	0	0	1	0	1
	Erythrocytosis/hemorrhage: sinusoidal	0	0	1	0	0	0	1
	Total	0	1	1	1	1	1	5

Tissues	Findings	1-F (10-Females)	1-M (10-Males)	4-F (10-Females)	4-M (10-Males)	5-F (10-Females)	5-M (10-Males)	Total
Lymph node, Mediastinal	Erythrocytosis/ hemorrhage, sinusal	0	0	0	2	0	1	3
	Total	0	0	0	2	0	1	3
Lymph node, Mesenteric	Accumulation: histiocyte/macrophage, foamy, sinusal	0	0	0	1	0	0	1
	Total	0	0	0	1	0	0	1
Optic Nerve	Atrophy: neural	0	0	0	0	1	0	1
	Total	0	0	0	0	1	0	1
Ovaries	Accumulation: histiocyte/macrophage, foamy, ovarian	9	0	9	0	0	0	18
	Accumulation: histiocyte/macrophage, foamy, sinusoidal	0	0	1	0	0	0	1
	Total	9	0	10	0	0	0	19
Parathyroids	Atrophy: glandular	0	0	0	1	0	0	1
	Congestion: blood vessel/vascular	1	0	0	0	0	0	1
	Total	1	0	0	1	0	0	2
Pituitary	Congestion: sinusoidal	2	0	0	0	0	0	2
	Erythrocytosis/hemorrhage: sinusoidal	1	0	0	0	1	0	2
	Dilatation: sinusoidal	1	0	0	0	0	0	1
	Total	4	0	0	0	1	0	5
Prostate	Inflammation: interstitial	0	1	0	0	0	0	1
	Total	0	1	0	0	0	0	1
Skin & Subcutis (Inguinal)	Cyst, single, subcutis, with bacteria	1	0	0	0	0	0	1
	Hyperplasia: glandular, with fatty droplets and light purple homogenous	1	0	0	0	0	0	1
Skin & Subcutis (Inguinal)	Tumor: adenomatous, shoulder	0	0	0	0	1	0	1
	Tumor: adenomatous, with fibrin and RBC	0	0	1	0	0	0	1
	Total	2	0	1	0	1	0	4
Spleen	Accumulation: histiocytic/macrophage, red pulp, with light brown/pale tan pigmenhtation	0	0	0	1	0	0	1
	Accumulation: histiocyte/macrophage, foamy, red and white pulps, with light brown/pale tan pigmentation	0	2	0	0	0	0	2
	Erythrocytosis/hemorhage: sinusal	0	1	0	0	0	0	1
	Total	0	3	0	1	0	0	4
Sternum & Marrow	Hypocellularity: hematopoietic	0	0	0	1	0	0	1
	Total	0	0	0	1	0	0	1
Stomach	Congestion: mucosal	0	1	0	0	0	0	1
	Hemorrhage: mucosal	0	0	0	1	1	0	2
	Total	0	1	0	1	1	0	3
Thymus	Hemorrhage: interstitial	2	8	4	5	3	6	28
	Hypocellularity/atrophy: lymphoid	0	0	0	1	0	0	1
	Total	2	8	4	6	3	6	29
Thyroids	Hypocellularity/atrophy: glandular	1	0	0	0	0	0	1
	Total	1	0	0	0	0	0	1
Urinary bladder	Dilatation	0	0	1	0	0	0	1
	Total	0	0	1	0	0	0	1

Reference ID: 3813066

Toxicokinetics:

- Toxicokinetic data for irinoteccan and its active metabolite, SN-38 were determined for animals receiving both PEP02 and free CPT-11.
- Males and females showed a dose dependent increase in exposure though females typically showed a slightly lower Cmax, AUC, and half life than males.
- Cmax and AUC of SN-38 were highest in both males and females at the mid dose level.
- PEP02 Cmax, AUC, and half life were significantly increased over CPT-11 at all doses in both sexes.
- There were increases in exposure and decreases in clearance in irinotecan between days 1 and 106. These changes were not seen in SN-38.

Irinotecan (CPT-11) Day 1

Group #	Sex	Treatment Article	Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-t} (ng×h/mL)	$AUC_{0-\infty}$ (ng×h/mL)	$T_{1/2}$ (h)	CL (mL/h/kg)	V_{ss} (mL/kg)
2	M	PEP02	30	680687.667	1.5	19614731.7	19766846.1	15.84	1.52	37.6
3	M	PEP02	75	1726086.000	1.5	51302953.8	52863768.3	20.52	1.42	41.4
4	M	PEP02	190	4217100.000	4.0	116672588.3	123299278.9	23.34	1.54	47.4
5	M	CPT-11	75	10698.213	1.5	38544.5	38588.9	9.79	1943.56	4601.9
2	F	PEP02	30	575923.000	4.0	14137698.3	14806388.9	15.72	2.03	43.8
3	F	PEP02	75	1549373.333	1.5	45859924.0	48131168.7	21.07	1.56	46.2
4	F	PEP02	190	3981660.833	1.5	114271250.8	119378897.2	21.11	1.59	45.5
5	F	CPT-11	75	9716.787	1.5	44308.9	44449.0	9.93	1687.33	4913.6

Irinotecan (CPT-11) Day 106

Group #	Sex	Treatment Article	Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-t} (ng×h/mL)	$AUC_{0-\infty}$ (ng×h/mL)	$T_{1/2}$ (h)	CL (mL/h/kg)	V_{ss} (mL/kg)	AF[a]
2	M	PEP02	30	1003821.667	1.5	37688133.8	41217028.4	28.40	0.73	28.8	1.92
3	M	PEP02	75	2170757.667	1.5	90277680.9	104729583.3	35.12	0.72	35.1	1.76
4	M	PEP02	190	5733162.500	1.5	251655061.8	294244954.8	34.58	0.65	31.4	2.16
5	M	CPT-11	75	21181.500	1.5	82610.2	82620.7	4.79	907.76	2396.4	2.14
2	F	PEP02	30	827747.667	1.5	24292002.3	25060713.9	17.98	1.20	33.0	1.72
3	F	PEP02	75	2160715.167	1.5	70220620.8	80266479.5	30.02	0.93	41.1	1.53
4	F	PEP02	190	5402740.833	1.5	221167056.7	248874586.5	31.30	0.76	33.2	1.94
5	F	CPT-11	75	18529.883	1.5	82885.0	82898.9	5.02	904.72	2298.7	1.87

[a]AF (Accumulation Factor) = Day 106 AUC_{0-t} / Day 1 AUC_{0-t}

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SN-38 Day 1

Group #	Sex	Treatment Article	Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-t} (ng×h/mL)	$AUC_{0-\infty}$ (ng×h/mL)	$T_{1/2}$ (h)	CL (mL/h/kg)	V_{ss} (mL/kg)
2	M	PEP02	30	3525.470	0.5	213117.4	289357.1	45.50	103.68	7664.7
3	M	PEP02	75	5855.203	1.5	341765.4	1137255.0	184.54	65.95	17588.8
4	M	PEP02	190	3804.020	24.0	317036.6	1558211.5	263.74	121.93	47342.9
5	M	CPT-11	75	2199.283	4.0	14687.7	14722.4	10.40	5094.28	19188.3
2	F	PEP02	30	3184.027	12.0	194456.4	207619.6	19.14	144.50	6493.1
3	F	PEP02	75	3935.330	48.0	293782.5	488596.2	59.83	153.50	15549.9
4	F	PEP02	190	2991.883	48.0	251931.9	791771.7	155.01	239.97	55635.5
5	F	CPT-11	75	1211.147	1.0	5246.3	5274.2	9.36	14220.22	65825.2

SN-38 Day 106

Group #	Sex	Treatment Article	Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-t} (ng×h/mL)	$AUC_{0-\infty}$ (ng×h/mL)	$T_{1/2}$ (h)	CL (mL/h/kg)	V_{ss} (mL/kg)	AF[a]
2	M	PEP02	30	3568.013	1.5	292359.8	1881793.8	347.05	15.94	8144.8	1.37
3	M	PEP02	75	4864.767	4.0	292338.2	1380878.5	278.55	54.31	21830.6	0.86
4	M	PEP02	190	5074.575	1.0	334862.4	NC	NC	NC	NC	1.06
5	M	CPT-11	75	1520.200	1.0	10477.7	10508.7	7.68	7136.97	32337.6	0.71
2	F	PEP02	30	3041.077	0.5	168939.8	NC	NC	NC	NC	0.87
3	F	PEP02	75	4435.217	48.0	310389.7	2452007.3	366.50	30.59	16871.8	1.06
4	F	PEP02	190	4102.653	72.0	319691.6	NC	NC	NC	NC	1.27
5	F	CPT-11	75	1251.663	1.0	4623.0	4625.4	4.50	16214.90	83788.9	0.88

[a]AF (Accumulation Factor) = Day 106 AUC_{0-t} / Day 1 AUC_{0-t}

NC: Not Calculated

Study title: A 6-Cycle (3 Weeks Per Cycle) Infusion Toxicity Study in Beagle Dogs Followed by a 6-week Recovery Period

Key study findings:

- There was increased exposure to irinotecan (Cmax and AUC) with PEP02 administration compared to test article alone (CPT-11).
- At the high dose of 36 mg/kg there was high morbidity and mortality for both the PEP02 and CPT-11 treated animals.
- The major toxicities were gastrointestinal and hematological.
- The gastrointestinal toxicity was the major cause of mortality and was similar to that seen in the reference article
- There was a greater loss of white blood cells in general in the PEP02 treated animals compared to the reference article, CPT-11, treated animals. This decrease was most obvious specifically in the lymphocyte population.

Study no.: PEP02-NC-G-Tx-011
Volume #, and page #: Vol.7 p. 799
Conducting laboratory and location: (b) (4)
Date of study initiation: Dec. 7, 2006

Reference ID: 3813066

GLP compliance: yes
QA report: yes () no (x)
Drug, lot #, and % purity: PEP02, LIIAS5001,
PEP02, LIIAS4002,
CPT-11, 70495AA010, 99.9%
PEP02 Placebo, LIIPS5001,
Diluent 5% dextrose for injection,
W5L20C2, W7C02C1,W7C05C2

Methods

Treatment Group	Dose Level* (mg/kg)	[Dose] (mg/mL)	Dose Rate (mg/kg/hr)	Infusion Period (hr.)	Number of Animals			
					Main		Recovery	
					M	F	M	F
1. Control Article	0	0	10	1.5	4	4	2	2
2. Low Dose	9	.06	10	1.5	4	4	2	2
3. Mid Dose	15	1	10	1.5	4	4	2	2
4. High Dose	36	2.4	10	1.5	4	4		
5. Ref. Article High (free CPT-11)	36	2.4	10	1.5	4	4		
6. Mid-High Dose	21	1.4	10	1.5	4	4	2	2
7. Ref Article Mid.-High (free CPT-11)	21	1.4	10	1.5	4	4	2	2

*These were not the intended dose levels. The infusion rate and infusion period were wrong resulting in these doses which were 50% higher than intended. Included here are the actual doses used in the study.

Doses: 0, 9, 15, 36, and 21 mg/kg given at days 1, 22, 43, 64, 85, and 106
Species/strain: Beagle dogs
Number/sex/group or time point (main study): 4/sex/group
Route, formulation, volume, and infusion rate: Control article, low, mid and high dose levels were given IV over 1.5 hours on days 1, 22, 43, 64, 85, and 106 according to the table above.
Satellite groups used for toxicokinetics or recovery: 2/sex/group
Age: 7-8 months
Weight: 6.3-9.2 kg (M), 5.6-9.4 kg (F)
Sampling times: TK samples were taken on Days 1 and 106 at 0.5,1, 1.5 6, 12, 24, 36, 48, 72, and 168 hours post infusion.

Observation and Times:
Body weights: Taken upon arrival, at randomization, weekly starting one week before dosing, one day prior to necropsy, at necropsy.
Food consumption: Measured daily starting one week pretreatment.
Ophthalmoscopy: Performed once pre-treatment, once during week 18 (days 120-126).
EKG: Once pre-treatment, on days 2 and 107 (23-25 hrs. after dosing).

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Hematology: Prior to start of treatment, at Days 7, 21, 63, and at necropsy.
Clinical chemistry: Prior to start of treatment, at Days 7, 21, 63, and at necropsy.
Urinalysis: Prior to start of treatment, Days 7, 21, 63, and at necropsy.
Gross pathology: Performed on all animals regardless of cause EXCEPT 3003C, 4003D, and 4502C
Organ weights : Taken at necropsy, see histopathology table
Histopathology: specify groups examined, special stains, etc
Adequate Battery: yes (x), no ( )
Peer review: yes ( ), no (x) not stated

Results:

Mortality:

Animal #	Group	Sex	Day of Death	Observations		
				Reason for Removal	Cause of Mortality	General
3003C	3 (15mg/kg)	M	1-5	Found dead, no necropsy	**	Day 23 euthanized animals were in generally poor health similar to the moribund animals sacrificed earlier or found dead. Clinical signs included decreased appetite and activity, severe weight loss, hunched posture, slow capillary fill time, slow skin turgor , soft dark fees and recumbency. Dark areas/discoloration in gut mucosa. Changes in bone marrow, GI tract, and lymphoid tissue
4001A	4 (36mg/kg)	M	5	Found dead	BM, GIT, LYM	
4002B	4 (36mg/kg)	M	5	Found dead	BM, GIT, LYM	
4003D	4 (36mg/kg)	M	5	Found dead, no necropsy	**	
4004E	4 (36mg/kg)	M	23	Euthanized	*	
4501B	4 (36mg/kg)	F	7	Euthanized	BM, LYM	
4502C	4 (36mg/kg)	F	6	Euthanized, no necropsy	**	
4503D	4 (36mg/kg)	F	23	Euthanized	*	
4504E	4 (36mg/kg)	F	5	Euthanized	BM, GIT, LYM	
5001B	5 CPT-11	M	7	Euthanized	BM, GIT, LYM	
5002C	5 CPT-11	M	23	Euthanized	*	
5003D	5 CPT-11	M	4	Euthanized	BM, GIT, LYM	
5004E	5 CPT-11	M	4	Euthanized	BM, GIT, LYM	
5501A	5 CPT-11	F	23	Euthanized	*	
5502C	5 CPT-11	F	4	Euthanized	BM, GIT, LYM	
5503D	5 CPT-11	F	23	Euthanized	*	
5504E	5 CPT-11	F	23	Euthanized	*	
6506G	6 (21mg/kg)	F	5	Euthanized	BM, GIT, LYM	
7005F	7 CPT-11	M	5	Euthanized	BM, GIT, LYM	

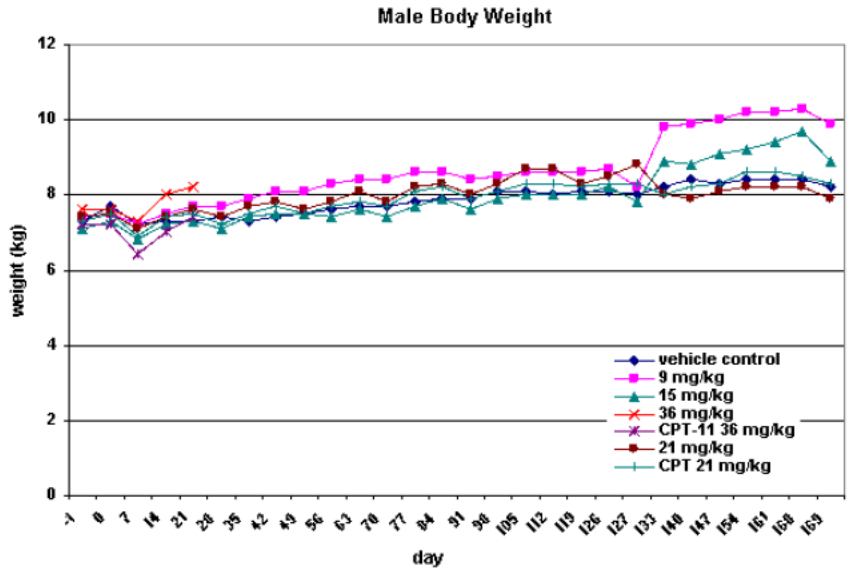
* Removed at the end of the first cycle (Day 23) due to high group mortality
** Animal discarded due to oversight
BM-bone marrow; GIT- gastrointestinal tract change; LYM-lymphoid tissue change

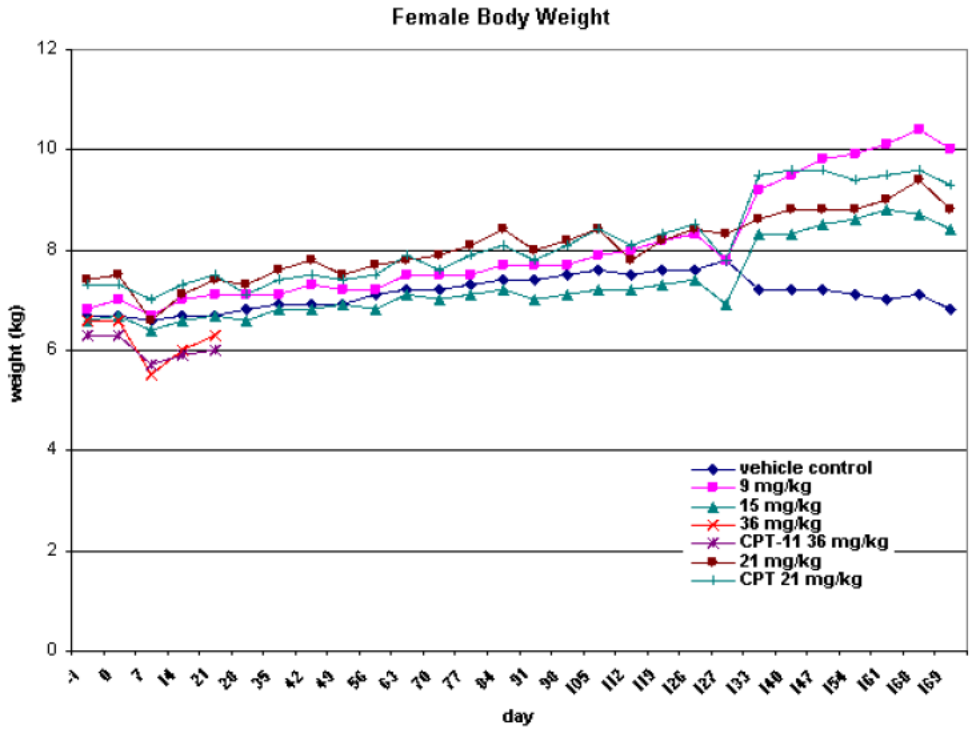
Reference ID: 3813066

Clinical signs:

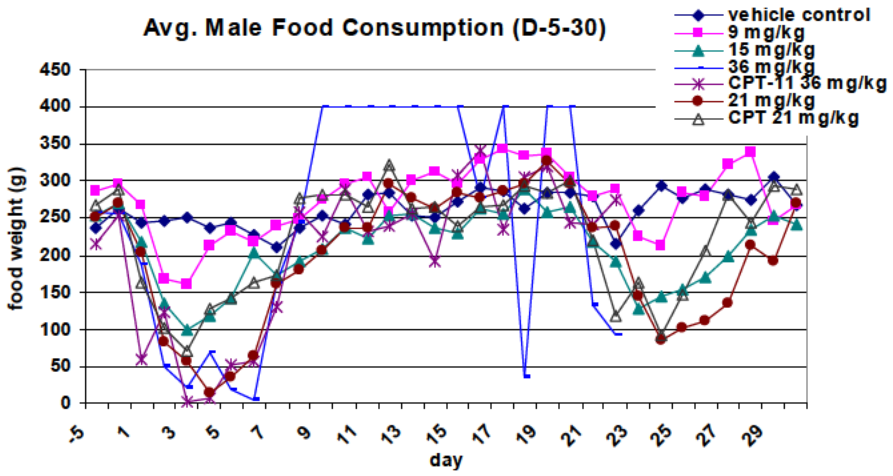
	Vehicle control		Group 2 (9 mg/kg)		Group 3 (15 mg/kg)		Group 4 (36 mg/kg)		Group 5 CPT-11 (36 mg/kg)		Group 6 (21 mg/kg)		Group 7 CPT-11 21 mg/kg)	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Decreased activity/appetite	1						4	2	3	2		2	3	3
Cold to touch							3	1	3	1		1		
Hunched/lying on floor							3	2				1	1	1
Slow capillary refill							3	1	3	2		2	2	2
Changes in respiration							2		1			1	1	1
Hot to touch								1			1			2
Thin body	1			2		3	2	2			1		2	4
Slow skin turgor							4	3		3	2	5	5	5
Lacrimation		1		4		2	2	3	1	1		2	1	3
Salivation		1	2	2		2	3		2	3	1	1	4	5
Liquid feces	3	4	5	5		6	3	4	3	2	6	5	6	4
Gait changes					2		1				1		1	

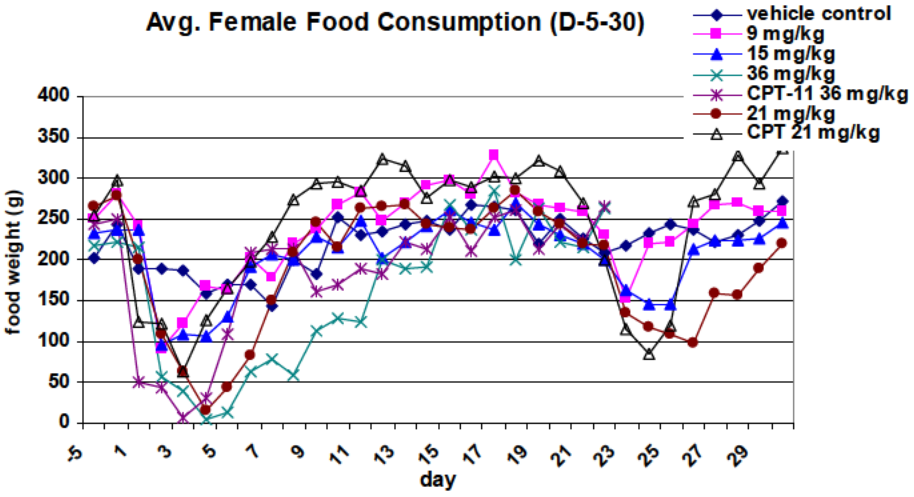
Body weights:

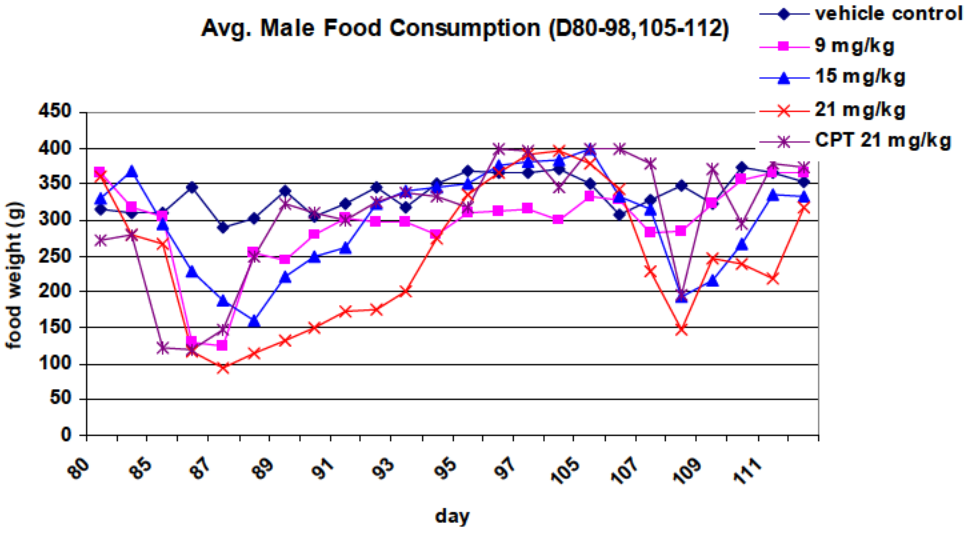


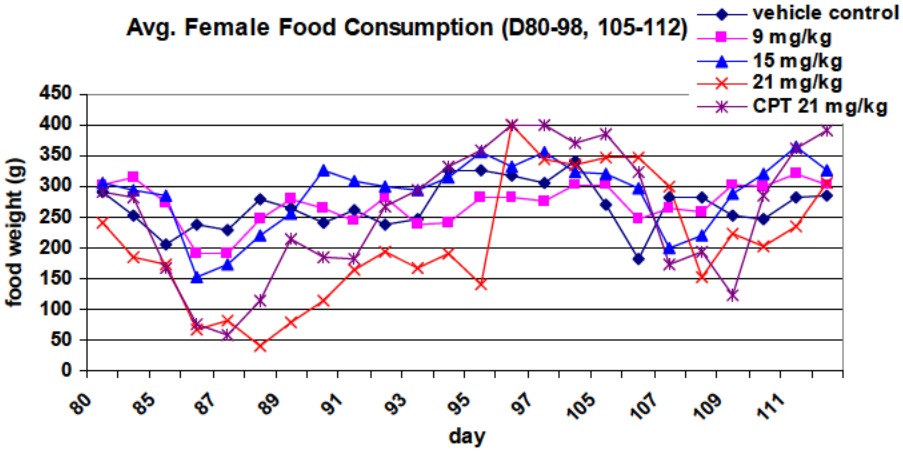


Food consumption:
There was a dose-dependent decrease in food consumption in all dose groups for several days after infusion. The decrease was marginally more sustained in animals receiving the test article PEP02 than in those receiving the reference article CPT-11. Food consumption returned to control levels in all dose groups within 7 days of dosing.









Ophthalmoscopy: There were no test article related changes.

EKG: There were no test article related changes.

Hematology:

- There was a significant decrease in platelets and white blood cells after administration of PEP02.
- This decrease was dose-dependent; males appear to be slightly more sensitive than females.
- PEP02 caused a greater depletion of WBCs than CPT-11 alone. This difference was most obvious in the lymphocyte population where CPT-11 alone had no noticeable effect.
- Toxicities were reversible, as cell numbers increased at later time points in the study.
- With the exception of Day 7, pre-recovery hematological values were taken at the end of treatment cycles and animals showed increased production of white blood cells, suggesting a rebound in bone marrow function and its ability to compensate for losses due to earlier drug dependent toxicity.
- Coagulation tests did not reveal any differences between the dose groups over time, suggesting that the platelet decrease was recoverable.

Percent Change from Vehicle Control in Hematology Values

	Group 2 (9 mg/kg)		Group 3 (15 mg/kg)		Group 4 (36 mg/kg)		Group 5 CPT-11 (36 mg/kg)		Group 6 (21 mg/kg)		Group 7 CPT-11 (21 mg/kg)	
	M	F	M	F	M	F	M	F	M	F	M	F
RBC 7 21 63 NEC REC					↓18% ↓21%							
PLT 7 21 63 NEC REC	↓26 % ↓19 % ↓14 % ↓18 %	↑23% ↑29% ↑12%	↓38 % ↑53 % ↓24 %	↑57 % ↑68 % ↑89 %	↓34% NA	↓42% ↑43% NA	↓44 % NA	↑68% NA	↓49 % ↑86 % ↓21 %	↓38% ↑51% ↑39% ↑30%	↓24% ↓17% ↑22% ↓19%	↓38% ↑11%

	Group 2 (9 mg/kg)		Group 3 (15 mg/kg)		Group 4 (36 mg/kg)		Group 5 CPT-11 (36 mg/kg)		Group 6 (21 mg/kg)		Group 7 CPT-11 (21 mg/kg)	
	M	F	M	F	M	F	M	F	M	F	M	F
WBC 7 21 63 NEC REC	↓28% ↑20% ↑12% ↑16%	↓11% ↑18% ↑18%	↓53% ↑44% ↑30% ↑17%	↓36% ↑22% ↑30% ↑55%	↓76% ↓11% NA	↓67% ↑34% NA	↓51% NA	↓52% ↑12% NA	↓71% ↑19% ↑15% ↑17%	↓66% ↑22% ↑14%	↓34%	↓19% ↑12% ↓21%
NEUT 7 21 63 NEC REC	↓29% ↑33% ↑15% ↑36% ↑18%	↓13% ↑16% ↑19%	↓59% ↑60% ↑36% ↑19% ↑84%	↓31% ↑28% ↑38% ↓20%	↓76% NA	↓67% ↑60% NA	↓74% NA	↓52% ↑20% NA	↓79% ↑25% ↑17% ↑38%	↓79% ↑27% ↑19% ↓15%	↓52% ↑14%	↓31% ↑27% ↑11% ↓29%
LYMP 7 21 63 NEC REC	↓16% ↑12% ↑13%	↑18% ↑13% ↑15% ↑12%	↓27% ↑25% ↑21%	↓37% ↓23% ↑40%	↓61% ↓17% NA	↓67% ↓15% NA			↓46% ↑14% ↑16%	↓40% ↑13% ↑36%	 ↑15%	 ↓25%
MON7 21 63 NEC REC	↓60% ↑14% ↑51% ↑31%	↓46% ↑33% ↑25% ↑28%	↓89% ↑45% ↑18% ↑13% ↑52%	↓72% ↑90% ↑84% ↑81% ↑21%	↓95% ↑15% NA	↓96% ↑67% NA	↓18% NA	↑75% NA	↓96% ↑40% ↑41% ↑33% ↓19%	↓93% ↑55% ↑47% ↑44% ↓29%	↓34% ↓21% ↓27%	 ↑35% ↓29%

	Group 2 (9 mg/kg)		Group 3 (15 mg/kg)		Group 4 (36 mg/kg)		Group 5 CPT-11 (36 mg/kg)		Group 6 (21 mg/kg)		Group 7 CPT-11 (21 mg/kg)	
	M	F	M	F	M	F	M	F	M	F	M	F
BAS 7 21 63 NEC REC	↓13% ↑29% ↑44%	 ↑11 %	↓50 % ↑14 % ↑78 %	↓38% ↓20% ↓11% ↓32%	↓88% ↓14% NA	↓75% NA	 NA	↑38% NA	↓63% ↓14% ↑11%	↓75% ↓30% ↓33% ↓26%	↓13 % ↓29 % ↑78 %	↓13% ↓30% ↑38% ↓47%
EOS 7 21 63 NEC REC	↓21% ↓39% ↓17% ↓52%	↑44 % ↑82 % ↑76 % ↑38 %	↓29 % ↓25 % ↑14 % ↓20 %	 ↑27% ↓47% ↓13%	↓50% ↓61% NA	↓56% ↑18% NA	↓21 % ↓53 % NA	↓33% ↓45% NA	↓21% ↓53% ↓32% ↑188 % ↓35%	↓22% ↑15% ↑42% ↓24%	↓29 % ↓61 % ↓47 %	↑33% ↑18% ↑46% ↓16% ↑20%
RETI C7 21 63 NEC REC	↑12% ↓13% ↑20%	↓29 % ↑13 % ↓29 %	↓26 % ↑19 % ↓21 %	↓61% ↑15% ↑14% ↓22% ↑30%	↓79% ↓38% NA	↓78% NA	↓55 % ↓16 % NA	 NA	↓51% ↓32% ↑27% ↓60%	↓63% ↑20% ↓24% ↓37%	↑85 % ↑33 % ↑37 %	 ↑14% ↓16% ↑20% ↓11%

Clinical chemistry: There were no test article related changes

Urinalysis: There were no test article related changes.

Gross pathology: The most common macroscopic findings were discoloration or dark material in the intestinal tract. These findings were more common with higher drug doses, but were independent of sex or test article vs. reference article.

Macroscopic Findings		Males							Females						
Dose (mg/kg)		0	9	15	36	36 CPT	21	21 CPT	0	9	15	36	36 CPT	21	21 CPT
Stomach	dark foci	2	3		1				1	1	1	1		1	
Colon/cecum	dark foci	1	2		2	1			1	3	1	1	2	1	
	dark material					1						1		1	
thymus	small				1	2		1				1			
	dark foci	1			1		1			2	1			1	
	gelatinous											1			
Lymph node	dark foci/ discoloration	1	1	1	1					1					
Small intestine	dark foci	1	1		3			1		1				1	1
	dark material					1						1		1	
Lungs	adhesion		1						1						1
	dark /pale foci			3					1	1	1				
	small			1											
Spleen	enlargement		1												
	adhesion								1						
	dark/pale area							1	1		1			1	2
	small												1		
Kidneys	depressed area			1											
Testes	soft			1											
Skin	dark area						1							3	
	thickening								1	1					
Liver	dark/ pale foci										1		1	1	1
Eyes	dark area				2									1	
Salivary gland	dark discoloration												1		
Infusion sight	dark area					3	3				1			3	
Esophagus	dark discoloration							1						1	
Mammary gland	enlargement								2	1					
Blood	thick/clotting												1		

Organ weights: The Applicant mentions that there are significant **decreases** in the organ weights for the spleen, thymus, testes, prostate, ovaries, and uterus in group 4 and 5 animals; however, they do not include these weights in their table because these animals all died or were euthanized early and data from other dose groups actually shows increases in some of these organs, particularly the uterus and spleen. Averages organ weights in groups that were euthanized or found dead were calculated by the reviewer and some organs from these groups showed wide variability.

Percent Change in Absolute Weight from Vehicle Control

	Group 2 (9 mg/kg)		Group 3 (15 mg/kg)		Group 4 (36 mg/kg)		Group 5 CPT-11 (36 mg/kg)		Group 6 (21 mg/kg)		Group 7 CPT-11 (21 mg/kg)	
	M	F	M	F	M*	F	M	F	M	F	M	F
Spleen	↑*192 %*	↓10 %	↓21 % ↑*230 %*	↓15 %	↓24 % *NA*	↓29 % *NA*	↓43 % *NA*	↓43 % *NA*	↑22 % ↑*130 %*	↑10 %	↑28 % ↑*165 %*	↑26 %
Thymus	↑*49 %*		↑*46 %*	↓35 %	*NA*	↓78 % *NA*	↓62 % *NA*	↓57 % *NA*	↑*69 %*		↑*66 %*	
Testes					↑13 % *NA*		↓57 % *NA*					
Prostate	↑114 %		↑80 %		↑40 % *NA*		↓73 % *NA*		↑143 %		↑40 %	
Ovaries				↓20 %		↓64 % *NA*		↓52 % *NA*				
Uterus		↑29 % ↑*143 %*		↑9% ↑*59 %*		↓92 % *NA*		↓79 % *NA*		↑35 % ↑*69 %*		↓17 %
Lungs										↑36 %		↑14 %

top=main group *bottom=recovery group* *only 1 animal from this group had organ weights reported

Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes ( ), no (x) not stated

Reference ID: 3813066

Microscopic Findings		Males							Females						
Dose (mg/kg)		0	9	15	36	36 CPT	21	21 CPT	0	9	15	36	36 CPT	21	21 CPT
Adrenals	Cortical hemorrhage					1							1		
	Neutrophil infiltrate											1	1		
	Cortical vacuolation				2	2							1		
Cecum	Lymphoid atrophy/necrosis			1	1		1				2				
	Mucosal congestion/hemorrhage				2	3						1	1		
	Cryptal dilation											1			
	Epithelial necrosis				2	2						1	1		
	Epithelial regeneration											1			
Colon	Lymphoid atrophy/necrosis				2	2	1					1			
	Mucosal congestion/hemorrhage				2	2						1	1		
	Cryptal dilation											1			
	Epithelial necrosis				2	2						1	1		
	Epithelial regeneration				1							1			
Epididymus	Single cell necrosis				2	3									
	Oligo/aspermia		1		1	1									
	Cellular debris				2	3		1							
Femur/sternum marrow	Pancellular hypocellularity				2	3						2	1		
Liver	Extramedullary hematopoiesis			2		1	4	2			1	1		3	2
	Capsular lipidosis													1	
Lungs	Pleural fibrosis		1												1
	Granuloma			1							1				
	Foreign body granuloma													1	1
	Alveolar hemorrhage			1											
	Mixed cell infiltrate			2		1					1			1	
	Alveolar inflammation		1	3	1		3	2			1			1	
Lymph node (mediastinal)	Sinusal hemorrhage/erythrocytosis	1			1					1					
Lymph node (mandibular)	Lymphoid necrosis/atrophy			1	2	3	1	1			1	2	1	2	
	Sinusal histiocytosis												1		
	Eosinophil infiltrate														1
Lymph node (mesenteric)	Lymphoid necrosis/atrophy			1	2	2					2	2	1		
	Sinusal hemorrhage/erythrocytosis		1												
	Sinusal histiocytosis				2	1		1			1	1			

Reference ID: 3813066

Microscopic Findings		Males							Females						
Dose (mg/kg)		0	9	15	36	36 CPT	21	21 CPT	0	9	15	36	36 CPT	21	21 CPT
	Lymphoid hyperplasia													1	
Mammary Gland	Acinar hyperplasia								2	1					
Ovaries	Reduced follicles											2	2		
Pituitary	cyst		1					1		3			1	1	2
Prostate	Acinar atrophy				2	3									
	Immature	1				1									
	Inflammation						1								
Salivary Gland	Acinar atrophy				2	2						1	1		
Small Intestine (duodenum)	Mucosal atrophy				2	1						1	1		
	Mucosal congestion/ Hemorrhage					1									
	Cyst											1	1		
	Epithelial necrosis				2	3						1	1		
	Epithelial regeneration				1	1						1	3		
Small Intestine (Ileum)	Lymphoid necrosis/atrophy			1	1	3	2	1			2	1	1	3	
	Mucosal atrophy			1	2	3						1	1		1
	Mucosal congestion/ Hemorrhage				1	1						1			
	Epithelial necrosis				2	3						1	1		
	Epithelial regeneration				1	1						1	1		
Small Intestine (jejunum)	Lymphoid necrosis/atrophy			1		2	1				1	1	1	3	1
	Mucosal atrophy				2	2						1	1		1
	Mucosal congestion/ Hemorrhage				2	1						1			
	Epithelial necrosis				2	3						1	1		
	Epithelial regeneration				1							1	1		
Spleen	Lymphoid necrosis/atrophy				2	3	1				2	2	1	2	1
	Extramedullary hematopoiesis			1	1	1	4	2			3	1	1	4	3
	Capsular fibrosis														1
	Foamy cell histiocytosis	4	4	4	3		4		4	4	3	2	1	4	
	Brown/green pigment				1							1			
	Siderotic placque								1		1				
Stomach	Lymphoid atrophy/necrosis	2		1	1	2						1		1	1
	Mucosal congestion/hemorrhage					1									
	Mucosal inflammation	2													

Microscopic Findings		Males							Females						
Dose (mg/kg)		0	9	15	36	36 CPT	21	21 CPT	0	9	15	36	36 CPT	21	21 CPT
Testes	Degeneration/necrosis seminiferous epithelium				2	4		1							
	Degeneration/necrosis seminiferous tubules		1	2	1										
	Tubular hypoplasia	1	1			1		2							
	Immature					3									
Thymus	Lymphoid atrophy/necrosis				2	3						2	1		
	Congestion/hemorrhage				1	1				1		1			
	Lymphoid involution	1	3	1			4	1			1	1	2		
Uterus	Transmural atrophy											3	1	1	
	Endometrial inflammation													1	

Toxicokinetics:

- Toxicokinetic data for irinoteccan and its active metabolite, SN-38 were determined for animals receiving both PEP02 and free CPT-11.
- Males and females showed a dose-dependent increase in exposure
- Cmax and AUC of SN-38 were highest in both males and females at the mid dose level.
- PEP02 Cmax, AUC, and half life were significantly increased over CPT-11 at all doses in both sexes. Clearance was much faster in CPT-11 than PEP02.
- Clearance was not reported for the SN-38 metabolite

Day 1 Irinotecan

Group	Sex	Treatment Article	Dose (mg/kg)	Cmax (ug/mL)	Tmax (hr)	AUC_{0-t} (hr*ug/mL)	$AUC_{0-\infty}$ (hr*ug/mL)	$T_{1/2}$ (hr)	CL (mL/hr/kg)
2	M	PEP02	9	147±11.1	1.5±0	2075±279	2113±314	5.3±1.4	4.28±.599
3	M	PEP02	15	308±83.7	1.5±.01	4088±1072	4207±1057	6.2±1.8	3.99±1.11
4	M	PEP02	36	550±129	1.51±.01	10198±4083	10376±4151	7.5±2.3	3.78±1.52
5	M	CPT-11	36	21.9±4.48	1.38±.25	141±28	162±39.8	4.4±1.4	247±77
6	M	PEP02	21	332±24.4	1.5±0	5750±1154	5800±1183	5.4±1.3	3.98±.892
7	M	CPT-11	21	13.6±0.864	1.5±0	47.7±11	55±11.8	3.3±1.2	407±76.7
2	F	PEP02	9	150±11.1	1.5±0	2080±531	2133±541	6.1±1.3	4.37±1.03
3	F	PEP02	15	227±15.3	1.51±.02	3446±294	3509±300	6.1±0.8	4.56±.375
4	F	PEP02	36	527±92.4	1.5±0	12663±1114	12842±1318	8.0±3.8	2.73±.283
5	F	CPT-11	36	19.8±2.59	1.5±0	98.4±21.1	124±25.4	4.1±1.6	313±54.7
6	F	PEP02	21	317±14.7	1.5±0	5394±651	5548±652	6.6±1	4.06±.479
7	F	CPT-11	21	13.8±2.22	1.5±0	54.5±13.9	61±15.1	3.6±1.7	373±94.6

Day 106 Irinotecan

Group	Sex	Treatment Article	Dose (mg/kg)	Cmax	Tmax	AUC_{0-1}	$AUC_{0-\infty}$	$T_{1/2}$	CL
2	M	PEP02	9	142±19.9	1.5±0	2344±385	2364±393	5.8±.8	3.07±.579
3	M	PEP02	15	226±27	1.51±.01	4660±1218	4717±1199	7.2±2.2	3.02±.733
6	M	PEP02	21	340±20.3	2.63±2.25	9423±1666	9852±2335	11.6±7.1	2.02±.428
7	M	CPT-11	21	14.6±3.66	1.38±.25	57.3±17.1	66.9±17.8	2.5±.8	308±72.7
2	F	PEP02	9	136±17.8	1.5±0	1748±609	1797±584	6.4±1.2	4.28±1.43
3	F	PEP02	15	207±15.2	1.51±.01	4127±624	4193±635	7.1±1.9	3.3±.49
6	F	PEP02	21	320±4.57	1.5±0	8775±1832	9098±2031	11.4±4	2.2±.574
7	F	CPT-11	21	16.6±3.19	1.5±0	61.8±8.94	73.2±5.64	3.0±1	269±21.8

Day 1 SN-38

Group	Sex	Treatment Article	Dose (mg/kg)	Cmax	Tmax	AUC_{0-1}	$AUC_{0-\infty}$	$T_{1/2}$
2	M	PEP02	9	11.2±2.16	1.5±0	135±15.6	143±16.7	16.6±1.8
3	M	PEP02	15	21.6±2.78	1.51±.01	287±73	295±72.3	29.9±10
4	M	PEP02	36	47.4±6.17	1.51±.01	527±138	571±157	25.3±9.2
5	M	CPT-11	36	43.9±16	1.51±.01	294±103	297±103	7.8±1.2
6	M	PEP02	21	27±2.86	1.5±0	339±31.4	347±33.4	34.1±4.5
7	M	CPT-11	21	27.6±9.21	1.5±0	131±68.1	133±68.1	5.8±2.6
2	F	PEP02	9	10.1±1.36	1.38±.25	126±13.6	131±13.2	16.1±1.6
3	F	PEP02	15	20.2±1.55	1.38±.26	250±43.5	260±41.1	20.6±9.1
4	F	PEP02	36	45.1±3.66	1.5±0	589±70.9	678±160	28.3±9.3
5	F	CPT-11	36	31.3±5.62	1±0	172±36.2	174±36.2	7.5±1.9
6	F	PEP02	21	25.7±5.891.5	1.5±0	316±115	326±124	30.8±12.4
7	F	CPT-11	21	26.5±4.06	1.63±.94	143±65.4	146±64.4	8±2.3

Day 106 SN-38

Group	Sex	Treatment Article	Dose (mg/kg)	Cmax	Tmax	AUC_{0-1}	$AUC_{0-\infty}$	$T_{1/2}$
2	M	PEP02	9	10.3±2.1	1.5±0	135±15.6	148±15.2	19.8±2.2
3	M	PEP02	15	17.1±2.18	1.38±.25	267±64.8	278±67.8	38.6±9.4
6	M	PEP02	21	23.2±3.23	1.5±0	456±126	493±178	40.3±21.5
7	M	CPT-11	21	34.8±16.2	1.13±.25	154±50.8	157±51.6	4.6±1.7
2	F	PEP02	9	11.1±1.92	1.38±.25	119±12.6	125±10.7	16.5±4
3	F	PEP02	15	18.2±2.89	1.51±.01	269±48.8	277±49.2	36±6.3
6	F	PEP02	21	21.1±5.8	1.5±0	382±95.5	401±87.9	30.3±4.8
7	F	CPT-11	21	42.9±13.3	1.38±.25	211±92.8	212±93.7	5.6±1.8

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/s/

MARGARET E BROWER
08/28/2015

WHITNEY S HELMS
08/28/2015

I concur with Dr. Brower's conclusion that the pharmacology/toxicology information submitted is sufficient to support this 505(b)(2) application. There are no outstanding issues from a pharmacology/toxicology perspective that would prevent the approval of ONIVYDE in combination with 5-fluorouracil and leucovorin for the treatment of patients with metastatic adenocarcinoma of the pancreas who have received a prior gemcitabine-containing regimen.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 207,793 **Applicant: Merrimack Pharmaceuticals** **Stamp Date: April 23, 2015**

Drug Name: Onivyde (irinotecan liposome injection) **NDA Type: Standard review**

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

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

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

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

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

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

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

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

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

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

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/s/

MARGARET E BROWER
05/20/2015

WHITNEY S HELMS
05/21/2015