CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

204026Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Pomalyst (pomalidomide)

Date: December 13, 2012
To: File for NDA 204026
From: John K. Leighton, PhD, DABT
Acting Director, Division of Hematology Oncology Toxicology
Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting review of Drs. Gehrke and Del Valle and labeling and secondary memorandum provided by Dr. Saber. I concur with Dr. Saber's conclusion that Pomalyst may be approved and that no additional nonclinical studies are needed for the proposed indication.

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/s/

JOHN K LEIGHTON
12/13/2012

MEMORANDUM

Date: December 12, 2012
From: Haleh Saber, Ph.D.
Pharmacology/Toxicology Supervisor
Division of Hematology Oncology Toxicology (DHOT)
Office of Hematology Oncology Products (OHOP)
Re: Approvability for Pharmacology and Toxicology
NDA: 204026
Drug: POMALYST; pomalidomide (capsule)
Indication: Patients with relapsed and refractory multiple myeloma who have received at least 2 prior regimens, including both lenalidomide and bortezomib and have demonstrated disease progression on the last therapy
Applicant: Celgene Corporation

Pomalidomide is an immune-modulator and analogue of thalidomide, developed to treat patients with relapsed/refractory multiple myeloma. Thalidomide and lenalidomide (also a thalidomide analogue) have been approved for treatment of multiple myeloma. Thalidomide, lenalidomide, and pomalidomide are structurally related. Previously the mechanism of action of thalidomide and lenalidomide were not fully characterized, as also mentioned in the label for these drugs. The Applicant conducted studies to characterize the pharmacology of pomalidomide, while using thalidomide and lenalidomide as comparators in several pharmacology studies. Pomalidomide targets the protein cereblon, which is involved in poly-ubiquitination of proteins. The activity of pomalidomide was dependent on the presence of cereblon. Expression of cereblon in activated human T cells was needed for induction of interleukin-2 (IL-2) and tumor necrosis factor-alpha (TNF-α) by pomalidomide. In cell based assays, pomalidomide modulated the production of several cytokines, e.g. decreased the production of IL-12, IL-6, TNF-α, and GM-CSF and increased the production of IL-10. Pomalidomide inhibited the expression of COX-2 in the assay tested.

Safety pharmacology, pharmacokinetic/ADME (absorption, distribution, metabolism and excretion), and toxicology studies were also conducted. Animal toxicology studies were conducted in appropriate species, using the administration route and dosing regimens that adequately addressed safety concerns in humans. Pomalidomide-related toxicities were more evident in monkeys and included: reduction in platelet and WBC counts, lymphoid depletion, inflammation in the GI tract, and infection (likely related to lymphoid depletion). In the chronic toxicology study, one of the 12 monkeys in the high-dose arm developed acute myeloid leukemia (AML) when animals were treated for 9 months. An association between pomalidomide treatment and development of AML cannot be ruled out at this time. While pomalidomide was negative in the battery of genetic toxicology studies, secondary malignancies with immune-modulatory agents have been reported. The following statement is from the label from Revlimid (lenalidomide), a thalidomide analogue:

> Patients with multiple myeloma treated with lenalidomide in studies including melphalan and stem cell transplantation had a higher incidence of second primary malignancies, particularly acute myelogenous leukemia (AML) and Hodgkin lymphoma, compared to patients in the control arms who received similar therapy but did not receive lenalidomide.

Pomalidomide was teratogenic in rats and rabbits. In the embryo-fetal developmental study conducted in rabbits, thalidomide was used as a comparator. Teratogenic and embryo-fetal toxic effects of pomalidomide were similar to those seen with thalidomide. A pregnancy Category X has been assigned to pomalidomide because of the teratogenic effects of this drug in animals and to be consistent with thalidomide and lenalidomide labels. Pomalidomide did not affect the fertility index in male or female rats, when tested in a fertility and early embryonic study. However, the number of viable embryos was reduced, which is likely secondary to the increase in post-implantation loss and the increase in resorption, as described in this study. This effect was seen when male and female rats treated with pomalidomide were mated. The reduction in the number of embryos was attributed to the exposure of females to pomalidomide, since treating male rats with pomalidomide and mating them with untreated females did not affect the viability of embryos.

The pharmacologic class assigned to pomalidomide is "thalidomide analogue". This is consistent with the pharmacologic class assigned to lenalidomide and is also based on similarities between pomalidomide and thalidomide in regard to chemical structures, and pharmacologic/ toxicologic effects.

The nonclinical studies were reviewed by Dr. Brenda Gehrke and Dr. Pedro Del Valle. The nonclinical findings are summarized in the "Executive Summary" of the NDA review and reflected in the product label.

Recommendation: I concur with Drs. Gehrke and Del Valle that from a nonclinical perspective, POMALYST may be approved for the proposed indication. No additional nonclinical studies are needed to support approval of POMALYST for the proposed indication.

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/s/

HALEH SABER
12/13/2012

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:	204026
Supporting document/s:	1
Applicant's letter date:	April 10, 2012
CDER stamp date:	April 10, 2012
Product:	Pomalidomide
Indication:	Multiple myeloma
Applicant:	Celgene Corporation
Review Division:	Division of Hematology Oncology Toxicology
	(for Division of Hematology Products)
Reviewers:	Brenda J. Gehrke, Ph.D.
	Pedro L. Del Valle, Ph.D.
Supervisor/Team Leader:	Haleh Saber, Ph.D.
Division Director:	John Leighton, Ph.D., DABT
	Ann Farrell, M.D. (DHP)
Project Manager:	Amy C. Baird

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 204026 are owned by Celgene Corporation or are data for which Celgene Corporation has obtained a written right of reference.
Any information or data necessary for approval of NDA 204026 that Celgene Corporation does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 204026.

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1 Executive Summary

1.1 Introduction

Celgene Corporation has submitted NDA 204026 for pomalidomide, a new molecular entity that is a thalidomide analogue and immunomodulatory agent. The proposed clinical dose of 4 mg once daily on Days 1-21 of 28-day cycles is administered orally as a capsule. Nonclinical pharmacology, pharmacokinetic, and toxicology studies have been submitted to support the approval of pomalidomide in combination with dexamethasone for the treatment of patients with relapsed and refractory multiple myeloma who have received at least two prior regimens, including both lenalidomide and bortezomib, and have demonstrated disease progression on the last therapy.

1.2 Brief Discussion of Nonclinical Findings

In support of the commercial development program for pomalidomide, *in vitro* studies and animal studies in the mouse, rat, rabbit, dog, and/or monkey were conducted to evaluate the pharmacology, pharmacokinetics, general toxicology, genotoxicity, reproductive and developmental effects of pomalidomide.

Pomalidomide is a thalidomide analogue and immunomodulatory drug. In general, immunomodulatory drugs may affect the immune system in several ways including inducing immune responses, enhancing activity of immune cells, and altering and modulating the induction of pro- and anti-inflammatory cytokines. The pharmacology of pomalidomide was evaluated using a series of *in vitro* cell based studies and *in vivo* animal models. The results include the following findings:

- Pomalidomide binds cereblon, a protein that modulates polyubiquitination of proteins. Cereblon expression was required for pomalidomide to exert its effects including the induction of interlukin 2 (IL-2) and tumor necrosis factor alpha (TNF-α) in myeloma cell lines. Decreased levels of cereblon correlated with acquisition of resistance to lenalidomide.
- Antiproliferative effects of pomalidomide are exerted through cell cycle arrest in G_1 and apoptotic induction with concomitant down regulation of cyclin-dependent kinases CDK4 and CDK6, decreases in retinoblastoma protein phosphorylation and modulation of other important proteins in the cell cycle.
- Immunomodulatory effects of pomalidomide include inhibition of pro-inflammatory cytokines and chemokines IL-12, IL-1β, IL-6, TNF-α, granulocyte macrophage colony-stimulating factor (GM-CSF) production, inhibition of monocyte chemoattractant protein (MCP)-1 and macrophage inflammatory protein (MIP)-1α, increased production of IL-10, and inhibition of COX-2 expression.

Safety pharmacology studies were conducted to assess the effects of pomalidomide on neurological, pulmonary, and cardiovascular function. No clear dose-dependent effects of pomalidomide were observed on neurological function in rats, cardiac function in telemeterized monkeys or anesthetized dogs, or pulmonary function in conscious rats.

In a cardiovascular and respiratory study in anesthetized dogs following intravenous infusion of pomalidomide, an increased rate of respiration accompanied with decreases in blood pressure and femoral artery blood flow occurred in one dog administered the highest dose tested (25 mg/kg; 500 mg/m^2). In a cardiovascular study in telemeterized monkeys following oral administration of 10.0 mg/kg (120 mg/m^2) pomalidomide, mean pulse pressure was slightly lower (~ 10%) compared with vehicle control during the first seven hours after treatment. In an *in vitro* hERG channel assay in HEK293 cells, pomalidomide at concentrations of 7.9 or 87.5 µM produced less than 1% inhibition of hERG current, suggesting a low potential to block *in vivo* cardiac I_{Kr}. This finding is supported by the absence of QT prolongation in the *in vivo* animal studies with electrocardiographic measurement.

Pharmacokinetic parameters were measured in single dose (intravenous or oral) studies using rats and monkeys, as part of the oral repeat-dose toxicology studies using rats and monkeys, and in *in vitro* models for protein binding, metabolism (human and rabbit hepatocytes and recombinant CYP450), and drug-drug interactions including P-gp transport. Pomalidomide was absorbed rapidly with a T_{max} of 4 and 2 hours after single oral dosing in rats and monkeys, respectively. The oral bioavailability was 13% in rats and 15% in monkeys. The terminal plasma half-life of pomalidomide after intravenous adminstration was 6-7 hours in rats and monkeys. Interconversion between enantiomers was detected after intravenous or oral administration of pomalidomide or the individual enantiomers. Plasma protein binding ranged from 15% to 40% and from 16% to 55% for the pomalidomide R- and S-enantiomers, respectively, when pomalidomide was incubated with plasma from human, monkey, rat, mouse and rabbit. A tissue distribution study using [^{14}C]-pomalidomide in rats demonstrated that pomalidomide is widely distributed throughout the body with the highest concentrations in the alimentary canal (GI tract) and organs of excreta (renal cortex, medulla, and urinary bladder). Pomalidomide is extensively metabolized through hydroxylation, glucuronidation, hydrolysis, and N-dealkylation-deamination. The percent distribution of the major metabolites was comparable in plasma samples from monkey and human studies. Pomalidomide was excreted primarily in the urine (~72%) with some excretion in the feces (~12%) following either intravenous or oral administration to monkeys. In a lacteal transfer study following a single oral dose of pomalidomide (10 mg/kg; 60 mg/m^2) in rats, the mean milk:plasma concentration ratios ranged from 0.63 to 1.5 for up to 24 hours after dosing, indicating that pomalidomide is excreted into milk.

Repeat-dose toxicology studies with daily oral administration were conducted in both rats and cynomolgus monkeys in order to fully characterize pomalidomide-induced toxicities. In the rat studies, pomalidomide was well tolerated and did not produce any clear toxicity at doses up to 2000 mg/kg/day (12,000 mg/m^2/day) for 28 days, 1500 mg/kg/day (9000 mg/m^2/day) for 90 days, or 1000 mg/kg/day (6000 mg/m^2/day) for 180 days (6 months). Effects of pomalidomide observed in cynomologus monkeys included decreases in platelets, white blood cell count, and lymphocytes in serum, lymphoid hypocellularity in the bone marrow, and lymphoid depletion or atrophy of various lymphoid tissues (mandibular and mesenteric lymph noses, Peyer's patch, spleen and thymus). Pomalidomide produced moribundity and mortality related to hemopoietic and

lymphoid system toxicity in multiple repeat-dose monkey studies at doses as low as 1 mg/kg/day (12 mg/m^2/day).

In the pivotal 9-month monkey study, pomalidomide (0.05, 0.1, or 1 mg/kg/day; 0.6, 1.2, or 12 mg/m^2/day) was administered by nasogastric gavage daily for 39 weeks with an 8-week recovery period. Treatment at the highest dose (12 mg/m^2/day) produced morbidity caused by chronic inflammation of the large intestine with or without villous atrophy of the small intestine, *Staphylococcus aureus* infection, and acute myeloid leukemia. The acute myeloid leukemia observed in the monkey was attributed to the effects of pomalidomide based on the rarity of this type of neoplasm in nonhuman primates. Irreversible proliferation of intrahepatic bile ducts was also observed at 12 mg/m^2/day in the 9-month study. Cynomolgus monkeys were more sensitive to pomalidomide than rats. This finding is consistent with the general toxicology results for lenalidomide, which suggests that rodents are not as sensitive as non-human primates to the effects of lenalidomide or pomalidomide assessed in general toxicology studies, and that there is a class effect for immunomodulatory agents.

Pomalidomide was tested for mutagenicity in an *in vitro* reverse mutation (Ames) assay and an *in vitro* mouse lymphoma assay and tested for clastogenicity in an *in vitro* chromosomal aberrations assay in cultured human peripheral blood lymphocytes and an *in vivo* rat bone marrow micronucleus assay. At the concentrations and doses tested, pomalidomide was not mutagenic or clastogenic.

Reproductive and developmental toxicology studies were conducted in rats and rabbits to assess the effects of pomalidomide on fertility and embryo-fetal development. In the fertility and early embryonic development study conducted in rats, pomalidomide had no effect on premating estrous cyclicity, reproductive performance, or fertility. In pomalidomide treated females paired with treated males, the number of viable embryos was significantly decreased and the post-implantation loss and total number of resorptions (early + late) were significantly increased compared to controls at all doses (≥25 mg/kg/day; ≥150 mg/m^2/day). There were no effects of pomalidomide on embryo viability in untreated females paired with the same treated males, indicating that the increase in post-implantation loss seen in the pairing with treated males and treated females was not attributable to the treatment of the males.

The embryo-fetal development effects of pomalidomide were studied in the rat and rabbit. Pomalidomide produced post-implantation loss and was clearly teratogenic in both species. In the rat, effects were observed at all doses of pomalidomide including significant increases in the mean number of resorptions and post-implantation loss, significant decreases in the number of viable fetuses, significantly decreased fetal weights, and increases in visceral and skeletal malformations and variations. Visceral malformations of absent urinary bladder and absent thyroid were observed at all doses. Increases in aortic arch malformations (right-sided aortic arch, dilated arch, retroesophageal arch, extra azygous vein, and small pulmonary trunk) were observed at the highest dose of 1000 mg/kg/day (6000 mg/m^2/day). Skeletal malformations

included fused centra, fused neural arches, and misaligned neural arches of the lumbar and thoracic vertebrae.

In the rabbit, pomalidomide produced similar effects to those observed in the positive control, thalidomide, including decreases in maternal body weight gain, increased post-implantation loss, and increases in gross external, visceral, and skeletal malformations and variations. Pomalidomide was maternally toxic in rabbits at the mid and high doses (100 and 250 mg/kg/day; 1200 and 3000 mg/m^2/day). Pomalidomide produced significant increases in the litter and fetal incidences of gross external, visceral, and skeletal malformations, including limb malformations similar to those observed in the thalidomide positive control group. External malformations included flexed and/or rotated fore and/or hindlimbs, unattached or absent digit of the fore and/or hindlimbs, and short tail. Visceral malformations were observed in the heart, vessels, diaphragm, kidney, and ureter. Increases in fetal cardiac anomalies, principally the malformation of interventricular septal defect, were observed at all doses of pomalidomide. There were numerous skeletal malformations including those associated with the external malformations of short tail and limb anomalies.

As a thalidomide analogue, the teratogenic effects of pomalidomide were expected and observed in both rats and rabbits at all doses tested. The teratogenicity observed in the rat with pomalidomide has not been observed with either lenalidomide or thalidomide. Based on teratogenicity findings, pregnancy category X is recommended. Celgene Corporation has submitted a risk evaluation and mitigation strategies (REMS) plan for pomalidomide that includes a restricted distribution program similar to those for thalidomide and lenalidomide.

1.3 Recommendations

1.3.1 Approvability

Recommended for approval. The non-clinical studies submitted to this NDA provide sufficient information to support the use of pomalidomide for the proposed indication.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

The content for the labeling of pomalidomide is contained in this review. Based on the teratogenicity findings and to be consistent with the labeling for thalidomide and lenalidomide, pregnancy category X is recommended. The human AUC (AUC_{24h}) of 402 ng•h/mL on week 4 at the recommended clinical dose of 4 mg/day was used for the animal:human conversions for pomalidomide labeling.

2 Drug Information

2.1 Drug

CAS Registry Number 19171-19-8

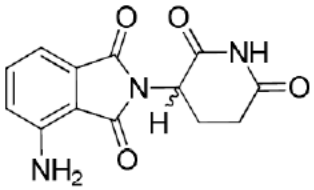
Generic Name Pomalidomide

Code Name CC-4047

Chemical Name 4-amino-2-([(3RS)-2,6-dioxopiperidin-3-yl)2*H* isoindole-1,3-dione

Molecular Formula/Molecular Weight $C_{13}H_{11}N_3O_4$ / 273.24 g/mol

Structure or Biochemical Description



Pharmacologic Class Thalidomide analogue

2.2 Relevant INDs, NDAs, BLAs and DMFs:

IND 66188, IND (b) (4) DMF (b) (4), DMF (b) (4) DMF (b) (4), DMF (b) (4)

2.3 Drug Formulation

Pomalidomide is available in 1, 2, 3 or 4 mg hard gelatin capsules packaged in a high density polyethylene bottle with tamper evident induction seal and (b) (4) child resistant closure. Each capsule contains pomalidomide as the active ingredient, and mannitol, pregelatinized starch, and sodium stearyl fumarate as inactive ingredients. The composition for each strength is presented in the table below provided by the CMC reviewer, Dr. William Adams.

Unit Composition

Strength	***Grade***	***Function***	***1mg***	***2mg***	***3mg***	***4mg***
			[mg/capsule]			
Pomalidomide (CC-4047, API)	In-house	Active	(b) (4)			
Mannitol	USP/NF/EP	(b) (4)				
Pregelatinized Starch (PGS)	NF/EP					
Sodium Stearyl Fumarate (SSF)	NF/EP					
Fill Weight (b) (4)						
HG Capsules*			A	B	C	D

A = (b) (4), dark blue/yellow opaque, imprinted; black "POML" on body & white "1 mg" on cap
B = (b) (4) dark blue/orange opaque, imprinted; white "POML" on body & white "2 mg" on cap

C = (b) (4) dark blue/green opaque, imprinted with white "POML" on body & white "3 mg" on cap
D = (b) (4) dark blue/blue opaque, imprinted with white "POML" on body & white "4 mg" on cap
* capsule shells are supplied by (b) (4) refer to NDA section 3.2.P.4.

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

None

2.6 Proposed Clinical Population and Dosing Regimen

The proposed clinical population is patients with relapsed and refractory multiple myeloma who have received at least two prior regimens of established benefit, including both lenalidomide and bortezomib, and have demonstrated disease progression on the last therapy. Pomalidomide (4 mg capsule) is to be administered once daily on Days 1-21 of 28-day cycles.

2.7 Regulatory Background

Pomalidomide has been developed as an anti-cancer drug by Celgene Corporation and clinical trials have been conducted since December 2002 under IND 66188. Pomalidomide was granted an orphan drug designation (No. 02-1614) for treatment of multiple myeloma on January 15, 2003 and granted Fast Track for multiple myeloma on December 15, 2011. A pre-NDA meeting with the FDA was held on September 13, 2011. NDA 204026 was submitted and received on April 10, 2012.

Pomalidomide is an analogue of thalidomide. Thalidomide is a known human teratogen that can cause severe birth defects such as amelia (absence of limbs), phocomelia (short limbs), hypoplasticity of the bones, absence of bones, external ear abnormalities (including anotia, micropinna, small or absent external auditory canals), facial palsy, eye abnormalities (anophthalmos, microphthalmos), and congenital heart defects. Thalidomide (Thalomid®) is approved for the acute treatment of the cutaneous manifestations of moderate to severe erythema nodosum leprosum (ENL), as maintenance therapy for prevention and suppression of the cutaneous manifestations of ENL recurrence, and in combination with dexamethasone for the treatment of newly diagnosed multiple myeloma. The first approved thalidomide analogue, lenalidomide (Revlimid®), is indicated for the treatment of multiple myeloma in combination with dexamethasone in patients who have received at least one prior therapy and patients with transfusion-dependent anemia due to low- or intermediate-1-risk myelodysplastic syndromes (MDS) associated with a deletion 5q abnormality with or without additional cytogenetic abnormalities. Lenalidomide caused limb abnormalities in a developmental monkey study similar to birth defects caused by thalidomide in humans. Both thalidomide and lenalidomide have REMS and are only available under restricted distribution programs. In the submission for NDA 204026, Celgene Corporation has submitted a REMS plan for pomalidomide that includes a restricted distribution program similar to those for thalidomide and lenalidomide.

3 Studies Submitted

3.1 Studies Reviewed

Primary Pharmacology

Study#	Title	Module
DM2528	Cereblon is required for anti-proliferative and immunomodulatory effects of lenalidomide and pomalidomide and decreased cereblon expression correlates with acquisition of lenalidomide resistance in myeloma cells	4.2.1.1
1110-038	The effect of IMiDs on multiple myeloma cell lines	4.2.1.1
7596-01	Effects of lenalidomide, pomalidomide, and CC-122 on multiple myeloma cell lines that have been made resistant to lenalidomide or pomalidomide	4.2.1.1
1785-4047	Evaluation of anti-proliferative effect of CC-4047 in cellular models of multiple myeloma	4.2.1.1
5127-132	Screening of IMiDs and PDE4 inhibitor compounds for anti-angiogenic activity in the human umbilical cord vessel ring assay	4.2.1.1
CGN-04	Effect of test compounds on human myeloid, erythroid and megakaryocytic progenitors	4.2.1.1
1110-028	Effects of CC-4047 on CD34+ hematopoietic progenitor differentiation and maturation to dendritic cells (DC)	4.2.1.1
5374-10	Multiple cytokine and chemokine profiling for IMiDs CC-5013, CC-4047, CC-2001, CC-11006 and CC-10015 in LPS-stimulated human PBMC	4.2.1.1
(b) (4)-03162010	Evaluation of lenalidomide and pomalidomide using SCID-hu models of multiple myeloma	4.2.1.1
CLG-4-Raji-B-e200	Evaluation of (b) (4), PB19, and PB20, in combination with rituximab, against Raji B human lymphoma in a survival study with C.B-17 SCID mice	4.2.1.1
(b) (4)-P10.0101	Evaluation of Celgene compounds using *in vivo* angiogenesis matrigel plug assay	4.2.1.1

Safety Pharmacology

Study #	Title	Module
CC-4047-TOX-011	Acute oral (gavage) central nervous system (CNS) safety pharmacology study of CC-4047 in rats	4.2.1.3
CC-4047-TOX-014	Respiratory assessment following oral gavage administration of CC-4047 to plethysmograph-restrained male Sprague-Dawley rats	4.2.1.3
CC-4047-TOX-009	Effect on cloned hERG channels expressed in human embryonic kidney (HEK293) cells	4.2.1.3
1398/110	Cardiovascular and respiratory effects in the anaesthetised dog following intravenous infusion	4.2.1.3
CC-4047-TOX-012	Cardiovascular safety pharmacology evaluation of CC-4047 administered by oral gavage to telemetry-instrumented conscious cynomolgus monkeys	4.2.1.3

Pharmacokinetics

Study #	Title	Module
Absorption		
1398/72	A study to determine the oral bioavailability of (b) (4) and CC-4047 in the rat	4.2.2.2
1398/73	A study to determine the oral bioavailability of (b) (4) and CC-4047 in the cynomolgus monkey	4.2.2.2
CC-4047-DMPK-021	Single dose intravenous and oral pharmacokinetics of CC-4047 and two enantiomers in monkeys	4.2.2.2
Distribution		
CC-4047-DMPK-015	*In vitro* protein binding study in rat, mouse, monkey, rabbit, and human plasma	4.2.2.3
CC-4047-DMPK-005	Quantitative tissue distribution of compound-related material using whole-body autoradiography following a single oral dose of [^{14}C]CC-4047 (100 mg/kg) to male Long-Evans rats	4.2.2.3
CC-4047-DMPK-038	Determination of the lacteal transfer of C-4047 following a single 10 mg/kg oral administration in rats	4.2.2.3
Metabolism		
CC-4047-DMPK-010	[^{14}C]CC-4047: Biotranformation following intravenous or oral administration to cynomolgus monkeys	4.2.2.4
CC-4047-DMPK-022	[^{14}C]CC-4047: P450 reaction phenotyping	4.2.2.4
CC-4047-DMPK-042	Determination of relative plasma exposures of CC-4047 metabolites in rat and monkey plasma to human plasma	4.2.2.4
Excretion		
CC-4047-DMPK-009	[^{14}C]CC-4047: Pharmacokinetics and mass balance following intravenous or oral administration to cynomolgus monkeys	4.2.2.5
Pharmacokinetic drug interactions		
CC-4047-DMPK-023	*In vitro* evaluation of CC-4047 as an inducer of cytochrome P450 expression in cultured human hepatocytes	4.2.2.6
CC-4047-DMPK-024	*In vitro* evaluation of CC-4047 as an inhibitor of human cytochrome P450 enzymes	4.2.2.6
CC-4047-DMPK-037	*In vitro* assessment of CC-4047 as a substrate or inhibitor of p-glycoprotein	4.2.2.6
Other pharmacokinetic studies		
CC-4047-DMPK-030	*In vitro* stability and interconversion of CC-4047 enantiomers in PBS and monkey/human plasma	4.2.2.7

Repeat-Dose Toxicology

Study#	Title	Module
1398/114	7 day oral (gavage) range-finding toxicity study in the rat	4.2.3.2
1398/115	28-day oral (gavage administration) toxicity study in the rat	4.2.3.2
CC-4047-TOX-001	A 90-day oral toxicity and toxicokinetics study in Sprague-Dawley rats	4.2.3.2
CC-4047-TOX-013	A 6-month toxicity study of CC-4047 administered by oral gavage to rats with a 1-month recovery period	4.2.3.2
1398/116	Maximum tolerated dose (MTD) followed by a fixed dose oral (gavage) toxicity study in the monkey	4.2.3.2
1398/117	28 day oral (gavage administration) toxicity study in the monkey	4.2.3.2
1398/126	CC-4047 & CC-5013: 28 day oral (gavage administration)	4.2.3.2

	toxicity study in the monkey	
CC-4047-TOX-002	A 13-week oral toxicity study in cynomolgus monkeys	4.2.3.2
CC-4047-TOX-006	A 9-month oral toxicity study of CC-4047 administered by nasogastric gavage to cynomolgus monkeys, with an 8-week recovery period	4.2.3.2

Genetic Toxicology

Study#	Title	Module
CC-4047-TOX-015	Evaluation of pomalidomide (CC-4047) in the bacterial reverse mutation with a confirmatory assay	4.2.3.3.1
1398/82	Mutation at the thymidine kinase (*tk*) locus of mouse lymphoma L5178Y cells (MLA) using the Microtitre fluctuation technique	4.2.3.3.1
CC-4047-TOX-016	Evaluation of pomalidomide (CC-4047) in the chromosomal aberrations assay in cultured human peripheral blood lymphocytes	4.2.3.3.1
CC-4047-TOX-017	Evaluation of pomalidomide (CC-4047) in the *in vivo* rat bone marrow micronucleus assay	4.2.3.3.2

Reproductive Toxicology

Study#	Title	Module
CC-4047-TOX-020	A fertility and early embryonic development study in rats administered pomalidomide (CC-4047) orally	4.2.3.5.1
CC-4047-TOX-021	An embryo-fetal development study in rats administered pomalidomide (CC-4047) orally	4.2.3.5.2
0329DC35.001	Developmental and reproductive toxicity screening study for effects on embryo-fetal development in rabbits	4.2.3.5.2
0329DC35.002	Oral screen study for effects on embryo-fetal development in New Zealand white rabbits (SEG II)	4.2.3.5.2
CC-4047-TOX-008	Oral (stomach tube) developmental toxicity study of CC-4047 in rabbits	4.2.3.5.2

Special Toxicology

Study#	Title	Module
CC-4047-TOX-019	A 28-day immunotoxicity study of CC-4047 administered by nasogastric gavage to cynomolgus monkeys followed by a 30-day recovery period	4.2.3.7.2

3.2 Studies Not Reviewed

Primary Pharmacology

Study#	Title	Module
5286-186	Combination studies of CC-5013 with chemotherapeutic agents on $FGFR3^{+}$ and $FGFR3^{-}$ multiple myeloma cell proliferation	4.2.1.1
5232-59-76 (Revised)	Anti-proliferative activity and mechanism of action of thalidomide, CC-4047, CC-5013 and CC-11006 in chromosome 5 deleted cells Namalwa and KG-1 and control cell lines MUTZ-5 and UT-7 *in vitro*	4.2.1.1
5783-003	*In vitro* comparison of CC-4047 and CC-5013 on proliferation and gene expression in multiple myeloma cell lines	4.2.1.1
5286-14	Anti-proliferative activity of IMiDs in 5q-solid tumor cell lines PC-3, HN, LCLC103H, SW480, and SK-MES-1	4.2.1.1

5196-141-155	Anti-proliferative activity of CC-4047, CC-5013, CC-5079, and CC-10004 against the non-Hodgkins B lymphoma cell line Farage *in vitro*	4.2.1.1
PD385	Effects of IMiDs on proliferation of breast cancer, NSCLC, CML and NHL cell lines *in vitro*	4.2.1.1
PD365	Cytokine profiling for five classes of IMiDs in primary human PBMCs and CD4+ T lymphocytes	4.2.1.1
5043-152-5119-172	Inhibition of TNF-α production by PBMC and evaluation of IL-2 and MIP-3α production by T cells by CC-4047, CC-5013 and CC-11006 *in vitro*	4.2.1.1
5304-79	Effect of IMiDs CC-4047, CC-10015, CC-11006 and CC-5013 on PBMC and T cell proliferation *in vitro*	4.2.1.1
5197-189-5226-016	Elevation of IL-2 production by CC-047, CC-5013, and CC-11006 from human and rat whole blood stimulated with Concanavalin A	4.2.1.1
PD522	IMiD increases T-BET expression mediated by TCR signaling in CD4+ T cells	4.2.1.1
PD465	Evaluation of NSCLC/PBMC co-culture models for their sensitivities to IMiDs	4.2.1.1
PD466	Effects of IMiDs on prostate tumor cell apoptosis in co-culture models	4.2.1.1
PD467	Development and validation of K562/PBMC co-culture model	4.2.1.1
PD468	Effects of IMiDs on Raji cell apoptosis in co-culture model	4.2.1.1
PD469	Effects of IMiDs on ovarian cancer cell apoptosis in co-culture models	4.2.1.1
5422-8-CC-4047	CC-4047 in combination with the therapeutic antibodies, trastuzumab, cetuximab and rituximab, enhances *in vitro* antibody-dependent cellular cytotoxicity (ADCC in breast cancer, colorectal cancer and NHL cell lines, respectively	4.2.1.1
5071-180	Lenalidomide inhibits angiogenesis *in vitro* and reduces lung metastasis of mouse melanoma cells in an animal model	4.2.1.1
5239-92-5239-188	Inhibition of endothelial cell migration by thalidomide, CC-4047, and CC-5013, Summary of experiments 5239-92 ~5239-188	4.2.1.1
CLG-10	Effect of test compounds on human megakaryocrytic progenitors from three distinct normal bone marrow donor cells as assessed in the colony forming cell assay	4.2.1.1
5196-175	Inhibition of tumor necrosis factor alpha (TNF-α) production by CC-4047, CC-5013, and CC-11006 from human and rat whole blood stimulated with lipopolysaccharide	4.2.1.1
5127-53	Anti-inflammatory effects of CC-4047, CC-5013, and CC-11006 on G-CSF, IL-10, and COX-2 expression by LPS-stimulated PBMC (revised)	4.2.1.1
5197-130	Effect of IMiDs, PDE4 inhibitors, and tubulin inhibitors on COX-2 expression and PGE_2 production by human PBMC	4.2.1.1
5116-86	Immunomodulatory drugs (IMiDs) inhibit expression of cyclooxygenase-2 from TNF-α, IL-1β and LPS stimulated human PBMC in a partially IL-10-dependent manner	4.2.1.1
PD408	Role of IMiDs in B cell signaling	4.2.1.1
UCONN 07192008HS	Vaccination and challenge model of intracerebral B16 melanoma tumors in mice	4.2.1.1

Secondary Pharmacology

Study#	Title	Module
(b) (4)		4.2.1.2
		4.2.1.2
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Pharmacodynamic Drug Interactions

Study #	Title	Module
SALA-151008	Efficacy of lenalidomide and pomalidomide in combination with dexamethasone and bortezomib in the MM.1S xenograft mouse model of multiple myeloma	4.2.1.4
1066-033	Role of CC-4047 in hematopoietic stem cell (HSC) expansion	4.2.1.4

Pharmacokinetics

Study #	Title	Module
Analytical methods and validation reports		
1398/78	Validation for the determination of CC 4047 in primate plasma (heparin anticoagulant) using solid phase extraction and liquid chromatography with mass spectrometric detection	4.2.2.1

1398/80	Validation for the determination of CC 4047 in rat plasma (heparin anticoagulant) using solid phase extraction and liquid chromatography with mass spectrometric detection	4.2.2.1
CC-4047-DMPK-001	Partial method validation of an LC-MS/MS assay for the determination of CC-4047 in K_3EDTA monkey plasma	4.2.2.1
CC-4047-DMPK-013	Method validation of an LC-MS/MS assay for the determination of CC-4047 in K_3EDTA rat plasma	4.2.2.1
CC-4047-DMPK-018	Method validation of an LC-MS/MS assay for the determination of CC-4047 enantiomers CC-6016 and CC-5083 in monkey plasma	4.2.2.1
CC-4047-DMPK-027	Partial method validation of an LC-MS/MS assay for the determination of CC-4047 in K_3EDTA rabbit plasma	4.2.2.1
CC-4047-DMPK-032	Method validation of an LC-MS/MS assay for the determination of CC-4047 enantiomers CC-6016 and CC-5083 in rat plasma	4.2.2.1
Distribution		
AP1505/AP1506	*In vivo* microdialysis of CC-4047 in male CD-IGS rats, and brain and spinal cord availability of CC-4047 in male CD-1 mice	4.2.2.3
Metabolism		
CC-4047-DMPK-004	CC-4047/[^{14}C]CC-4047: Comparative biotransformation in rabbit and human cryopreserved hepatocytes	4.2.2.4
CC-4047-DMPK-007	Metabolism of [^{14}C]CC-4047 and excretion, mass balance and pharmacokinetics of radioactivity after a single oral or intravenous administration of [^{14}C]CC-4047 to male and female Sprague-Dawley rats	4.2.2.4
Pharmacokinetic drug interactions		
CC-4047-DMPK-043	CC-4047: Inhibition potential for BCRP, OAT1, OAT3, OCT2, OATP1B1 and OATP1B3	4.2.2.6

Single-Dose Toxicology

Study#	Title	Module
1398/50	Single dose oral toxicity study in the mouse	4.2.3.1
1398/52	Single dose intravenous toxicity study in the mouse (approximation of the minimum lethal dose level)	4.2.3.1
1398/49	Single dose oral toxicity study in the rat (approximation of the minimum lethal dose level)	4.2.3.1
1398/51	Single dose intravenous toxicity study in the rat	4.2.3.1

Genetic Toxicology

Study#	Title	Module
1398/20	Reverse mutation in four histidine-requiring strains of *Salmonella typhimurium* and two tryptophan-requiring strains of *Escherichia coli*	4.2.3.3.1
1398/68	Induction of chromosome aberrations in cultured human peripheral blood lymphocytes	4.2.3.3.1
1398/86	Induction of micronuclei in the bone marrow of treated rats	4.2.3.3.2

Reproductive Toxicology

Study#	Title	Module
CC-4047-TOX-007	Oral (stomach tube) dosage-range developmental toxicity study of CC-4047 in rabbits	4.2.3.5.2

3.3 Previous Reviews Referenced

None

4 Pharmacology

4.1 Primary Pharmacology

Study title: Cereblon is required for anti-proliferative and immunomodulatory effects of lenalidomide and pomalidomide and decreased cereblon expression correlates with acquisition of lenalidomide resistance in myeloma cells

Study No.:	DM2528
Report Date:	September 1, 2011
Study report location:	eCTD 4.2.1.1
Conducting Laboratory:	Celgene Corporation (San Diego, CA)
GLP:	No

Introduction

The antitumor activity of thalidomide and its analogues lenalidomide and pomalidomide is exerted through multiple overlapping effects including anti-proliferative activities, increased immune surveillance, and decreased stromal cell support of tumor growth. Cereblon (CRBN) was shown to bind a thalidomide analogue and its presence required for thalidomide to exert its teratogenic effects in zebra fish and chicken embryos. In this study, the sponsor wanted to investigate the role of CRBN in the antitumor efficacy of lenalidomide and pomalidomide and the resistance to lenalidomide and pomalidomide in myeloma cells.

Key Study Findings

- Lenalidomide-resistant H929 cells that showed reduced expression of cereblon remained responsive to inhibition by pomalidomide.
- Pomalidomide-resistant DF15R myeloma cells lacking CRBN protein were resistant to the anti-proliferative effects of both lenalidomide and pomalidomide.
- CRBN expression in activated human T cells was required for induction of interleukin-2 (IL-2) and tumor necrosis factor-alpha (TNF-α) by lenalidomide and pomalidomide.
- CRBN is a direct target of lenalidomide and pomalidomide and its expression in cells is necessary for the antitumor and immunomodulatory effects of lenalidomide and pomalidomide.

Thermal melt assays to measure binding of compounds to recombinant cereblon

Thermal melt shift studies were conducted to show binding affinities of thalidomide, lenalidomide and pomalidomide. Figure 1 shows the chemical structures of phthalimide, which lacks the glutarimide ring, and thalidomide, lenalidomide, and pomalidomide whose structures contain a glutarimide ring.

Figure 1: Chemical structures of pomalidomide, lenalidomide, thalidomide, and phthalidomide

(excerpted from Applicant's submission)

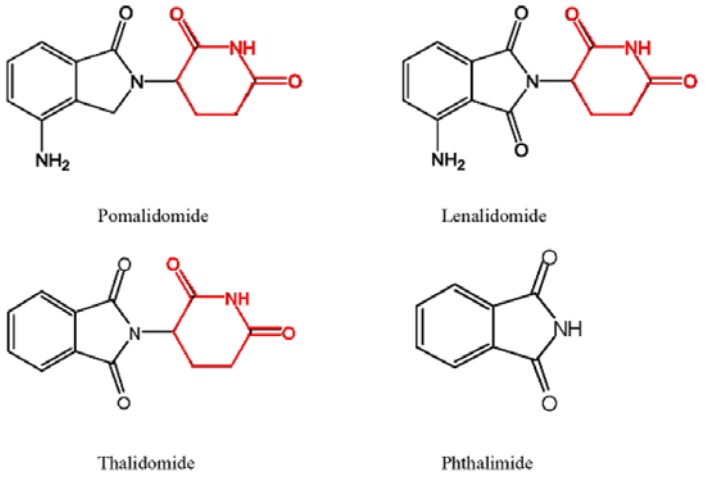


Figure 2: Fluorescence-based thermal assays with CRBN-DDB1 complex

(excerpted from Applicant's submission)

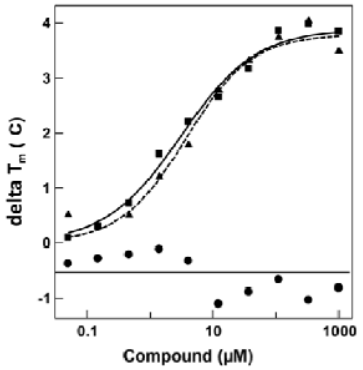


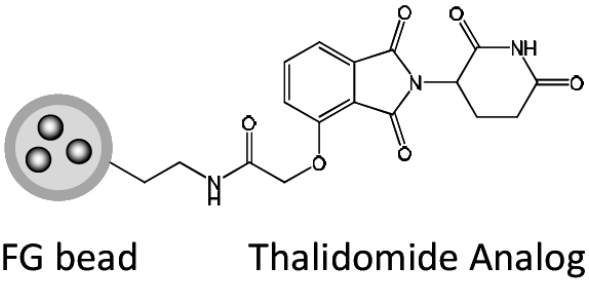
Figure 2 shows similar dose-dependent binding affinities of lenalidomide (■) and pomalidomide (▲) to CRBN-DDB1 complex using the thermal shift assay. On the contrary, phthalimide (●) showed no binding since it lacks the glutarimide ring.

Thalidomide analogue bead assay to measure compound binding to endogenous CRBN

FG beads are magnetic nanoparticles readily separable and recoverable by a magnet that are specially designed for receptor fishing (Tamagawa Seiki Co). Thalidomide analogue-coupled FG beads (Figure 3) were used to demonstrate binding to endogenous CRBN.

Figure 3: Thalidomide analogue coupled to FG magnetic nanoparticles bead

(excerpted from Applicant's submission)



Lenalidomide-resistant NCI-H929 cells (H929R) and pomalidomide-resistant DF15 cells (DF15R) were generated by supplementing the culture medium with increasing amounts of lenalidomide or pomalidomide and re-plating cells until they were fully resistant to lenalidomide up to 30 μM or pomalidomide up to 100 μM. CRBN bound to FG thalidomide analogue-coupled beads, but preincubation of U266 (myeloma cell) protein extract with lenalidomide prevented CRBN binding to FG thalidomide analogue-coupled beads.

Human T cells, CRBN knockdown and activity assays

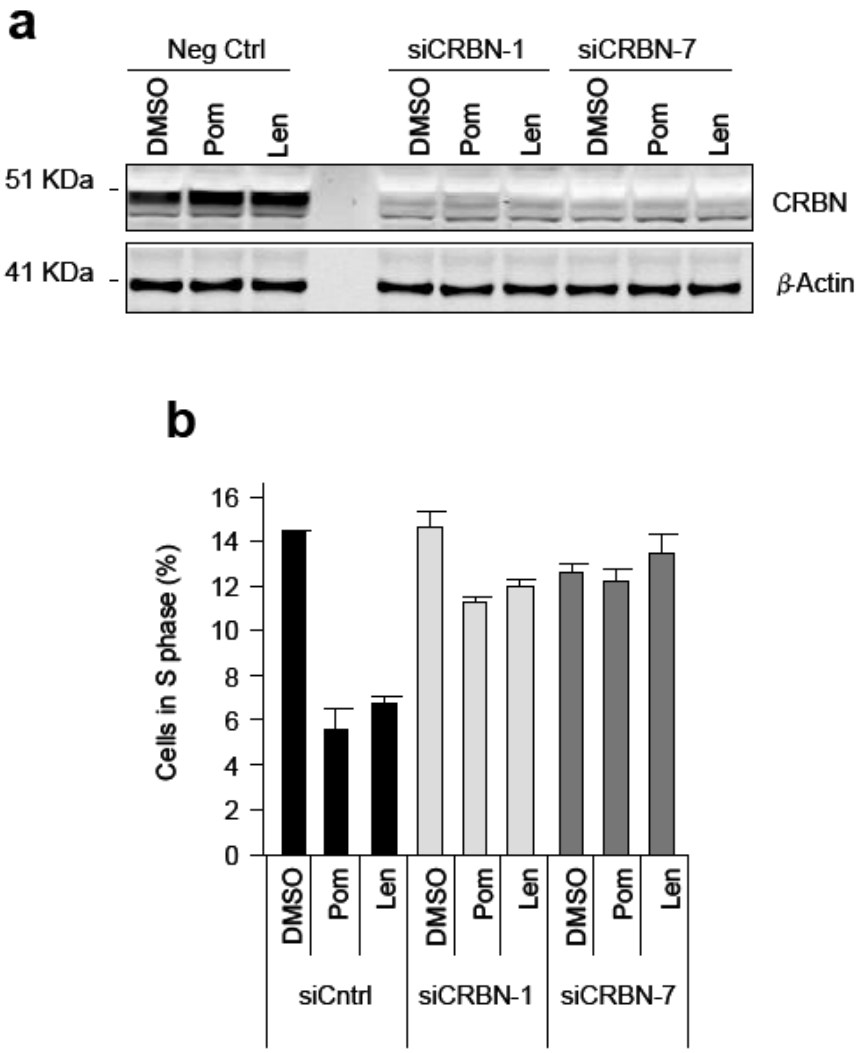
Purified T cells were transfected with small interfering ribonucleic acid (siRNA) to inhibit CRBN, cells were seeded on prebound OKT3 96-well plates and treated with either DMSO or compounds in duplicate at 37ºC for 48 hours. Low guanine-cytosine (GC) content negative siRNA was also transfected and used as control siRNA. Supernatant of drug-treated cells were collected and IL-2 or TNFα production was measured using ELISA.

CRBN expression in human T cells was reduced by transfection with siRNA CRBN as compared to controls. Activation of T cells with 1 and 10 μM of lenalidomide and pomalidomide resulted in 11- to 14-fold increase in IL-2 and 5- to 10-fold increase in TNF-α. These increases were substantially reduced in siRNA CRBN transfected T cells indicating that some of the immunomodulatory effects of these compounds are mediated by initial binding to CRBN.

Expression of CRBN in U266 myeloma cells was silenced with two separate siRNAs and the decrease in CRBN protein expression was confirmed, but depletion of CRBN affected neither cell cycle nor proliferation of U266 cells (Figure 4, panel a). However, knockdown of CRBN significantly reduced lenalidomide- and pomalidomide-induced delay of cell cycle progression measured as percent of cells in S phase (Figure 4, panel b).

Figure 4: Knockdown of cereblon gene expression in U266 myeloma cells modulates anti-proliferative response of lenalidomide and pomalidomide

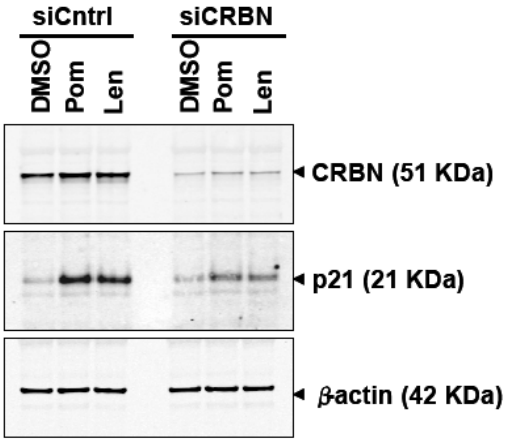
(excerpted from Applicant's submission)



Other forms of knockdown CRBN such as the use of lentiviral vectors expressing shRNA were also effective in depleting CRBN expression in U266, H929 and DF15 myeloma cell lines.

Figure 5: Lenalidomide and pomalidomide induced gene expression changes that were prevented by CRBN knockdown

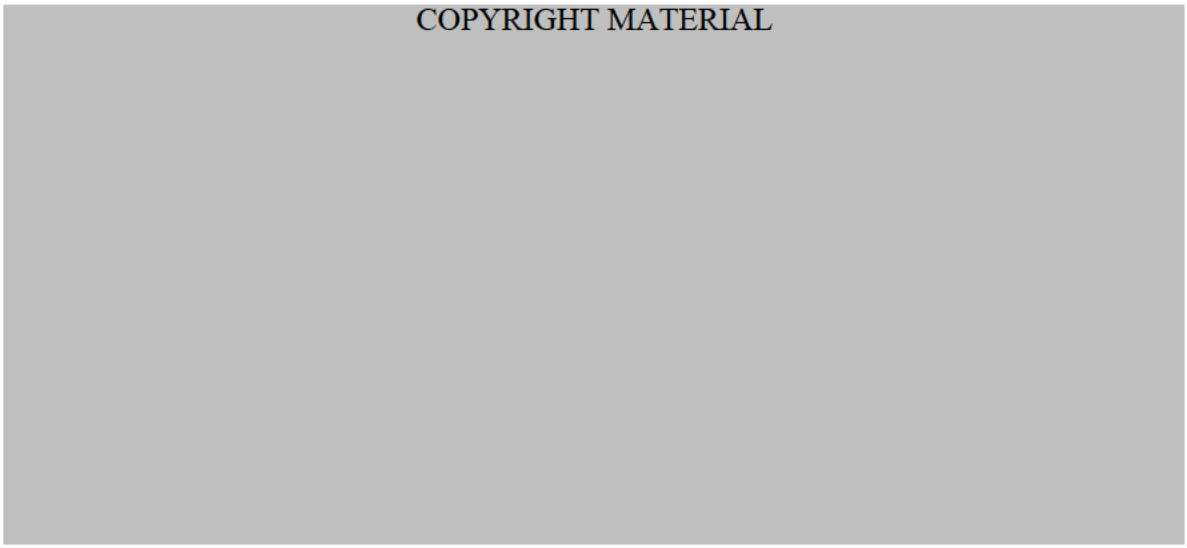
(excerpted from Applicant's submission)



Lenalidomide-resistant H929R cells showed decreased CRBN mRNA determined by reverse transcription-PCR after exposure to 1 μM and further decreases over 2 to 6 months at 10 μM lenalidomide (Figure 6, panel a). CRBN protein expression in four separate H929R cell lines was decreased compared to the parental cell line (Figure 6 panel b). The proliferation of the H929R cell lines was not inhibited by lenalidomide, but the proliferation was slightly responsive to pomalidomide. Parental DF15 myeloma cells showed dose-dependent inhibition of proliferation by lenalidomide and pomalidomide, but the lenalidomide- and pomalidomide-resistant DF15R cells were insensitive to lenalidomide or pomalidomide proliferative inhibition effects up to 10 μM. CRBN protein was fully expressed in DF15 myeloma parental cells but absent in DF15R cells. These results corroborated that CRBN is essential for lenalidomide and pomalidomide proliferative inhibition effects.

Figure 6: Chronic exposure to lenalidomide induces resistance to anti-proliferative effect of lenalidomide correlating with decreases in cereblon levels

(excerpted from Lopez-Girona et al 2012)



Study title: The effect of IMiDs on multiple myeloma cell lines

Study No.:	1110-038
Report Date:	April 21, 2006
Study report location:	eCTD 4.2.1.1
Conducting Laboratory:	Celgene Corporation (San Diego, CA)
GLP:	No

Introduction

The objective of this study was to determine the overall effect of CC-4047 on different multiple myeloma cell lines by monitoring cell proliferation, cell cycle progression, and apoptosis. The effect on cell-cycle regulatory proteins in selected cell lines (including those sensitive and resistant to CC-4047) and the effect of CC-4047 on CCAAT element binding protein-β (C/EBP-β), a protein that regulates cell growth and differentiation, were also examined. Twelve multiple myeloma cell lines (Arp-1, U266, Norway U-266, UUN, ARK, CAG, DF-15, H929, RPMI 8226, ANBL-6, W182, and MM-1S) were screened for sensitivity to CC-4047 defined as G1 arrest, usually Day 1 or Day 2, and apoptosis, usually Day 3 or Day 4. Cell lines DF15, H929 and MM-1S were determined to be sensitive to CC-4047.

Key Study Findings

- Three multiple myeloma cell lines, DF-15, H929, and MM-1S were determined to be sensitive to CC-4047.
- CC-4047 down-regulated cell-cycle regulatory proteins CDK4 and CDK6 with no effect on CDK2 within 24 hours, and this was associated with a decrease in retinoblastoma protein phosphorylation in responsive multiple myeloma cell lines.

- CC-4047 decreased CCAAT element binding protein-β levels (a regulator of cell growth and differentiation) within 24 hours in the DF15 multiple myeloma cell line.

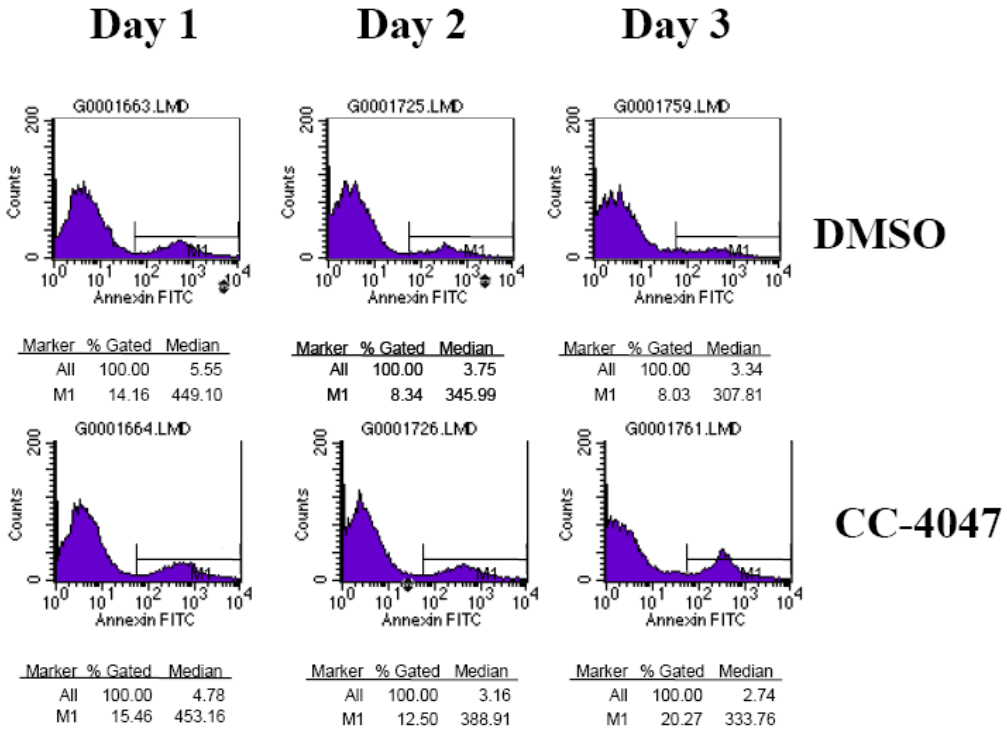
Effect of CC-4047 on DF-15

Plated DF15 myeloma cells were treated with DMSO (control) or 1 μM CC-4047 for specified number of days. Harvested cells after treatment were resuspended in 1 mL of digitonin:PI staining solution, incubated for 1 hour at room temperature protected from light, and analyzed using flow cytometry. CC-4047 arrested cell growth of DF15 myeloma cells in G1 phase within 2 days, 77% CC-4047 vs. 52% DMSO control.

CC-4047 at 1 μM also induced apoptosis within 3 days, 32% CC-4047 vs. 7% DMSO control and 20% CC-4047 vs. 8% DMSO control (Figure 7). Apoptosis was also assessed determining phosphatidyl serine (PS) exposure using Annexin V-FITC staining in combination with propidium iodide (BD Pharmingen Apoptosis Detection Kit I). Cells undergoing apoptosis stained positive for both FITC and PI, and are grouped within the lines in the bottom right-hand corner of individual panels (M1 Marker) in Figure 7. CC-4047 induced apoptosis in 20.3% of cells compared to 8% DMSO control on Day 3.

Figure 7: Apoptosis in multiple myeloma DF15 cells induced by 1 μM CC-4047

(excerpted from Applicant's submission)



Cyclin-dependent kinases (CDKs) are a family of protein kinases involved in regulating the cell cycle, transcription, mRNA processing, and the differentiation of nerve cells. CDKs bind to specific cyclins forming the cyclin-CDK complexes that regulate the cell

cycle. CDK2 binds to cyclin E and CDK4 and CDK6 bind to cyclin D for regulation of the G1/S transition and/or G1 phase. G1 arrest by CC-4047 in DF-15 cells was characterized by down regulation of CDK4 and CDK6 with no changes in CDK2 within 24 hours and this down regulation was associated with a decrease in retinoblastoma protein (Rb) phosphorylation (Figure 8).

Figure 8: Down regulation of CDK4 and CDK6 with concomitant Rb phosphorylation in DF-15 cells treated with 1 µM CC-4047

(excerpted from Applicant's submission)

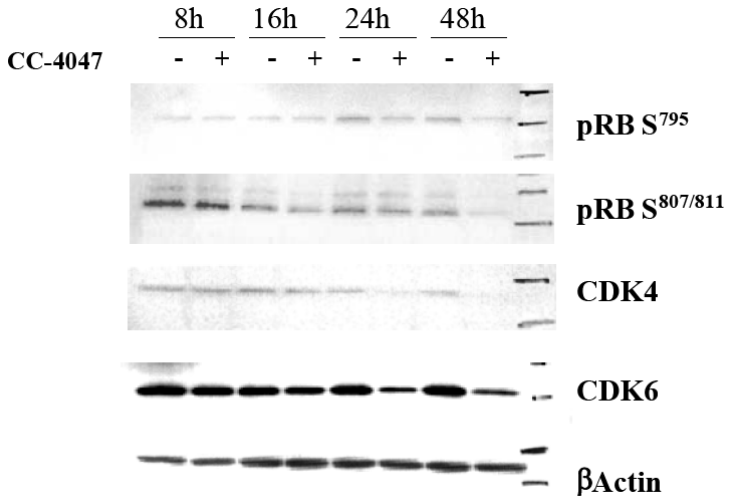
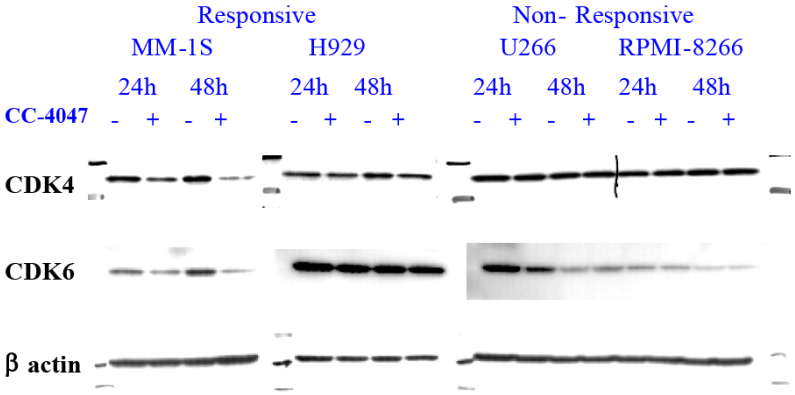


Figure 9: Down regulation of CDK4 and CDK6 with concomitant Rb phosphorylation occurred in responsive multiple myeloma cell lines

(excerpted from Applicant's submission)

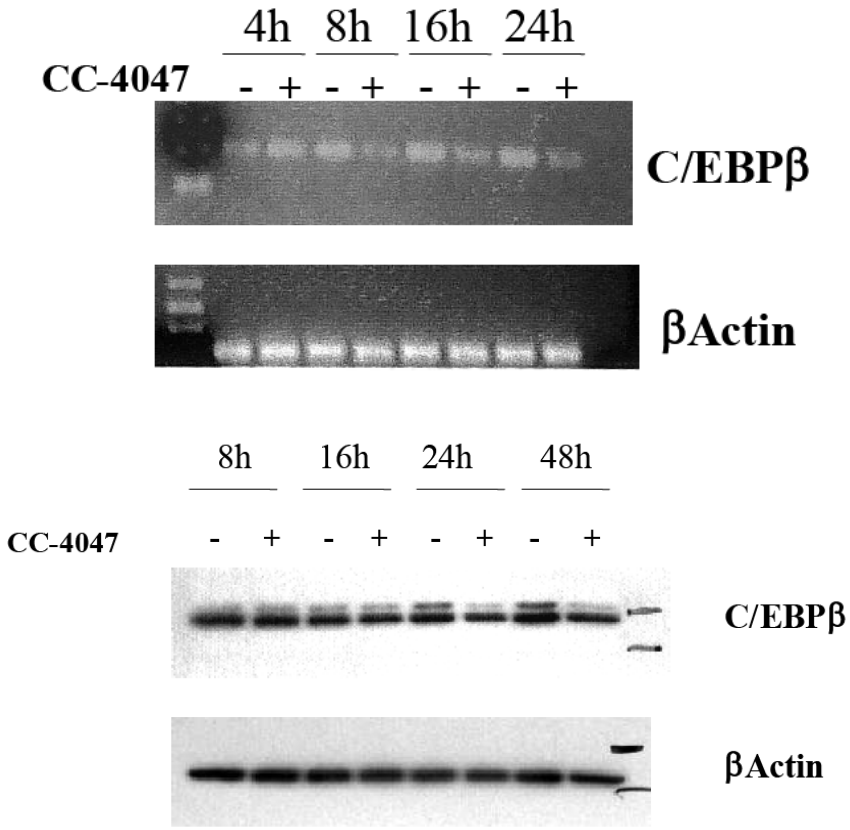


Levels of CDK4, CDK6 and Rb phosphorylation was further determined in two responsive multiple myeloma cell lines, MM-1S and H929, and two non-responsive multiple myeloma cell lines, U-266 and RPMI-8266. Down regulation of CDK4 and CDK6 and reduced Rb phosphorylation was observed only in the responsive multiple myeloma cell lines MM-1S and H929 (Figure 9).

CCAAT-enhancer-binding proteins (or C/EBPs) are a family of transcription factors, composed of six members called C/EBP α to C/EBP ζ that are involved in dimerization and DNA binding. They promote the expression of certain genes through interaction with their promoter. C/EBPβ function is regulated via multiple mechanisms: phosphorylation, acetylation, activation and repression via other transcription factors, oncogenic elements or chemokines, and autoregulation among others. Phosphorylation of C/EBPβ can have an activation or a repression effect. CC-4047 greatly decreased C/EBPβ mRNA expression and protein levels within 24 hours (Figure 10).

Figure 10: Decrease in C/EBPβ mRNA (top panel) and protein (bottom panel) Levels in DF-15 multiple myeloma cells after CC-4047 treatment

(excerpted from Applicant's submission)



Study title: Effects of lenalidomide, pomalidomide, and CC-122 on multiple myeloma cell lines that have been made resistant to lenalidomide or pomalidomide

Study No.:	7596-01
Report Date:	August 18, 2011
Study report location:	eCTD 4.2.1.1
Conducting Laboratory:	Celgene Corporation (Summit, NJ)
GLP:	No

Introduction
Resistance to multiple drugs and combinations often occurs in the later course of multiple myeloma. Resistance in an incurable hematological malignancy may be overcome with new drugs and/or combinations that re-sensitize the tumor. Clinical data suggested that the pomalidomide-dexamethasone combination is active in relapsed/refractory multiple myeloma patients who have previously been treated with lenalidomide-dexamethasone. The objective of this study was to evaluate the direct anti-proliferative effects of CC-5013 (lenalidomide), CC-4047 (pomalidomide), CC-2001 (thalidomide), and CC-122 (listed as a pleiotropic pathway modulator in clinical development by Celgene; no additional information on the drug was provided) on cells continuously grown in CC-5013 or CC-4047 that have become moderately or highly resistant to those agents. KMS-12-BM and H929 myeloma cell lines were continuously grown with lenalidomide (CC-5013; 1 μM), pomalidomide (CC-4047; 0.1 μM), or DMSO (0.01%) for 3 or 4 months, respectively.

Key Study Finding
- Combination treatment of CC-4047 (pomalidomide) with dexamethasone reduced the growth of lenolidamide-resistant and CC-4047-resistant myeloma cells.

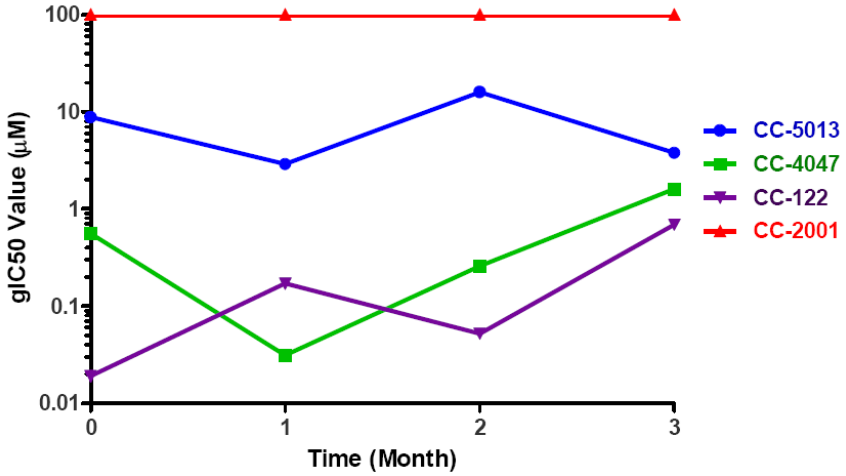
Cell growth assays
Cell proliferation was evaluated using the Cell Titer-Glo Luminescent Cell Assay (Promega, Madison, WI). The method quantifies the amount of ATP present, which is directly proportional to the number of cells present in culture. Percent inhibition of cell growth was calculated by averaging all triplicates, subtracting original ATP counts from Day 1 and normalizing to the DMSO control (0% inhibition) and to 1 μM staurosporine control (100% inhibition). Resistance Ratios (RR) were calculated by dividing the gIC_{50} of the indicated compounds in the continuously cultured cells by the gIC_{50} in the parental cells prior to continuous exposure to either CC-5013 or CC-4047.

KMS-12-BM and H929 myeloma cell lines were continuously cultured with 1 μM CC-5013 to make CC-5013-resistant cells, 0.1 μM CC-4047 to make CC-4047-resistant cells or 0.01% DMSO for control. Resistant cells were treated for 3 days with CC-5013, CC-4047 or CC-2001 at 100, 10, 1, 0.1, 0.01, 0.001, 0.0001 and 0 μM, or with CC-122 or Staurosporine at 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001, or 0 μM, in a final concentration of 0.25% DMSO for all samples. Staurosporine is an alkaloid isolated from the culture broth of Streptomyces stauroporine and used as a cell permeable protein kinase C and other kinases inhibitor. Combination treatment with dexamethansone was performed with the same concentrations of CC-5013, CC-4047, or CC-2001 with constant dexamethasone concentrations at either 10 nM or 100 nM.

KMS-12-BM cell line
Initial growth inhibition with different potencies remained relatively constant over a three month period except for CC-122, in which resistance went up 36-fold (Figure 11).

Figure 11: Growth inhibition of KMS-12-BM-control cells

(excerpted from Applicant's submission)



KMS-12-BM-CC-5013 resistant cells (lenalidomide-resitant cells) showed partial responses to the combination of CC-4047 (pomalidomide)-dexamethasone with a gIC_{50} of 0.18 µM by month 2 (Table 1, top panel). The combination of CC-5013 (lenalidomide)-dexamethasone had partial responses for the first two months but was ineffective by month 3, and the combination of CC-2001 (thalidomide)-dexamethasone did not overcome the resistance in KMS-12-BM-CC-5013 resistant cells.

KMS-12-BM-CC-4047 resistant cells (pomalidomide-resistant cells) had partial responses to the combination of CC-4047-dexamethasone with a gIC_{50} of 0.17 µM by month 3 (Table 1, bottom panel). The combination of CC-5013 (lenalidomide)-dexamethasone had partial responses for the first two months but was ineffective by month 3, and the combination of CC-2001 (thalidomide)-dexamethasone did not overcome the resistance in KMS-12-BM-CC-4047 resistant cells.

Table 1: Time course of gIC50 values of KMS-12-BM-CC-5013 (top) and KMS-12-BM-CC-4047 (bottom) resistant cells

(excerpted from Applicant's submission)

Test Article	KMS-12-BM-Control cells (gIC_{50} [µM])	KMS-12-BM-CC-5013-Resistant Cells (gIC_{50} [µM])		
		Month 1	Month 2	Month 3
CC-5013	8.8	>100	>100	>100
CC-4047	0.56	12.3	4.9	5.7
CC-2001	>100	>100	>100	>100
CC-122	0.019	0.56	0.38	0.81
CC-5013 + Dex (100 nM)	3.3	6.8	4.8	>100
CC-4047 + Dex (100 nM)	0.0019	0.099	0.18	48.3
CC-2001 + Dex (100 nM)	0.22	>100	>100	>100
Stauro	0.096	0.0035	0.00040	0.0012

Dex = dexamethasone; Stauro = Staurosporine.
gIC_{50} = 50% inhibition of growth after 72 hour drug treatment. KMS-12-BM Original cells gIC_{50} values were obtained prior to cell growth in CC-5013.
Combined results for monthly cell growth assays (6 experiments; n = 1 for the original, Month 1 and Month 2 gIC_{50}s; n=3 for Month 3 gIC_{50}s) are summarized.

Test Article	KMS-12-BM-Original cells (gIC_{50} [µM])	KMS-12-BM CC-4047-Resistant Cells (gIC_{50} [µM])		
		Month 1	Month 2	Month 3
CC-5013	8.8	>100	48.2	>100
CC-4047	0.56	0.20	0.41	6.5
CC-2001	>100	>100	>100	>100
CC-122	0.019	0.085	0.014	2.1
CC-5013 + Dex (100 nM)	0.55	30.5	54.4	>100
CC-4047 + Dex (100 nM)	0.0013	0.46	0.19	0.17
CC-2001 + Dex (100 nM)	0.22	>100	>100	>100
Stauro	0.096	0.008	0.0020	0.0013

Dex = dexamethasone; Stauro = Staurosporine
gIC_{50} = 50% inhibition of growth after 72 hour drug treatment. KMS-12-BM Original gIC_{50} values were obtained prior to cell growth in CC-4047.
Combined results for monthly cell growth assays (6 experiments; n = 1 for the original, Month 1 and Month 2 gIC_{50}s; n=3 for Month 3 gIC_{50}s) are summarized.

H929 cell line

H929-CC-5013 resistant cells (lenalidomide-resistant cells) had partial responses to the combination CC-4047-dexamethasone with a gIC50 of 0.048 µM by month 3 (

Table 2, top panel), but its resistance increased and became ineffective by month 4 when compared to the gIC50 in H929-control cells. The combination of CC-2001 (thalidomide) and CC-5013 (lenalidomide) with dexamethasone had partial responses for the first and three months, respectively.

Table 2: Time course of gIC50 values of H929-CC-5013 (top) and H929-CC-4047 (bottom) resistant cells

(excerpted from Applicant's submission)

Test Article	H929-Control cells (gIC_{50} [µM])	H929-CC-5013-Continuously Cultured Cells (gIC_{50} [µM])			
	Naïve	Month 1	Month 2	Month 3	Month 4
CC-5013	0.024	1.5	35.5	65.4	>100
CC-4047	0.0026	0.089	2.1	0.82	11.3
CC-2001	0.37	>100	>100	>100	>100
CC-122	0.0017	0.011	0.52	0.67	2.7
CC-5013 + Dex (100 nM)	0.0037	0.068	56.5	3.7	>100
CC-4047 + Dex (100 nM)	0.0016	0.0023	0.082	0.048	3.9
CC-2001 + Dex (100 nM)	0.0018	0.88	>100	>100	>100
Stauro	0.00012	0.044	0.00055	0.00011	0.00014

Dex = dexamethasone; Stauro = Staurosporine

gIC_{50} = 50% inhibition of growth after 72 hour drug treatment. H929 Original gIC_{50} values were obtained prior to cell growth in CC-5013.

Combined results for monthly cell growth assays (6 experiments; n = 1 for the original, Month 1 and Month 2 gIC_{50}s; n=3 for Month 3 gIC_{50}s) are summarized.

Test Article	H929-Original cells (gIC_{50} [µM])	H929-CC-4047-Continuously Cultured Cells (gIC_{50} [µM])			
		Month 1	Month 2	Month 3	Month 4
CC-5013	0.024	0.66	60.5	40.4	>100
CC-4047	0.0026	0.057	1.8	7.9	17.9
CC-2001	0.37	20.7	>100	>100	>100
CC-122	0.0017	0.083	0.15	0.92	1.2
CC-5013 + Dex (100 nM)	0.0037	0.011	1.1	0.15	36.6
CC-4047 + Dex (100 nM)	0.0016	0.00077	0.029	0.054	0.67
CC-2001 + Dex (100 nM)	0.0018	0.010	>100	10.2	>100
Stauro	0.00012	0.088	0.0011	0.00013	0.00022

Dex = dexamethasone; Stauro = Staurosporine.

gIC_{50} = 50% inhibition of growth after 72 hour drug treatment. H929 Original gIC_{50} values were obtained prior to cell growth in CC-4047.

Combined results for monthly cell growth assays (6 experiments; n = 1 for the original, Month 1 and Month 2 gIC_{50}s; n=3 for Month 3 gIC_{50}s) are summarized.

H929-CC-4047 resistant (pomalidomide-resistant) cells had partial responses to the combination of CC-4047-dexamethasone with a gIC_{50} of 0.67 µM by month 4 (Table 2, bottom panel). The combination of CC-5013 (lenalidomide) with dexamethasone had partial responses for the first 2 months, and the combination of CC-2001 (thalidomide) with dexamethasone did not overcome the resistance in KMS-12-BM-CC-4047 resistant cells.

Study title: Evaluation of anti-proliferative effect of CC-4047 in cellular models of multiple myeloma.

Study No.:	1785-4047
Report Date:	August 30, 2011
Study report location:	eCTD 4.2.1.1
Conducting Laboratory:	Celgene Corporation (San Diego, CA)
GLP:	No

Introduction

Lenalidomide is an orally active immunomodulatory and anti-proliferative agent with activity in hematologic disorders such as myelodysplastic syndromes and multiple myeloma. Most multiple myeloma patients eventually relapse or become refractory to their therapeutic regimens including lenalidomide. The objective of this study was to evaluate the use of CC-4047 alone or in combination with low-dose dexamethasone in the treatment of CC-5013 (lenalidomide)-sensitive and –resistant multiple myeloma cell lines.

Key Study Findings

- Both CC-5013 (lenalidomide) and CC-4047 (pomalidomide) inhibited cell proliferation of H929-CC-5013-sensitive (lenalidomide-sensitive) cell lines characterized by G_1 cell cycle arrest.
- Only CC-4047 (pomalidomide) inhibited cell proliferation of H929-CC-5013-resistant (lenalidomide-resistant) cell lines and the combination treatment of CC-4047 with dexamethasone was synergistic in both H929-CC-5013-sensitive and -resistant cell lines.
- Results suggest that the CC-4047 and dexamethasone combination may be considered for treatment of lenalidomide-resistant cases.

Anti-proliferative and viability (tumoricidal) effects in multiple myeloma cell lines *in vitro*

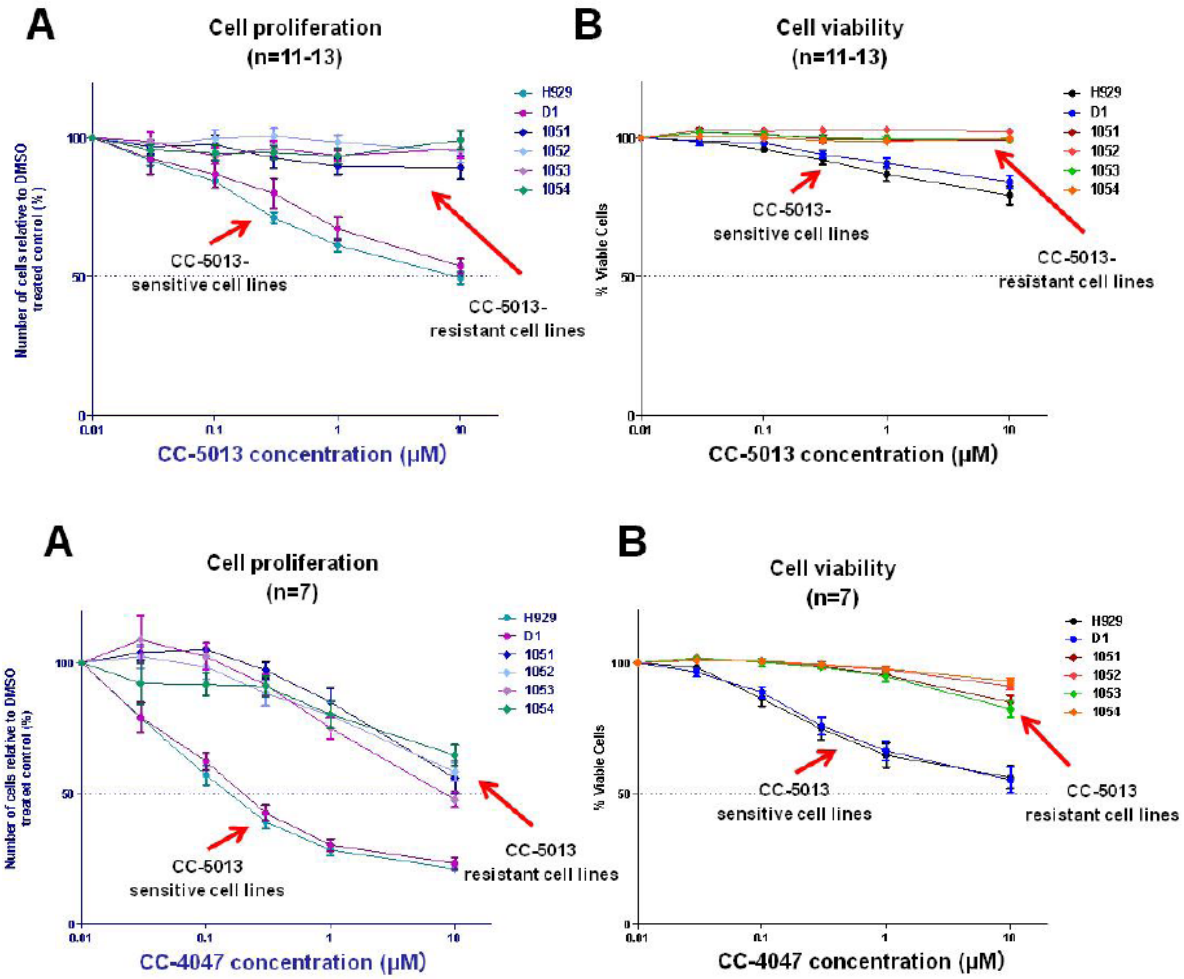
H929 cells were treated with either CC-4047 or CC-5013, and the cell count or percent of viable cells relative to vehicle (DMSO) was measured after 5 days. Results from seven to thirteen independent experiments were pooled and showed that the effects of CC-4047 on proliferation and viability in H929 multiple myeloma cells were greater than the effects of CC-5013.

CC-5013-sensitive (H929 and D1) and -resistant (1051, 1052, 1053, and 1054) cells were treated with serial dilutions of CC-5013 or CC-4047 for 5 days and then cell

proliferation and viability were evaluated. CC-5013 (lenalidomide) did not have an anti-proliferative or tumoricidal effect while CC-4047 showed anti-proliferative effects but no tumoricidal effects in H929-resistant cell lines were detected as shown in Figure 12.

Figure 12: CC-5013 had no effects (upper panels) and CC-4047 inhibited cell proliferation (bottom panels) on H929-CC-5013-resistant cell lines

(excerpted from Applicant's submission)



The combination of dexamethasone with CC-4047 (pomalidomide) but not with CC-5013 (lenalidomide) had strong synergistic effects in H929-sensitive and -resistant cell lines.

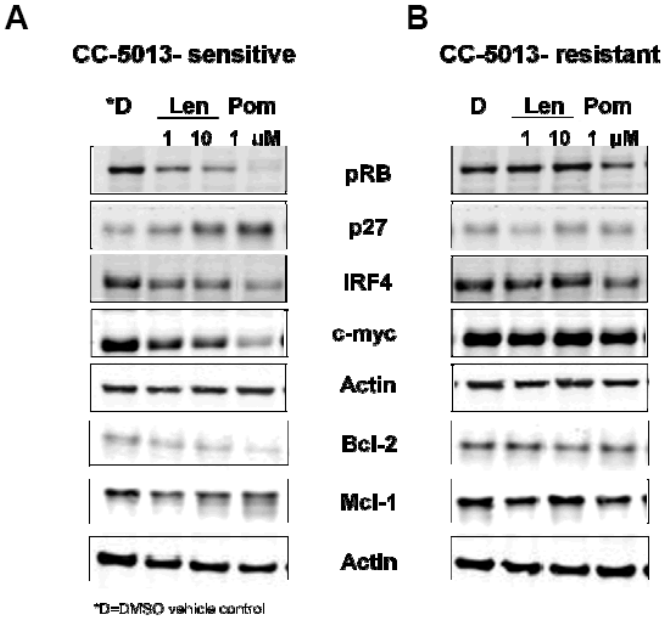
Pomalidomide molecular mechanisms of action in H929 multiple myeloma cell line

Treatment of H929 cells with CC-4047 (1 μM) and CC-5013 (1 and 10 μM) for 48 hours caused a reduction in pRB1 phosphorylation and the expression of IRF4, Bcl-2 and the transcription factor c-Myc and an increase in the expression of p27 in CC-5013-sensitive cells. The effects were more evident after treatment with CC-4047 when

compared to treatment with CC-5013. CC-4047 had a reduced effect on these proteins in H929-CC-5013-resistant cell lines (Figure 13). Pomalidomide induced G_1 cell cycle arrest by reducing phosphorylated retinoblastoma protein 1 (pRB1) phosphorylation, the expression of interferon regulatory factor 4 (IRF-4), B-cell lymphoma 2 (Bcl-2), myeloid cell leukemia sequence 1 (Mcl-1), and c-Myc and increasing the expression of p27.

Figure 13: Effects of pomalidomide and lenalidomide on cell cycle and apoptotic proteins in H929-CC-5013-sensitive and -resistant cell lines

(excerpted from Applicant's submission)



The combination of CC-4047 with dexamethasone induced a synergistically decreased expression in pRB1 phosphorylation, elevated p27 expression and decreased IRF-4 expression in both H929-CC-5013-sensitive and -resistant cell lines, which is consistent with an increase in G_1 cell cycle arrest induced by CC-4047. In addition, the CC-4047-dexamethasone treatment synergistically decreased anti-apoptotic factors Survivin and Bcl2 and increased the expression of pro-apoptotic factor BIM after 48 hours of treatment. These effects were reduced after treatment with CC-4047 alone and were not existent after treatment with CC-5013 alone or in combination with dexamethasone.

Study title: Screening of IMiDs® and PDE4 inhibitor compounds for anti-angiogenic activity in the human umbilical cord vessel ring assay

Study No.:	5127-132
Report Date:	June 28, 2006
Study report location:	eCTD 4.2.1.1
Conducting Laboratories:	Celgene Corporation (Summit, NJ)
GLP:	No

Introduction

Inhibition of endothelial cell growth, adhesion and migration, and growth factor expression are recognized anti-angiogenic targets. The objective of this study was to determine the anti-angiogenic activity *in vitro* of several IMiDs including thalidomide and lenalidomide, and thalidomide analogues in development (pomalidomide and CC-10015).

Key Study Findings

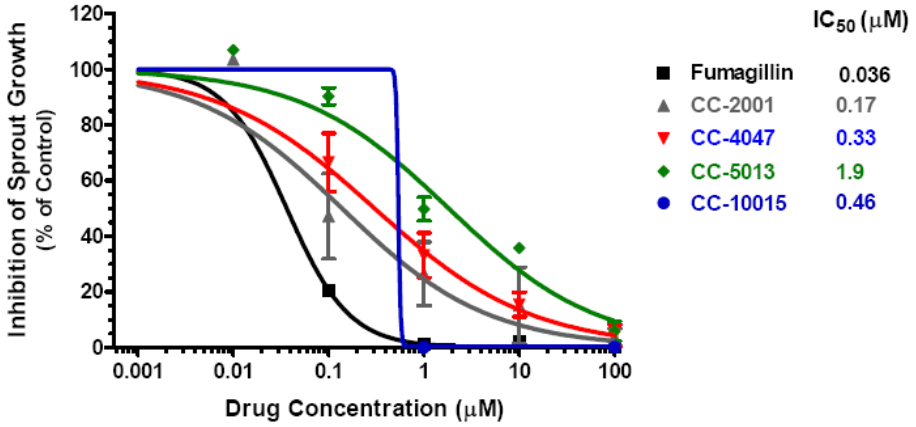
- The order of anti-angiogenic activity among the IMiDs tested was CC-2001/thalidomide (IC_{50} = 0.17 µM) > CC-4047/pomalidomide (IC_{50} = 0.33 µM) > CC-10015 (IC_{50} = 0.46 µM) > CC-5013/lenalidomide (IC_{50} = 1.9 µM).
- At the highest concentration tested (100 µM), CC-2001, CC-4047, CC-10015, and CC-5013 completely inhibited sprout formation.

Fresh human umbilical cord arteries were cleaned of connective tissue, vessel rings were cut cross-wise in a length of 1 mm and vessel rings were placed in the wells such that the lumen was horizontally oriented. After 24 hours of incubation, the rings were treated either with 0.1% DMSO as a control, the anti-angiogenic agent fumagillin (1 µg/ml) as a control, and the test compounds. Culture medium was changed twice per week for four weeks. The experiments were done in triplicates and the results are the average of three rings of the same experiment. Angiogenesis was quantified and compared to control cultures after 28 days. Test compounds were assayed for the ability to inhibit or enhance the growth of microvessels (sprout formation) as an indication of anti- angiogenic or pro-angiogenic activity.

Based on the calculated IC_{50}s, CC-4047 (pomalidomide) had >5-fold activity than CC-5013 (lenalidomide) as an anti-angiogenic agent in this assay (Figure 14). All PDE4 inhibitors inhibited sprout formation in a concentration-dependent manner. Hydrolysis products of lenalidomide were the least active inhibitors.

Figure 14: CC-4047 displayed inhibitory activity in the human angiogenesis assay

(excerpted from Applicant's submission)



Study title: Effect of test compounds on human myeloid, erythroid and megakaryocytic progenitors

Study No.:	CGN-04
Report Date:	June 28, 2006
Study report location:	eCTD Section 4.2.1.1
Conducting Laboratory:	(b) (4)
GLP:	No

Introduction

Severe bone marrow suppression effects (myeloid, erythroid and megakaryocytic progenitors) of cancer treatments increase the potential for infection and hemorrhagic complications. *In vitro* clonogenic assays are useful for evaluation of a compound's toxicity to the hematopoietic system by measuring the degree of inhibition of colony formation that results from drug exposure. In this study, clonogenic progenitors of the human erythroid (CFU-E, BFU-E), granulocyte-monocyte (CFUGM) and multipotential (CFU-GEMM) lineages were assessed in a semi-solid methylcellulose-based medium containing recombinant human (rh) SCF (50 ng/mL), rhIL-3 (10 ng/mL), rhGM-CSF (10 ng/mL), and rhEpo (3 U/mL).

Key Study Findings

- Pomalidomide inhibited proliferation of erythroid progenitor cells with an IC_{50} of 0.07 µM
- Pomalidomide did not inhibit myeloid progenitor proliferation in this study.

Test compounds were prepared in DMSO at 10, 1.0, 0.1, 0.01 and 0.001 µM using 5-fluorouracil at 1.0, 0.1 and 0.01 µg/mL as a positive control for progenitor proliferation (inhibition of colony growth). Test compounds were pomalidomide (CC-4047), another thalidomide analogue in development (CC-10015), and other compounds in early drug development/screening (b) (4) type of drugs not provided).

Table 3: Inhibition values of test compounds on human erythroid, myeloid and megakaryocyte progenitor proliferation

(excerpted from Applicant's submission)

Test Compound	Erythroid IC_{50} (µM)	Myeloid IC_{50} (µM)	Mk IC_{50} (µM)
CC-10015	0.06 ± 0.02	N/A	>10
(b) (4)			
CC-4047	0.07 ± 0.01	N/A	>10
(b) (4)			

MK = megakaryocyte.
Data represents the IC_{50} value ± 1 standard error (where possible).
CC-4047= pomalidomide; CC-10015 is a thalidomide analogue in development
(b) (4) are coumpounds in early drug development or screening

CC-10015, (b) (4) CC-4047, (b) (4) inhibited erythroid progenitor proliferation at various concentrations ranging from 0.01 – 10 µM, but did not inhibit myeloid progenitor proliferation even at the highest test concentration (Table 3). (b) (4)

Study title: Effect of CC-4047 on CD34+ hematopoietic progenitor differentiation and maturation to dendritic cells (DC)

Study No.:	1110-028
Report Date:	April 19, 2006
Study report location:	eCTD 4.2.1.1
Conducting Laboratory:	Celgene Corporation (San Diego, CA)
GLP:	No

Introduction

Modulation of myeloid specific lineages can affect immunological response to cancer and transplantation. In this study, human CD34+ cells were differentiated into myeloid dendritic cell (DC) progenitors by culture with growth factors in the presence or absence of IMiDs to evaluate their role in the differentiation pathway of myeloid cells.

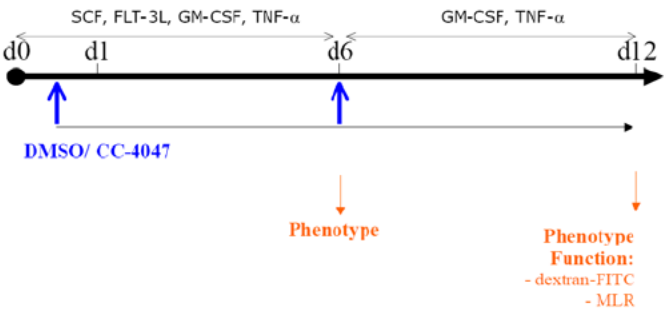
Key Study Findings

- IMiDs tested increased early hematopoietic progenitors (CD34+CD38- population) and modulated subsequent myeloid differentiation.
- IMiDs tested blocked the generation of DC progenitors and increased differentiation of CD34+ cells into the granulocytic lineage.
- The inhibitory effect of IMiDs on CD34+ cell-differentiation to DC was not due to an increased sensitivity of DC progenitors to apoptosis.

To study the effect of CC-4047 on the generation of DC, CD34+ progenitor cells were cultured with or without CC-4047 (1 µM) for a period of 12 days during the expansion and maturation phase (Days 1 to 12) or a period of 6 days during the maturation phase (Days 6 to 12; Figure 15).

Figure 15: Study design of CC-4047 effects on CD34+ differentiation and maturation to DC cells

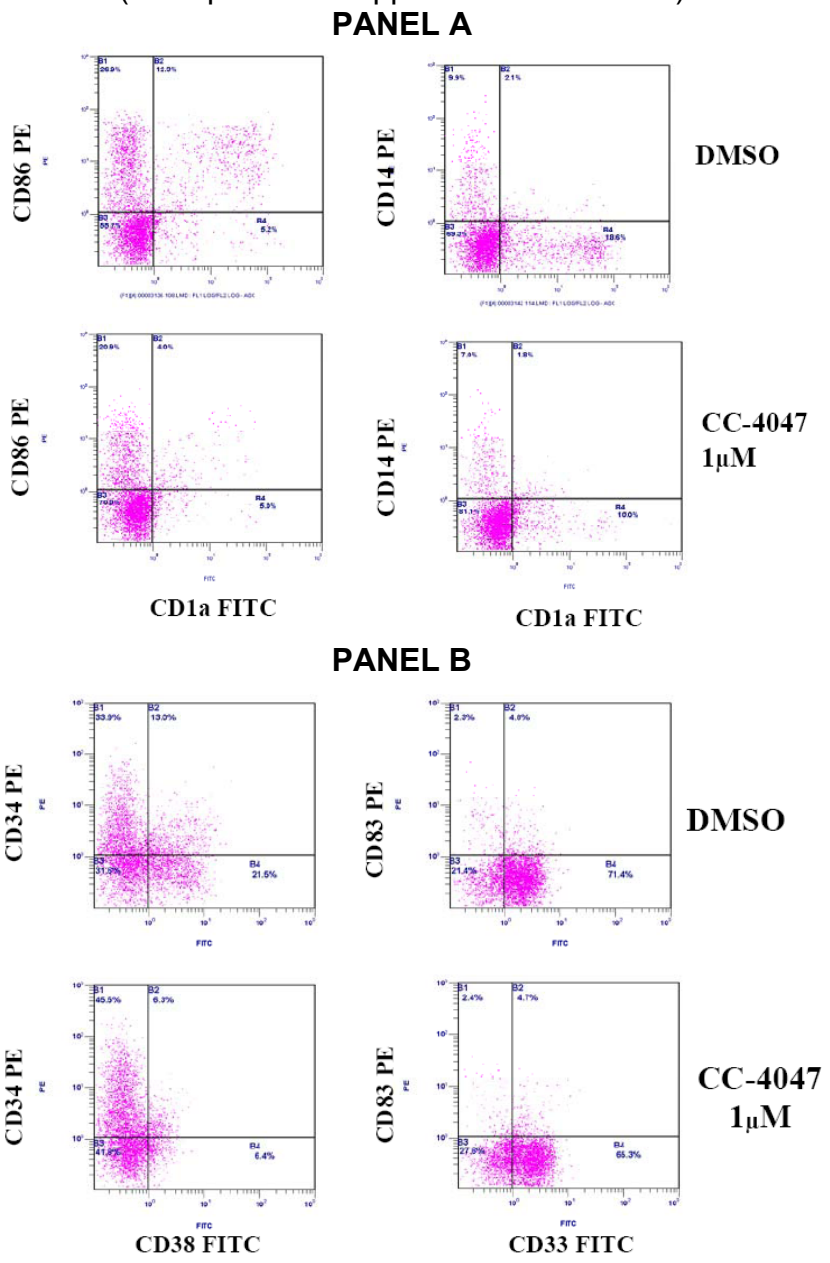
(excerpted from Applicant's submission)



The presence of CC-4047 at 1 µM from Day 1 to 12 (expansion and maturation phases) inhibited the acquisition of the dentritic cell phenotype that occurred in the absence of pomalidomide (Figure 16, Panel A). Additionally, the CD34+CD38- population increased and attenuated the differentiation of CD34+CD38- cells into CD34+CD38+ cells (Figure 16, Panel B). CC-4047-treated CD34+ cells acquired CD33 myeloid marker and presented the phenotype of CD34+CD38- and CD33+ cells at Day 6.

Figure 16: Phenotypic characterization at Day 6 of dentritic cells generated from CD34+ progenitor cells cultured with pomalidomide from Day 1

(excerpted from Applicant's submission)

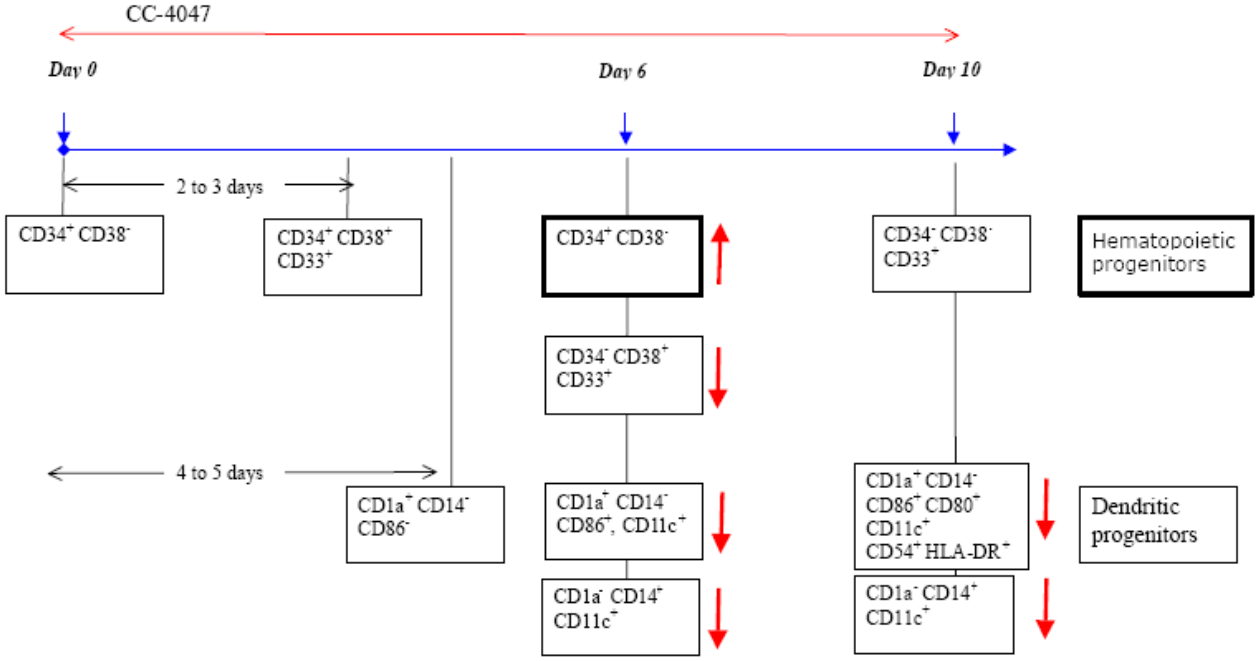


CC-4047 concentration-dependent increase in early progenitor population (CD34+CD38- cells) and the block in the myeloid DC progenitors (CD1a+CD14- and CD1a-CD14+ cells) reached a maximum at 1 µM. The effects were reversible and the interference with CD34+ differentiation pathways was observed only after at least 3 days of culture with CC-4047 (Figure 17).

CC-4047 decreased the phagocytic capacity of cells derived from CD34+ progenitors when added to the culture from Day 1 to 6 but it did not have the same effect when added to the culture from Day 6 to 12 (Figure 17). CC-4047 reduced the antigen presentation capacity of CD34+ cells when stimulation of T cells was reduced.
A positive correlation between the capacity of IMiDs to block DC differentiation (CD1a, CD14) and the ability to inhibit TNF-α was observed. TNF-α inhibition is a required component in culture for the differentiation and maturation of DC cells from CD34 progenitors.

Figure 17: Effects of CC-4047 on the differentiation pathway of CD34+ hematopoetic stem cells cultured in the presence of GM-CSF and TNF-α

(excerpted from Applicant's submission)



IMiDs increased early hematopoietic progenitors (CD34+CD38- population) and modulated lineage-specific expression and subsequent myeloid differentiation (Table 4).

Table 4: Summary of IMiDs profile in the CD34 myeloid dentritic cells differentiation assay

(excerpted from Applicant's submission)

	PBMC (LPS) **TNF-α IC50 (μM)**	T cells (OKT3) **IL-2 EC50 (μM)**	**expansion early progenitors** CD34+	**decrease erythroid precursors** CD36+	**Block DC differentiation** CD1a+ CD14+	**Increase erythroid marker** CD235a+	**Increase erythroid marker** CD71+
CC-4047	0.013	0.0084	++	-	++	++	-
CC-5013	0.1	0.153	++	-	+	+	-
(b) (4)	0.04	0.022	++	-	++	++	-
	8.7	0.037	++	-	+	+	-
	0.00724	11.5	++	+	++	-	+
	>100	>100	+	-	-	+	-
	>100	>100	-	-	-	-	-

Study title: Multiple cytokine and chemokine profiling for IMiDs CC-5013, CC-4047, CC-2001, CC-11006 and CC-10015 in LPS-stimulated human PBMC

Study No.: 5374-10
Report Date: August 31, 2005
Study report location: eCTD 4.2.1.1
Conducting Laboratory: Celgene Corporation (Warren, NJ)
GLP: No

Introduction

Cytokines are important immunoregulatory molecules produced by many cell populations. The anti-inflammatory activity profile of five IMiDs was evaluated using the Lipopolysaccharide-stimulated human Peripheral Blood Mononuclear Cells cytokine and chemokine production system (LPS-stimulated PBMCs). Thalidomide, lenalidomide, and pomalidomide were evaluated along with two other thalidomide analogues that are in drug development (CC-11006 and CC-10015).

Key Study Findings

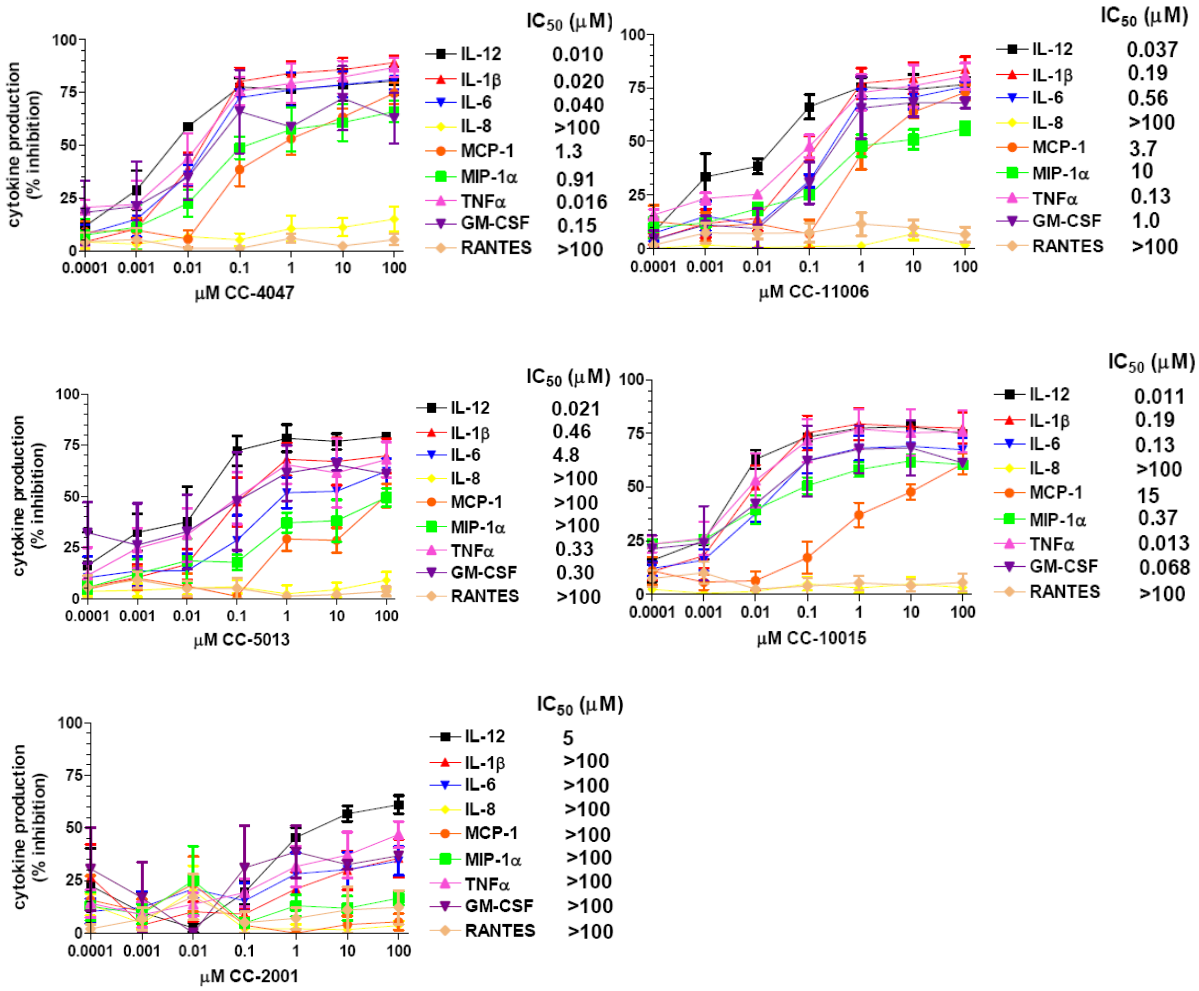
- IMiDs CC-5013 (lenalidomide), CC-4047 (pomalidomide), CC-11006 and CC-10015 inhibited IL-12, IL-1β, IL-6, TNF-α, and GM-CSF production.
- CC-4047 inhibited MCP-1 and MIP-1α while CC-5013 had a modest effect.
- All compounds tested enhanced IL-10 production but CC-2001 showed the least activity.
- None of the compounds tested had an effect on IL-8 or RANTES production.

Peripheral blood mononuclear cells (PBMCs) were isolated from three or four donors using Ficoll-Paque Plus (Amersham Bioscience). After re-suspension in media, PBMCs were treated with test compounds or DMSO as control and then stimulated with LPS.

Samples were analyzed for 10 cytokines (IL-12, IL-1β, IL-6, IL-8, MCP-1, MIP1α, TNFα, GM-CSF, RANTES and IL-10) using a Luminex IS100 (Linco Research). CC-5013, CC-4047, CC-11006 and CC-10015 inhibited IL-12, IL-1β, IL-6, TNF-α, and GM-CSF production. CC-4047, CC-11006 and CC-10015 inhibited MCP-1 and MIP-1α production. CC-5013 displayed modest inhibition of MCP-1 and MIP-1α production in the concentration range tested. CC-2001 has a modest inhibitory effect on IL-12 production. None of the tested compounds had an effect on IL-8 or RANTES production (Figure 18).

Figure 18: Inhibition of cytokine and chemokine production in LPS-stimulated PBMCs by various IMiDS

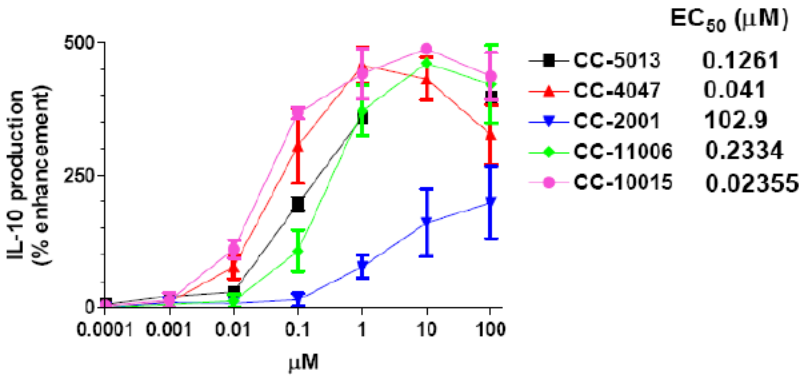
(excerpted from Applicant's submission)



All compounds tested enhanced IL-10 production but CC-2001 showed the least activity (Figure 19).

Figure 19: IMiDs enhance IL-10 production in LPS-stimulated PBMCs

(excerpted from Applicant's submission)



Study title: Evaluation of lenalidomide and pomalidomide using SCID-hu models of multiple myeloma

Study No.:	[redacted]-03162010
Report Date:	March 10, 2010
Study report location:	eCTD 4.2.1.1
Conducting Laboratory:	[redacted]
GLP:	No

Introduction

Severe combined immunodeficiency mice (SCID mice) have reduced ability to reject allogeneic or xenogeneic tissue grafts, and are therefore, good hosts for human cells and tissues. In this study, the Applicant evaluated the *in vivo* anti-myeloma effects of orally administered lenalidomide and pomalidomide using SCID mice bearing human multiple myeloma tumors (LAGκ-1A, LAGκ-1B, and LAGλ-1).

Key Study Findings

- LAGκ-1A-bearing mice treated with pomalidomide at 10 mg/kg showed lower IgG levels and smaller tumor volumes when compared to vehicle-treated mice.
- Neither lenalidomide nor pomalidomide doses up to 30 or 10 mg/kg/day, respectively, were effective in reducing human IgG levels and tumor volumes in LAGκ-1B- and LAGλ-1-bearing mice.

LAGκ-1A was generated from a female patient with multiple myeloma exhibiting progressive disease after treatment with lenalidomide. SCID mice surgically implanted with LAGκ-1A tumor fragments developed measurable tumors and detectable levels of serum human IgG levels at 7 days post-implantation. LAGκ-1B was generated from the same female patient with multiple myeloma as LAGκ-1A but at a later date after the patient had progressed from treatment with the combination of bortezomib and melphalan. The origin of the LAGλ-1-bearing mice was not specified in the report.

Mice were blindly assigned to treatment groups receiving daily oral doses of either the vehicle control (aqueous carboxymethylcellulose), or lenalidomide or pomalidomide prepared in the vehicle control from days 8 to 56 post tumor implantation.

SCID mice MM model	Dose (mg/kg/day)	
	Lenalidomide	Pomalidomide
LAGκ-1A, LAGκ-1B, and LAGλ-1	1, 3, 10 or 30	0.3, 1, 3 or 10

Pomalidomide treatment at 10 mg/kg/day in LAGκ-1A-bearing mice showed lower human IgG levels and a borderline statistically significant tumor volume growth inhibition when compared to mice receiving vehicle (data not shown in this review).

Lenalidomide doses up to 30 mg/kg/day or pomalidomide doses up to 3 mg/kg/day had mild effects on human IgG levels and tumor volume reduction when compared to vehicle-treated mice.

Neither lenalidomide nor pomalidomide doses up to 30 or 10 mg/kg/day, respectively, were effective in reducing human IgG levels and tumor volumes in LAGκ-1B- and LAGλ-1-bearing mice.

Study title: Evaluation of (b) (4) PB19, and PB20 in combination with rituximab against Raji B human lymphoma in a survival study with C.B-17 SCID mice

Study No.: CLG-4-Raji-B-e200
Report Date: December 14, 2007
Study report location: eCTD 4.2.1.1
Conducting Laboratory: (b) (4)
GLP: No

Introduction

Raji is the first continuous human cell line of hematopoietic origin derived from patients with Burkitt's lymphoma (BL). In this study, SCID mice were given Raji BL cells via a tail-vein injection to induce systemic lymphoma and then treated with rituximab alone or in combination with different IMiDs to evaluate the prolongation of survival provided by different treatments. Pomalidomide and other immunomodulatory compounds in early drug development/screening were tested. The codes for IMiDs were:

(b) (4)	
PB19	CC-4047 (pomalidomide)
PB20	CC-5013 (lenalidomide)

Key Study Findings

- Pomalidomide (PB19) at both doses of 0.5 mg/kg/day and 5 mg/kg/day was associated with median TTE of 103 days corresponding to ILS of 312%. However, since pomalidomide was used in combination with rituximab, the results obtained may be due to the effect of rituximab. Of note, rituximab as a single agent resulted in TTE of 103 days and ILS of 312%.

Study Design

Treatment regimens shown in Table 5 started on Day 3 after mice were inoculated with Raji cells. The survival study was terminated on Day 103 and the endpoints were death, or moribundity / hind limb paralysis.

Efficacy was calculated as percent increase lifespan (ILS) and percent median increase in time to end point (TTE) of drug-treated versus untreated mice. The median TTE of treated mice is expressed as a percentage of the median TTE of the control mice (%T/C). The increase in life-span (ILS) was calculated according to the formula ILS = %T/C – 100%, where T = median $TTE_{treated}$, and C = median $TTE_{control}$. Thus, if T = C, ILS = 0%.

Table 5: Study design for the survival study with Raji BL-inoculated SCID mice

(excerpted from Applicant's submission)

Group	n	Treatment Regimen 1				Treatment Regimen 2			
		Agent	mg/kg	Route	Schedule	Agent	mg/kg	Route	Schedule
1	10	No Treatment	-	-	-	No Treatment	-	-	-
2	10	Rituximab	10	iv	Days 5,10,15,20	Vehicle	0.0001	po	Days 3,4,8,9,13,14,18,19
3	10	Rituximab	10	iv	Days 5,10,15,20	PB19	0.5	po	Days 3,4,8,9,13,14,18,19
4	10	Rituximab	10	iv	Days 5,10,15,20	PB19	5	po	Days 3,4,8,9,13,14,18,19
5	10	Rituximab	10	iv	Days 5,10,15,20	PB20	5	po	Days 3,4,8,9,13,14,18,19
6	10	Rituximab	10	iv	Days 5,10,15,20	(b) (4)		po	Days 3,4,8,9,13,14,18,19
7	10	Rituximab	10	iv	Days 5,10,15,20			po	Days 3,4,8,9,13,14,18,19
8	10	Rituximab	10	iv	Days 5,10,15,20			po	Days 3,4,8,9,13,14,18,19

Drug-related early decedents were assigned a TTE value equal to the day of death. Acceptable toxicity for the MTD was defined as a group mean body weight loss of ≤20% during the test and not more than one treatment-related death among ten animals. Animals were examined for drug-related and adverse clinical signs and a necropsy was conducted to determine the causes. Animals determined to have died from non-drug-related causes were excluded from analysis.

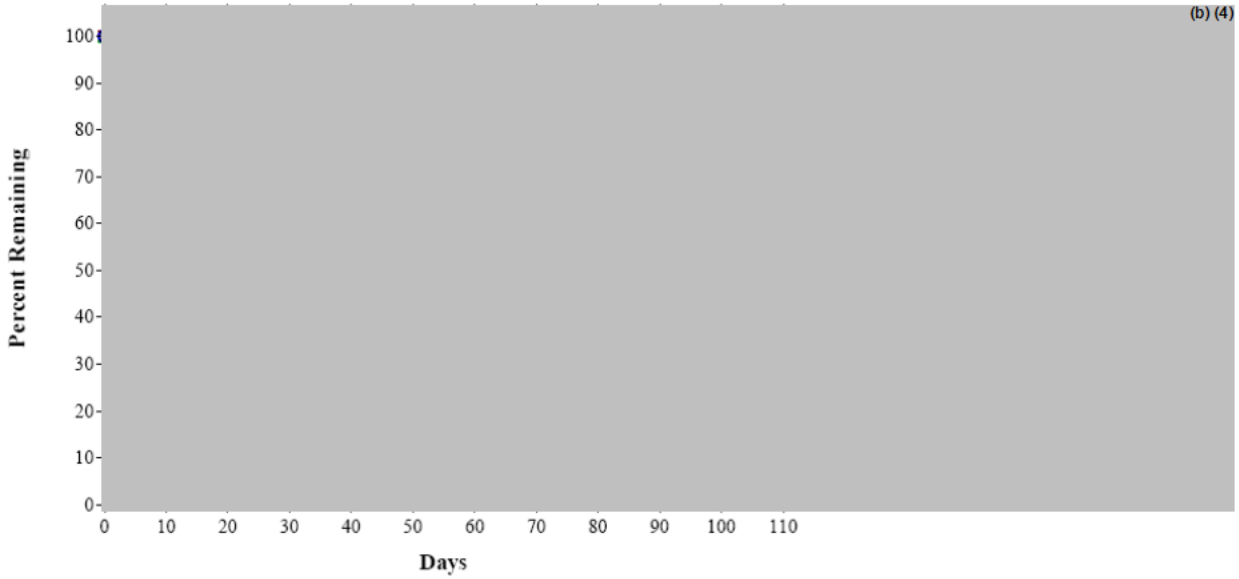
Results

All treatment combinations had activity (Figure 20). The control group had a median survival of 25.0 days. Rituximab had a median TTE of 103.0 days corresponding to ILS of 312%. Pomalidomide (PB19) at both doses of 0.5 mg/kg/day and 5 mg/kg/day was

associated with median TTE of 103.0 days corresponding to ILS of 312%. However, since pomalidimode was used in combination with rituximab, the results may be due to rituximab. Similar results were observed for (b) (4) PB20 had the lowest median TTE with 71.5 days and ILS of 186%. Positive modulation by drugs tested was not possible due to the magnitude of the rituximab response.

Figure 20: Kaplan-Meier plot for the Raji-BL study

(excerpted from Applicant's submission)



Study title: Evaluation of Celgene compounds using *in vivo* angiogenesis matrigel plug assay

Study No.:	(b) (4)-P10.0101
Report Date:	June 17, 2010
Study report location:	eCTD 4.2.1.1
Conducting Laboratory:	(b) (4)
GLP:	No

Introduction

Matrigel matrix provides the substrate necessary for the study of angiogenesis *in vivo*. It is a solubilized basement membrane preparation extracted from a mouse sarcoma, a tumor rich in extracellular matrix proteins. In this study, matrigel matrix was mixed with different growth factors and injected subcutaneously into mice to form a plug. The plug was removed after treatment and analyzed for the formation of blood vessels. IMiDs compounds including CC-5013 (lenalidomide), CC-4047 (pomalidomide) and thalidomide were tested for their potential anti-angiogenic activity. Avastin, Sutent, and AZ 2171 were used as reference compounds in this study; however, these data are not shown because they are not relevant to this indication.

Key Study Findings

- Thalidomide showed no anti-angiogenic activity at either of the doses tested.
- CC-5013 demonstrated significant anti-angiogenic activity at both doses tested.
- CC-4047 showed anti-angiogenic activity only at the highest dose tested.

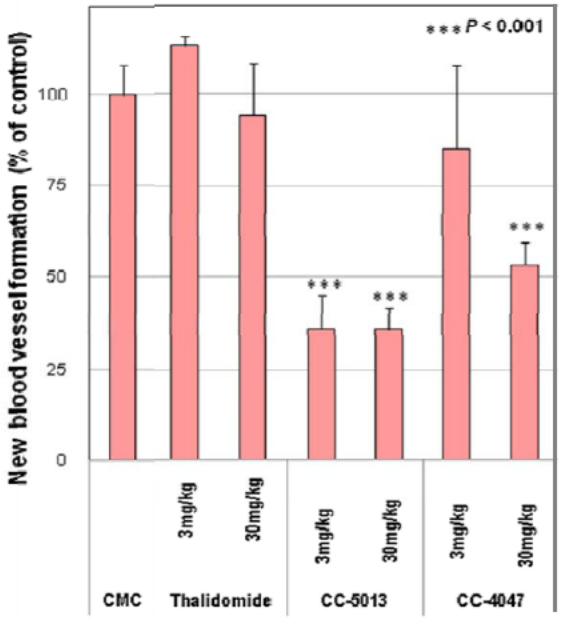
Methods

Growth factor reduced Matrigel Matrix High Concentration was mixed with VEGF, FGF-2 and heparin as angiogenic stimuli and implanted subcutaneously into female C57BL/6 mice bilaterally (in the right and left flanks) with a volume of 0.5 mL per plug. All plugs were collected 10 days later and processed for microvessel analysis. Microsections from the center range of each plug were processed with antigen retrieval and then immunostained with specific antibody against CD31 and counterstained with H&E. Numbers of CD31-positive blood vessels in one entire section of each plug sample were counted under the microscope and compared between drug-treatment groups, the vehicle (CMC) control group and the no treatment control group. Body weight changes in all groups were less than 10%. All compounds were tested at 3 and 30 mg/kg.

Results

Figure 21: Blood Vessel Formation in Matrigel Plugs

(modified from Applicant's submission)



CMC=carboxymethylcellulose CC-5013=lenalidomide CC-4047=pomalidomide

4.2 Secondary Pharmacology

No studies reviewed.

4.3 Safety Pharmacology

Neurological effects:

Study title: Acute oral (gavage) central nervous system (CNS) safety pharmacology study of CC-4047 in rats

Study No.:	CC-4047-TOX-011
Study report location:	eCTD 4.2.1.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 28 2008
GLP compliance:	Yes; US and OECD. Statement included and signed. Protocol Amendment 1 was never finalized nor included in the final report.
QA statement:	Statement included and signed
Drug, lot #, and % purity:	CC-4047, Lot # CMLW-174/06-CC2, Purity: 100.4 %

Key Study Findings

- No neurological effects were observed in Crl:CD(SD) rats after a single oral dose of CC-4047 up to 2000 mg/kg.

Methods

Doses:	0, 250, 1000, or 2000 mg/kg 0, 1500, 6000, or 12000 mg/m^2
Frequency of dosing:	Single dose
Route of administration:	Oral gavage
Dose volume:	12 mL/kg
Formulation/Vehicle:	Aqueous 1% w/v carboxymethylcellulose sodium salt, USP (CMC, medium viscosity)
Species/Strain:	Crl:CD(SD) rat
Number/Sex/Group:	10/sex/group
Age:	~59-61 days old
Weight:	Males: 236-260 g Females: 176-207 g

Results

No CC-4047-related deaths, changes in body weight, or statistically significant or biologically important differences in the measures of behavior, autonomic functions, appearance, grip strength or body temperature were observed at 3 or 24 hours after a single oral dose of 250, 1000, or 2000 mg/kg CC-4047 to Crl:CD(SD) rats. CC-4047-related discoloration of urine (green) was observed in 1/3 male and/or female rats in each dosing group that persisted in just one 2000 mg/kg male at the 24 hour evaluation and during observations on Day 2. This finding was not adverse or dose-dependent. No external gross lesions were identified at necropsy.

Respiratory effects:

Study title: Respiratory assessment following oral gavage administration of CC-4047 to plethysmograph-restrained male Sprague-Dawley rats

Study No.:	CC-4047-TOX-014
Study report location:	eCTD 4.2.1.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 5, 2008
GLP compliance:	Yes; US and OECD. Statement included and signed
QA statement:	Statement included and signed
Drug, lot #, and % purity:	CC-4047, CMLW-174/06-CC2, Purity: 100.4 %

Key Study Findings

- No effects on respiratory function were observed in Crl:CD(SD) male rats after a single oral dose of CC-4047 up to 2000 mg/kg.

Methods

Doses:	0, 250, 1000, or 2000 mg/kg 0, 1500, 6000, or 12000 mg/m^2
Frequency of dosing:	Single dose
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	1% w/v carboxymethylcellulose sodium salt, USP (CMC, medium viscosity) prepared in deionized water
Species/Strain:	Crl:CD(SD) rat
Number/Sex/Group:	8 males/group
Age:	~77 days old
Weight:	327-397 g

Results

No CC-4047-related deaths or effects on respiratory frequency, tidal or minute volume were observed after administration of a single oral dose of 250, 1000 or 2000 mg/kg CC-4047 to Crl:CD(SD) rats. CC-4047-related discoloration of urine (bright yellow) was noted in 1/8, 6/8 and 6/8 males and yellow feces was observed in 1/8, 5/8, and 6/8 males in the 250, 1000, and 2000 mg/kg groups, respectively. These findings were not adverse or dose-dependent. Body weights were measured prior to dosing and were not analyzed for differences compared to control.

Cardiovascular / Respiratory effects:

Study title: CC-4047: Effect on cloned hERG channels expressed in human embryonic kidney (HEK293) cells

Study No.:	CC-4047-TOX-009
Study report location:	eCTD 4.2.1.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 24, 2005
GLP compliance:	Yes; US and OECD. Statement included and signed
QA statement:	Statement included and signed
Drug, lot #, and % purity:	CC-4047, Lot # 61749-06, Purity: >99%

Key Study Findings

- CC-4047 does not block hERG potassium channels *in vitro*, suggesting a low potential to block cardiac Ikr.

Methods

The solubility of CC-4047 in vehicle control (Hepes Buffer-PS with 0.3% DMSO) was determined using nephelometric measurements. Cultured HEK293 cells were stably transfected with hERG cDNA and used to measure hERG current amplitude currents in the patch-clamp assay during application of vehicle control, a positive control (terfenadine - 60 nM) or CC-4047 at 7.9 and 87.5 µM prepared in vehicle control. Solution application sequence included vehicle control to record baseline measurements followed by either terfenadine or CC-4047. A supramaximal concentration of E-4031 (500 nM) was used as a reference substance to eliminate the contribution of HEK293 endogenous outward current to the recordings.

The following definition for a steady state was excerpted from Applicant's submission: "Steady state was defined by the limiting constant rate of change with time (linear time dependence). The steady state before and after test article application was used to calculate the percentage of current inhibited at each concentration."

Results

CC-4047 at 7.9 or 87.5 µM produced less than 1% inhibition of hERG current and the IC_{50} was not determined (Table 6).

Table 6: Percent hERG current by CC-4047 compared to Terfenadine

Drug	Mean % Inhibition	N
Vehicle control	0.3	3
7.9 µM CC-4047	0.8	3
87.5 µM CC-4047	0.9	4
60 nM Terfenadine	78.8	3

Study title: CC-4047: Cardiovascular and respiratory effects in the anaesthetised dog following intravenous infusion

Study No.:	1398-10
Study report location:	eCTD 4.2.1.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 19, 1998
GLP compliance:	Yes; OECD. Statement included and signed
QA statement:	Statement included and signed
Drug, lot #, and % purity:	CC-4047, Lot # 40753-10(40778-24-B), Purity: 99.62 %

Key Study Findings

- CC-4047-related adverse reactions included: an increase in respiratory rate from a pre-dose value accompanied by cardiovascular suppression leading to low values of blood pressure, dP/dt_{max} and femoral artery blood flow in 1 out of 4 dogs treated at 25 mg/kg.
- No CC-4047-related cardiovascular or respiratory effects were observed in anesthetized dogs after intravenous infusion of 2.5 and 10 mg/kg.

Methods

Dogs (2 males and 2 females in each group) were anesthetized and maintained under this condition with propofol and additional infusions of alfentanil to provide a more balanced anesthetic state. Dogs were set with a cuffed endotracheal tube, a rectal probe to monitor body temperature, a pulse oximeter to monitor the status of blood oxygen, an iv cannula in the femoral artery for constant monitoring of blood pressure, a cannula in the femoral vein for pharmacokinetic blood sampling, and a flow probe in the contralateral femoral artery to monitor systemic resistance. A catheter tip transducer was inserted in the left carotid artery so that the tip lay in the left ventricle to measure left ventricular pressure and its derivative dP/dt. Lead II ECG was obtained using subdermal electrodes and the left jugular vein was exposed and ligated for dose administration.

After allowing dogs to stabilize for at least 30 minutes, baseline readings were taken. Vehicle or test article was then infused intravenously at 200 mL per hour via a cannula placed in the jugular vein. Administration of vehicle or test article was given in ascending doses at intervals of at least 30 minutes after the end of the previous infusion (Table 7). Blood samples (1 mL) were taken before dose administration and at 2, 10, 15 and 30 minutes after dose administration. Additional samples were taken at 60, 90 and 120 minutes after administration of the 25 mg/kg dose.

Table 8 lists the hemodynamic and respiratory parameters monitored during the study.

Table 7: Study design with anesthetized dogs

(excerpted from Applicant's submission)

Group number	Group description	Dose number	Dose concentration (mg/mL)	Dose Volume (mL/kg)	Dose Level (mg/kg)	*Approximate Time of Infusion (min)
1	Vehicle treated group (n=4)	1	NA	0.5	NA	1.5
		2	NA	2	NA	6
		3	NA	5	NA	15
2	Test article treated group (n=4)	1	5	0.5	2.5	1.5
		2	5	2	10	6
		3	5	5	25	15

* the exact duration of infusion depended on the weight of the animal; the figures given are for a 10.0 kg animal

Table 8: Hemodynamic and respiratory parameters measured in anesthetized dogs

(excerpted from Applicant's submission)

Parameter	Variable
Arterial Blood Pressure	Systolic, Diastolic and mean blood pressure
Heart Rate	
Left ventricular pressure (LVP)	LVP dP/dt_{max} - Maximum rate of change in LVP. (This is an indicator of the force of ventricular contraction)
Peripheral blood flow	Maximum and mean femoral blood flow and femoral resistance.
Lead II ECG	RR, ST, QRS, PR, QT and QT_c-intervals, and the heights of the R, P and T-waves of the ECG complex
Respiration	Peak inspiratory and expiratory flow, tidal volume, minute volume and rate of respiration

Results

Adverse reactions occurred in a CC-4047-treated female (8F) during the infusion of the high dose of 25 mg/kg and the animal was artificially ventilated on several occasions. This female dog presented with a marked increase in respiratory rate from a pre-dose value of 33 breaths per minute (bpm) to 63 bpm. This was accompanied by cardiovascular suppression leading to extremely low values of blood pressure, dP/dt_{max} and femoral artery blood flow. Dosing was discontinued while all cardiovascular parameters stabilized and resumed with no further clinical signs. Gross pathology examination of the lungs revealed large red patches indicative of possible pulmonary embolus. According to the report, it is possible that these changes may be due to prolonged artificial ventilation rather than the effects of the anesthetics or the test article.

Cardiovascular parameters: Baseline values for systolic, diastolic and mean blood pressure, as well as heart rate and the maximum rate of change of left ventricular pressure, dP/dt_{max}, were similar in both groups. Changes occurring during dosing were of a similar trend and intensity in the control and CC-4047-treated dogs.

Respiratory parameters: Baseline values for peak inspiratory flow (PIF), peak expiratory flow (PEF), tidal volume (TV), minute volume (MV) and respiratory rate were similar in both groups. Changes occurring during dosing were of a similar trend and intensity in the control and CC-4047-treated dogs.

Pharmacokinetics: Analysis was conducted only with samples taken at 30 minutes postdose. Plasma concentration values from the F8 dog in the 25 mg/kg group was from the repeat administration. There was a slightly higher than dose-proportional increase in plasma levels of pomalidomide between 2.5, 10 and 25 mg/kg (Table 9).

Table 9: CC-4047 Plasma Concentrations in Anesthetized Dogs

Animal No.	CC-4047 Doses (mg/kg) 2.5	10	25
M 5	441	2925	8654
M 6	2737*	2197	5168
F 7	500	2772	7574
F 8	364	1791	5348
Mean (ng/mL)	435	2421	6686
N	3	4	4

*Excluded from this analysis

Study title: Cardiovascular safety pharmacology evaluation of CC-4047 administered by oral gavage to telemetry-instrumented conscious cynomolgus monkeys

Study No.:	CC-4047-TOX-012
Study report location:	eCTD 4.2.1.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 13, 2008
GLP compliance:	Yes; USA and OECD. Statement included and signed
QA statement:	Statement included and signed
Drug, lot #, and % purity:	CC-4047, Lot # CMLW-174/06-CC2, Purity: 100.4%

Key Study Findings

- No evidence of undesirable pharmacodynamic effects on cardiovascular function was observed following oral gavage administration of 0.2 or 2.0 mg/kg CC-4047 to cynomolgus monkeys.

- Pulse pressure was slightly lower (~ 10%) at 10.0 mg/kg CC-4047 compared with vehicle control during the first seven hours after treatment.

Methods

Male, naïve and nonnaïve cynomolgus monkeys were dosed with vehicle control [aqueous 1% (w/v) carboxymethylcellulose sodium salt (medium viscosity)] and CC-4047 at 0.2, 2.0, and 10.0 mg/kg (2.4, 24, and 120 mg/m^2) via oral gavage in a Latin square crossover design to evaluate potential effects of CC-4047 on respiration and cardiovascular function. Monkeys were acclimated to the study room and to oral gavage dosing twice with approximately 5.0 mL/kg of water during the predose phase. Each monkey received all treatments and treatment procedures with a 7-day washout interval between doses (Figure 10).

Table 10: Treatment design with jacketed monkeys

(excerpted from Applicant's submission)

Animal	Dose Level Designation on Specified Dosing Days			
Male	Day 1	Day 8	Day 15	Day 25
I08523	Low	Control	High	Mid
I08524	Mid	High	Control	Low
I08525	High	Low	Mid	Control
I08526	Control	Mid	Low	High

Dose Level Designation	Dose Level[a] (mg/kg)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)
Control	0.0[b]	5.0	0.00
Low	0.2	5.0	0.04
Mid	2.0	5.0	0.40
High	10.0	5.0	2.00

[a]Dose levels represent mg/kg of CC-4047.
[b]Control animals (0.0 mg/kg) received an equivalent dose volume of vehicle control article.

Results

No mortality, morbidity, or CC-4047-related clinical signs were observed. Pulse pressure was slightly lower (~ 10%) at 10.0 mg/kg (120 mg/m^2) CC-4047 compared with vehicle control during the first seven hours after treatment (Figure 11). No other CC-4047-related effects were noted for any other hemodynamic or electrocardiographic parameters, heart rate, respiratory rate, qualitative food consumption, body weight, or body temperature.

Table 11: Mean Arterial Pulse Pressure in Jacketed Monkeys

(excerpted from Applicant's submission)

Block 1
(mm Hg)

Dose Level (mg/kg)		Baseline	Time (Hours) 1	2	3	4	5	6	7
0.0	CAM	-	33	30	31	31	29	28	27
	MEAN	29	34	31	31	31	29	29	27
	SD	6.7	9.5	5.9	5.3	7.2	6.4	8.4	7.1
	N	4	4	4	4	4	4	4	4
0.2	CAM	-	32	30	29	30	29	27	26
	MEAN	28	32	29	29	29	28	27	25
	SD	5.6	8.1	7.2	5.8	6.1	4.8	4.8	5.4
	N	4	4	4	4	4	4	4	4
2.0	CAM	-	33	29	29	29	28	29	26
	MEAN	29	33	29	29	29	28	29	26
	SD	5.7	6.4	5.2	5.1	5.5	8.0	7.9	8.0
	N	4	4	4	4	4	4	4	4
10.0[a]	CAM	-	31	29	28	28	27	26	24
	MEAN	29	31	29	28	28	27	26	24
	SD	7.5	10.9	9.4	7.1	7.2	7.3	9.3	8.7
	N	4	4	4	4	4	4	4	4

a Across time points, the grand covariate-adjusted mean arterial pulse pressure values for animals administered 10.0 mg/kg were significantly lower than those of animals administered 0.0 mg/kg.
Grand covariate-adjusted means: 0.0 mg/kg = 30; 0.2 mg/kg = 29; 2.0 mg/kg = 29; 10.0 mg/kg = 27.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption

Study title: A study to determine the oral bioavailability of (b) (4) and CC-4047 in the rat

Study No.:	1398-72
Study report location:	eCTD 4.2.2.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 3, 1997
GLP compliance:	Yes; OECD. Statement included and signed
QA statement:	Statement included and signed
Drug, lot #, and % purity:	CC-4047, Lot # 40563-12-A, Purity: 95%. Drug certificate of analysis was not provided by the sponsor.

Key Study Findings

- Bioavailability of CC-4047 in rats was 13%.
- Mean C_{max} was 3185.7 ng/mL and 4896.7 ng/mL for the intravenous and oral dosing, respectively. C_{max} was reached at 4 hours after oral dosing.

- Mean half-lives occurred at 6.2 and 5.5 hours for the intravenous and oral dosing, respectively. The similar $t_{1/2}$ suggested comparable elimination phases for both routes of administration.

Methods

Doses:	Intravenous (IV): ~2.5 mg/kg (15 mg/m^2) Oral: ~100 mg/kg (600 mg/m^2)
Frequency of dosing:	Single dose
Route of administration:	IV and oral gavage
Dose volume:	2 mL/kg (IV) and 5 mL/kg (oral)
Time points for blood collection:	Predose, 5 (IV only), 10, 20 and 30 min and 1, 2, 4, 6, 8, 12, 24, and 48 hours post-dose
Formulation/Vehicle:	PEG400:Intralipi 20% (1:4 v/v) for IV dosing 1% w/v carboxymethylcellulose for oral dosing
Species/Strain:	Crl:CD®BR(SD) rat
Number/Sex/Group:	69 males; 3 males/time point/CC-4047 dose
Age:	Not specified
Weight:	217 - 360 g

Results

The initial IV dosing for CC-4047 at 10 mg/kg caused severe toxicity including 2 deaths. The dose was reduced to 2.5 mg/kg and administered to all rats assigned to IV dosing. No clinical signs were observed after 100 mg/kg oral or 2.5 mg/kg IV administration. The mean C_{max} was 3185.7 ng/mL and 4896.7 ng/mL for the IV and oral dosing, respectively (Table 12). C_{max} was reached at 4 hours after oral dosing. The similar $t_{1/2}$ suggested comparable elimination phases for both routes of administration. Plasma analysis for (b) (4) was not conducted as the sponsor terminated the development plan for that molecule.

Table 12: Summary of PK parameters for CC-4047 after oral (100 mg/kg) and intravenous (2.5 mg/kg) administration to male Sprague Dawley rats

(excerpted from Applicant's submission)

Dose Route (Dose (mg/kg))	AUC (0-t) (ng.h/mL)	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	Cl_{tot} (mL/h/kg)	V_d (mL/kg)	F (%)
PO (100 mg/kg)	45127.6	4896.7	4.0	5.5	NC	NC	13.0
IV (2.5 mg/kg)	8703.4	3185.7	0.083	6.2	285.5	2564.2	

NC - Not calculated

The bioavailability (F) of CC-4047 in rats was 13%. The absolute bioavailability was calculated as a ratio of areas under the curve according to the formula below:

$F_{abs} = 100 \cdot \frac{AUC_{po} * D_{iv}}{}$ $F_{abs} = 100 \cdot \frac{45127.6 * 2.5 \text{ mg/kg}}{}$ $F_{abs} = 13.0\%$

$AUC_{iv} * D_{po}$	8703.4 * 100 mg/kg

Study title: A study to determine the oral bioavailability of (b) (4) and CC-4047 in the cynomolgus monkey

Study No.:	1398-73
Study report location:	eCTD 4.2.2.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 3, 1997
GLP compliance:	Yes; OECD. Statement included and signed
QA statement:	Statement included and signed
Drug, lot #, and % purity:	CC-4047, Lot # 40563-12-A, Purity: 95%. Drug certificate of analysis was not provided by the sponsor.

Key Study Findings

- Bioavailability of CC-4047 in monkeys was 15%.
- Mean C_{max} was 6930.6 ng/mL and 3714.2 ng/mL for the intravenous and oral dosing, respectively. C_{max} was reached at 2 hours after oral dosing.
- Mean half-lives occurred at 6.7 and 25.0 hours for the intravenous and oral dosing, respectively.

Methods

Doses:	Intravenous (IV): ~10 mg/kg (120 mg/m^2) Oral: ~100 mg/kg (1200 mg/m^2)
Frequency of dosing:	Single dose
Route of administration:	Oral gavage and IV, 2-weeks washout period between doses
Dose volume:	5 mL/kg (oral) and 2 mL/kg (IV)
Time points for blood collection:	Predose, 5 (IV only), 10, 20 and 30 min, and 1, 2, 4, 6, 8, 12, 24, and 48 hours post-dose
Formulation/Vehicle:	1% w/v carboxymethylcellulose for oral dosing PEG400:Intralipi 20% (1:4 v/v) for IV dosing
Species/Strain:	Cynomolgus monkeys (*Macaca fascicularis*)
Number/Sex/Group:	4 males for CC-4047 dosing
Age:	15 - 20 months
Weight:	2.0 – 2.25 kg

Results

No clinical signs were observed after 100 mg/kg oral or 10 mg/kg IV administration. The mean C_{max} was 6930.6 ng/mL and 3714.2 ng/mL for the IV and oral dosing, respectively (Table 13). C_{max} was reached at 2 hours after oral dosing. The mean half-

live occurred at 6.7 and 25 hours for the IV and oral dosing, respectively. Plasma analysis for (b) (4) was not conducted as the sponsor terminated the development plan for that molecule.

Table 13: Summary of PK parameters for CC-4047 after a single oral and intravenous administration to male Cynomolgus monkeys

(excerpted from Applicant's submission)

Animal	Dose Route (Dose (mg/kg))	$AUC_{(0-t)}$ (ng.h/mL)	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	Cl_{tot} (mL/h/kg)	V_d (mL/kg)	F (%)
Animal 5	PO (100 mg/kg)	58749.7	3523.5	2.0	19.0	NC	NC	17.0
	IV (10 mg/kg)	34486.2	6906.5	0.5	5.5	288.3	2279.7	
Animal 6	PO (100 mg/kg)	71150.5	3049.1	2.0	28.2	NC	NC	15.1
	IV (10 mg/kg)	47053.5	7800.8	0.167	5.6	211.6	1708.0	
Animal 7	PO (100 mg/kg)	74980.7	3740.2	2.0	38.3	NC	NC	16.9
	IV (10 mg/kg)	44327.9	6305.0	0.333	6.9	224.3	2246.5	
Animal 8	PO (100 mg/kg)	47560.7	4543.9	2.0	14.4	NC	NC	11.2
	IV (10 mg/kg)	42467.3	6709.9	0.333	8.6	233.8	2899.1	
Mean data	PO (100 mg/kg)	63110.4	3714.2	2.0	25.0	NC	NC	15.0
	IV (10 mg/kg)	42083.7	6930.6	0.333	6.7	239.5	2283.3	

NC = Not calculated

The bioavailability (F) of CC-4047 in monkeys was 15%. The absolute bioavailability was calculated as a ratio of areas under the curve according to the formula:

$$F = 100 \times \frac{AUC_{po} * D_{iv}}{AUC_{iv} * D_{po}} \qquad F = 100 \times \frac{63110.4 * 10\ mg/kg}{42083.7 * 100\ mg/kg} \qquad F = 15.0\%$$

Study title: Single dose intravenous and oral pharmacokinetics of CC-4047 and two enantiomers in monkeys

Study No.:	CC-4047-DMPK-021
Study report location:	eCTD 4.2.2.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 17, 2007

GLP compliance:	Yes. Statement included and signed
QA statement:	Statement included and signed
Drug, lot #, and % purity:	CC-4047 (racemate, Lot # CMLW-174/06-CC2), Purity: 100.4% CC-5083 (S enantiomer, Lot # 5373-110-C), Purity: 98.6% CC-6016 (R enantiomer, Lot # 5423-3-B), Purity: 99.06%

Key Study Findings

- The median T_{max} for both enantiomers after oral administration of CC-4047 was 3 hours indicating rapid absorption.
- Mean half-lives after oral administration of CC-4047 were 8 and 5.5 hours for the S and R enantiomers, respectively, showing low to moderate systemic clearance.
- Mean half-lives after intravenous administration of CC-4047 were 4.9 and 3.5 hours for the S and R enantiomers, respectively.
- Exposure (AUC) to CC-6016 (R-enantiomer) was almost twice that of CC-5083 (S-enantiomer) after either intravenous or oral dosing of CC-4047 racemate or the individual enantiomers.

Methods

Doses:	CC-4047: ~1 mg/kg (IV), ~2 mg/kg (oral) Enantiomers: ~0.5 mg/kg (IV), ~1.0 mg/kg (oral)
Frequency of dosing:	Single dose
Route of administration:	IV and oral gavage, 14-day washout period between doses
Dose volume:	5 mL/kg (oral) and 2 mL/kg (IV)
Time points for blood collection:	0.083 (IV only), 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, and 48 hours post-dose
Formulation/Vehicle:	1% w/v carboxymethylcellulose for oral dosing 5% dimethylacetamide, 45% PEG-400, and 50% saline for IV dosing
Bioanalytical LLOQ	Enantiomers 1.0 ng/mL;
Species/Strain:	Cynomolgus monkeys (*Macaca fascicularis*)
Number/Sex/Group:	3 males/group
Age:	Young adult/adult
Weight:	3.0 – 3.9 kg

Results

Formulation preparations for intravenous dosing were within the 15% of the target concentration range; however, formulation preparations for oral dosing fell outside the 15% of the target concentration range for the CC-4047 racemate and the CC-6016 (R-enantiomer) preparations. Pharmacokinetic analysis for the oral dosing was conducted using 79.1 and 73.2% nominal doses of 2 mg/kg for the CC-4047 racemate and 1 mg/kg for the CC-6016 (R-enantiomer), respectively.

Following oral dosing of CC-4047 racemate, both enantiomers were absorbed at rapid to moderate rates with median Tmax values for CC-5083 and CC-6016 of 3 hr (Table 14). Interconversion between enantiomers occurred and exposure (AUC) to CC-6016 (R-enantiomer) was almost twice that of CC-5083 (S-enantiomer) after either IV or oral dosing of CC-4047 racemate or the individual enantiomers.

CC-4047 and its enantiomers exhibited low systemic clearance, with terminal half-lives ranging from 3.3 to 4.9 hours. Clearance values for CC-5083 (S-enantiomer) were approximately twice that of CC-6016 (R-enantiomer) following IV dosing of individual enantiomers (Table 15). The oral bioavailability for CC-4047 as well as its enantiomers was approximately unity with ranges between 10 to 16%.

Table 14: Summary of PK parameters for CC-4047 and its S and R enantiomers after oral administration to male cynomolgus monkeys

(excerpted from Applicant's submission)

Group	Analyte	C_{max} (ng/mL)	T_{max} (hr)	$t_{½}$ (hr)	AUC_t (hr*ng/mL)	AUC_∞ (hr*ng/mL)	MRT (hr)	F %
1	CC-5083 (S-enantiomer)	439	3.00	8.0	3060	3740	12.5	-
	CC-5083 + CC-6016 (S + R)	1060	3.00	5.0	9340	9350	10.4	116
	CC-6016 (R-enantiomer)	631	3.00	5.5	5720	5730	10.6	-
	S/R AUC_∞ Ratio	-	-	-	-	0.661	-	-
2	CC-5083 (S-enantiomer)	910	1.00	3.5	2980	3000	3.1	130
	CC-6016 (R-enantiomer)	132	3.00	4.1	990	1030	6.8	-
	S/R AUC_∞ Ratio	-	-	-	-	3.30	-	-
3	CC-5083 (S-enantiomer)	97.9	3.00	4.4	869	898	7.8	111
	CC-6016 (R-enantiomer)	780	2.00	3.9	4070	4120	5.2	-
	S/R AUC_∞ Ratio	-	-	-	-	0.215	-	-

S/R AUC_∞ Ratio = AUC_∞(CC-5083)/ AUC_∞(CC-6016)
Bioavailability (F) = {(AUC_∞ (CC-5083 + CC-6016) /Dose[PO])/(AUC_∞ (CC-5083 + CC-6016)/Dose [IV])} x 100%

Table 15: Summary of PK parameters for CC-4047 and its S and R enantiomers after IV doses to male Cynomolgus monkeys

(excerpted from Applicant's submission)

Group	Analyte	C_0 (ng/mL)	$t_{½}$ (hr)	AUC_t (hr*ng/mL)	AUC_∞ (hr*ng/mL)	CL (mL/hr/kg)	Vss (mL/kg)	MRT (hr)
1	CC-5083 (S-enantiomer)	743	4.9	1900	1920	NA	NA	3.8
	CC-5083 + CC-6016 (S + R)	1640	4.1	5470	5490	211	826	4.2
	CC-6016 (R-enantiomer)	896	3.5	3270	3290	NA	NA	3.9
	S/R AUC_∞ Ratio	-	-	-	0.587	-	-	-
2	CC-5083 (S-enantiomer)	698	4.1	1210	1230	457	1140	2.7
	CC-6016 (R-enantiomer)	25.4	3.8	556	576	NA	NA	6.1
	S/R AUC_∞ Ratio	-	-	-	2.14	-	-	-
3	CC-5083 (S-enantiomer)	15.3	3.3	541	553	NA	NA	5.9
	CC-6016 (R-enantiomer)	703	3.8	2450	2470	203	769	3.9
	S/R AUC_∞ Ratio	-	-	-	0.221	-	-	-

NA: Not applicable.
S/R AUC_∞ Ratio = AUC_∞(CC-5083)/ AUC_∞(CC-6016)

Distribution

Study title: CC-4047: *In vitro* protein binding study in rat, mouse, monkey, rabbit, and human plasma

Study No.: CC-4047-DMPK-015
Study report location: eCTD 4.2.2.3

Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 24, 2006
GLP compliance:	No
QA statement:	NA
Drug, lot #, and % purity:	[^{14C}]CC-4047, Lot # CFQ14361 (Batch 1); Specific activity 58 mCi/mmol; Radiopurity: 99.7%; chemical purity: 99.9%; (b) (4) CC-4047 (racemic, Lot # 61749-06), Purity: >99.9%, (b) (4) Reference Standards: CC-5083 (S enantiomer, Lot # 5373-110-C / 5157-133-A), Purity: 98.1%, Celgene CC-6016 (R enantiomer, Lot # 5423-3-B / 5364-179-F), Purity: 99.1%, Celgene

Key Study Findings

- Plasma protein binding ranged from 15 to 40% and from 16 to 55% for the CC-4047 R- and S-enantiomers, respectively, when CC-4047 was incubated with plasma from human, monkey, rat, mouse and rabbit origin.
- Protein binding of CC-4047 enantiomers was similar in mouse and rabbit plasma.
- Protein binding of R was higher than the S enantiomer in rat plasma.
- Protein binding of S was higher than the R enantiomer in monkey and human plasma. Protein binding of the R-enantiomer was comparable in monkey and human plasma.

Methods

Concentrations:	CC-4047: 30, 100, 300, 1000 and 3000 ng/mL
Incubation time/conditions:	15 minutes at 37°C with 5% CO_2
Method:	Ultrafiltration at ~2000 g for 30 minutes
Measurements:	Non-specific loss of [^{14C}]CC-4047 Concentration of R- and S-enantiomers in the protein-free filtrate Degradation of R- and S-enantiomers in plasma
Molecular weight cutt-off:	30000 daltons
Bioanalytical Method:	Chiral LC/MS/MS
Species/Strain:	Plasma with K_3 EDTA as anti-coagulant from Sprague-Dawley rats, mice (CD-1), New Zealand White rabbits, Cynomolgus monkeys and healthy human subjects from (b) (4)
Number of samples:	Pooled (N≥3, mixed gender)

Results

No significant differences were observed in the radioprofiles of [^{14C}]CC-4047 in the presence or absence of Amastatin. Non-specific loss of [^{14C}]CC-4047 to the ultrafiltration apparatus was not significant. The mean plasma protein binding ranged from 15 to 40% and from 16 to 55% for the CC-4047 R- and S-enantiomers, respectively, when CC-4047 at concentrations ranging from 30 to 1000 ng/mL was incubated with plasma from human, monkey, rat, mouse and rabbit origin (Table 16 and Table 17). Accurate protein binding values were not obtained at 3000 ng/mL CC-4047; however, the trend of protein binding values at this concentration were in general lower for all species except for monkey, suggesting saturation at higher concentrations.

Protein binding of CC-4047 R- and S-enantiomers was similar in mouse and rabbit plasma. Protein binding of R- was higher than the S-enantiomer in rat plasma, while the protein binding of S- was higher than the R-enantiomer in monkey and human plasma. No concentration dependency of protein binding was noted over the 30 to 1000 ng/mL concentration range of CC-4047 in human, monkey, rat, mouse or rabbit plasma.

Table 16: Summary of protein binding of CC-4047 R-enantiomer after incubation with human, monkey, rat, mouse and rabbit plasma samples

(excerpted from Applicant's submission)

	Human Plasma	Monkey Plasma	Rat Plasma	Mouse Plasma	Rabbit Plasma
Concentration	Average	Average	Average	Average	Average
(ng/mL)	% Bound	% Bound	% Bound	% Bound	% Bound
30	11.96	21.05	43.72	37.94	29.82
100	12.60	22.20	40.32	46.19	31.41
300	13.95	13.89	41.20	33.69	31.33
1000	24.85	11.97	35.60	25.14	32.29
Average[1]	15.84	17.28	40.21	35.74	31.21

Note that the results at a CC-4047 concentration of 3000 ng/mL were not included because an accurate assessment could not be made.
[1]Average %bound through the concentration range of 30 ng/mL to 1000 ng/mL.

Table 17: Summary of protein binding of CC-4047 S-enantiomer after incubation with human, monkey, rat, mouse and rabbit plasma samples

(excerpted from Applicant's submission)

	Human Plasma	Monkey Plasma	Rat Plasma	Mouse Plasma	Rabbit Plasma
Concentration	Average	Average	Average	Average	Average
(ng/mL)	% Bound	% Bound	% Bound	% Bound	% Bound
30	43.94	52.93	23.11	44.96	38.15
100	41.09	58.90	15.88	51.70	35.74
300	40.11	55.48	15.79	39.04	36.85
1000	43.65	53.47	12.37	25.61	40.45
Average	42.20	55.20	16.79	40.33	37.80

Note that the results at a CC-4047 concentration of 3000 ng/mL were not included because an accurate assessment could not be made.
[1]Average %bound through the concentration range of 30 ng/mL to 1000 ng/mL.

Study title: CC-4047: Quantitative tissue distribution of compound-related material using whole-body autoradiography following a single oral dose of [^{14C}]CC-4047 (100 mg/kg) to male Long-Evans rats

Study No.:	CC-4047-DMPK-005
Study report location:	eCTD 4.2.2.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 18, 2005
GLP compliance:	No
QA statement:	NA
Drug, lot #, and % purity:	[^{14C}]CC-4047, Lot # CFQ14361 (Batch 1); Specific activity 58 mCi/mmol; Radiopurity: 99.7%; chemical purity: 99.9%, (b) (4) (b) (4) CC-4047 (racemic, Lot # 61749-06), Purity: >99.9%, (b) (4)

Key Study Findings

- The highest concentrations of CC-4047 were found in the alimentary canal (GI tract) and organs of excreta (renal cortex, medulla, and urinary bladder).
- Moderate concentrations were found in the bile, liver, endocrine glands, secretory glands, brown adipose, pigmented skin, lymph nodes and thymus.
- Both the liver and kidney are involved in the excretion of CC-4047 based on radioactivity detected in the bile and urinary bladder. Radioactivity was higher in the kidney than liver.

Methods

Doses:	CC-4047: 100 mg/kg, 200 µCi/kg (oral)
Frequency of dosing:	Single dose
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Time points autoradiography:	0.5, 1, 3, 6, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours post-dose
Formulation/Vehicle:	1% w/v carboxymethylcellulose medium viscosity
Species/Strain:	Long-Evans pigmented rat
Number/Sex/Group:	13 males; 1 pigmented rat/time point
Age:	Approximately 8 weeks of age
Weight:	225 – 275 g

Results

CC-4047-derived radioactivity was widely distributed to most tissues and blood through 8 hours post-dose (Table 18). The highest concentrations were measured in the alimentary canal (GI tract) and organs of excreta (renal cortex, medulla, and urinary bladder). Moderate concentrations were found in the bile, liver, endocrine glands, secretory glands, brown adipose, and pigmented skin, lymph nodes and thymus. Minimal radioactivity was noted in the spinal cord and the brain.

CC-4047 radioactivity was distributed equally between the cellular and non-cellular portions of whole blood based on the blood to plasma ratios of approximately 1. Both the liver and kidney were involved in the excretion of CC-4047 based on evidence of radioactivity detected in the bile and urinary bladder. No radioactivity was detected in rats assigned to the 72, 96, and 168 hour time points and therefore, no image analysis was conducted.

Table 18: Concentration of Radioactivity in Tissues of Male Pigmented Rats

(excerpted from Applicant's submission)

Tissue	0.5 h	1 h	3 h	6 h	8 h	12 h	24 h	72 h	96 h	168 h
Blood (cardiac)	0.685	0.840	1.696	0.987	0.962	NI	NI	NI	NI	NI
Lymph nodes	0.499	0.585	0.883	1.106	1.008	NI	NI	NI	NI	NI
Thymus	1.069	0.783	1.376	0.914	0.787	NI	NI	NI	NI	NI
Bile (in duct)	6.063	6.486	9.962	9.486	1.777	NI	NI	NI	NI	NI
Liver	2.341	1.469	2.589	1.835	1.513	BQL	NI	NI	NI	NI
Renal Cortex	1.333	2.297	5.635	3.007	2.163	NI	NI	NI	NI	NI
Renal Medulla	1.091	5.140	5.477	1.940	2.480	NI	NI	NI	NI	NI
Urinary Bladder	0.762	4.388	6.729	28.466	9.005	19.362	NI	NI	NI	NI
Urinary Bladder (contents)	14.728	14.092	66.639	47.222	52.370	24.360	NI	NI	NI	NI
Spinal Cord	BQL	BQL	0.728	BQL	BQL	NI	NI	NI	NI	NI
Skin, Pigmented	0.725	1.037	2.106	1.407	1.431	0.606	NI	NI	NI	NI
Seminal Vesicles	0.559	0.698	1.684	0.939	1.214	NI	NI	NI	NI	NI
Testis	0.587	BQL	1.483	0.786	0.497	NI	NI	NI	NI	NI
Cecum	0.642	0.769	1.901	197.581	32.738	NI	NI	NI	NI	NI
Esophagus	2.182	0.725	1.547	0.849	0.740	NI	NI	NI	NI	NI
Large Intestine	0.784	1.392	1.308	1.006	1.007	NI	NI	NI	NI	NI
Stomach	12.492	9.615	2.891	1.284	1.612	NI	NI	NI	NI	NI
Small intestine	0.840	1.878	3.271	8.218	14.490	NI	NI	NI	NI	NI
Eye Uveal Tract	1.213	2.144	3.251	3.211	3.047	0.652	NI	NI	NI	NI
Eye Lens	BQL	BQL	0.433	BQL	0.490	BQL	NI	NI	NI	NI

BQL = Below Quantifiable Limit, value is below the lower limit of quantitation.
NI = Not Identified: the tissue to be quantified could not be visually identified on the autoradiogram. The amount of radioactivity was so low that an autoradiographic image could not be produced; thus the tissue could not be sampled during image analysis.

Study title: CC-4047: Determination of the lacteal transfer of CC-4047 following a single 10 mg/kg oral administration in rats

Study No.:	CC-4047-DMPK-038
Study report location:	eCTD 4.2.2.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 16, 2010
GLP compliance:	No
QA statement:	NA
Drug, lot #, and % purity:	CC-4047, Lot # CMLW-377/09-CC2, Purity: 100.8%
Drug lot expiration date:	CC-4047, June 2013

Key Study Findings

- Mean milk to plasma concentration ratios ranged from 0.63 to 1.5 for up to 24 hours after dosing, indicating that CC-4047 is absorbed and excreted into milk in rats.

Methods

Doses:	CC-4047: 10 mg/kg (60 mg/m^2)
Frequency of dosing:	Single dose
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Time points for milk and blood collection:	1, 4, 8, and 24 hours post-dose
Formulation/Vehicle:	1% w/v carboxymethylcellulose
Species/Strain:	Sprague-Dawley rat
Number/Sex/Group:	16 females with litters (culled to 4 pups per litter); 4 dams/time point
Age:	Approximately 7 to 9 weeks of age; 14-day postpartum
Weight:	225 – 275 g

Results

The mean CC-4047 concentration in milk was 1640, 4820, 3220 and 70.3 ng/mL, while plasma concentrations were 1390, 3880, 2290 and 113 ng/mL at 1, 4, 8 and 24 hours, respectively (Table 19). The mean milk/plasma concentration ratios were 1.2, 1.3, 1.5, and 0.63 at 1, 4, 8 and 24 hours, respectively. No CC-4047-related clinical signs occurred. Results provided evidence that CC-4047 is excreted into milk in rats. The bioanalytical LCMS-MS upper and lower limit of quantization for milk and plasma were 546 ng/mL and 0.82 ng/mL, respectively.

Table 19: Mean concentrations of CC-4047 in rat plasma and milk and mean milk to plasma concentration ratios

Time Point (H)	Mean Plasma Concentration (ng/mL) ± SD	Mean Milk Concentration (ng/mL) ± SD	Mean Milk / Plasma Concentration Ratios ± SD
1	1390 ± 136	1640 ± 430	1.2 ± 0.27
4	3880 ± 661	4820 ± 159	1.3 ± 0.22
8	2290 ± 549	3220 ± 727	1.5 ± 0.39
24	113 ± 38.6	70.3 ± 24.6	0.63 ± 0.071

Metabolism

Study title: [^{14}C]CC-4047: Biotransformation following intravenous or oral administration to cynomolgus monkeys

Study No.:	CC-4047-DMPK-010
Study report location:	eCTD 4.2.2.4
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 17, 2005

GLP compliance:	Yes. Statement included and signed
QA statement:	Statement included and signed
Drug, lot #, and % purity:	[^{14}C]CC-4047, Lot # CFQ14361, Specific activity 58 mCi/mmol; Radiopurity: 99.7%; chemical purity: 99.9%, (b) (4) CC-4047, Lot # 61749-06, Purity: >99%

Key Study Findings

- No significant differences were observed in the metabolic profiles from urine, feces and plasma when pomalidomide was given intravenously or orally, suggesting that minimal first pass metabolism occurred in the intestine after oral administration.
- CC-4047 was extensively metabolized based on the amount of parent compound radioactivity excreted in urine (≤10%) and feces (≤3%).
- More than 15 metabolites were identified in urine and feces; the contribution of the 6 major metabolites was not significant.

Methods

Doses:	[^{14}C]CC-4047: ~1 mg/kg (IV) and ~10 mg/kg (oral)
Frequency of dosing:	Single dose
Route of administration:	IV and oral gavage
Dose volume:	1 mL/kg and 5 mL/kg
Time points for blood collection:	Plasma samples from individual monkeys pooled by interval to prepare 11 pooled samples IV: 2 min, 1, 4, 10 and 24 hr Oral: 0.5, 1, 4, 10, 24 and 48 hr
Time points for urine collection:	Urine samples from each individual monkey were pooled by each group and interval at 0-8, 8-24, and 24-48 hr
Time points for feces collection:	Homogenized feces samples from individual monkeys were pooled by each group and interval at 0-48 hr
Formulation/Vehicle:	5% dimethylacetamide:95% PEG-400 (v/v) for IV dosing 1% w/v carboxymethylcellulose for oral dosing
Species/Strain:	Cynomolgus monkeys (*Macaca fascicularis*)
Number/Sex/Group:	3 males/group
Age:	Not specified
Weight:	3.1 – 5.7 kg

Samples for this study were collected from the study CC-4047-DMPK-009 reviewed under the excretion section.

Results

No significant differences were observed in the metabolic profiles from urine, feces and plasma between the two different routes of administration, suggesting that minimal first pass metabolism occurred in the intestine. CC-4047 was extensively metabolized based on the amount of parent compound radioactivity (≤10%) excreted in urine. The amount of radioactive CC-4047 excreted in feces was 0.2% for IV administration and 2.8% for oral administration (Table 20).

More than 15 metabolites were identified in urine and feces. Major pathways involved in CC-4047 metabolism included hydroxylation (M16 and M17), glucuronidation (M12 and M13), hydrolysis (M10 and M11) and N-dealkylation-deamination (M2). A metabolic pathway was proposed and metabolite chemical structures were identified, (Figure 22). CC-4047 was the major component in plasma samples analyzed up to 24 hours for IV administration and 48 hours for oral administration (Table 21). The contribution of the 6 major metabolites to the total exposure was not significant (Table 22).

Table 20: Distribution of CC-4047 and its major metabolites in urine and feces

(excerpted from Applicant's submission)

Metabolite ID[1]	R_t Range (min)	Group 1 (IV) Male 0-48 hr Urine % Dose	Group 1 (IV) Male 0-48 hr Feces % Dose	Group 1 (IV) Male 0-48 hr Total % Dose	Group 2 (Oral) Male 0-48 hr Urine % Dose	Group 2 (Oral) Male 0-48 hr Feces % Dose	Group 2 (Oral) Male 0-48 hr Total % Dose
M2	3.00-4.00	2.13%	0.75%	2.88%	2.74%	0.41%	3.15%
M10	15.50-16.25	2.69%	ND	2.69%	2.96%	0.04%	3.00%
M11	16.25-18.75	14.03%	0.35%	14.38%	16.69%	0.50%	17.19%
M12	19.50-20.25	11.48%	ND	11.48%	10.58%	ND	10.58%
M13	20.25-21.25	15.32%	ND	15.32%	13.38%	ND	13.38%
M16	27.50-28.75	7.00%	ND	7.00%	6.61%	ND	6.61%
M17	32.75-33.75	7.42%	6.21%	13.63%	5.66%	4.04%	9.70%
CC-4047	35.25-36.25	7.37%	0.21%	7.58%	6.08%	2.80%	8.88%
Sum[2]		67.44%	7.52%	74.96%	64.70%	7.79%	72.49%
Total[3]		69.66%	9.25%	78.91%	69.60%	9.51%	79.11%

1. Metabolite that accounted for >2% of the dose. Trace metabolites (<2% of the dose) were not included in the table.
2. Sum of the major metabolites listed above
3. Total dose recovered in the 0-48 hour urine or feces samples

Figure 22: Proposed metabolic pathway of CC-4047 in monkeys

(excerpted from Applicant's submission)

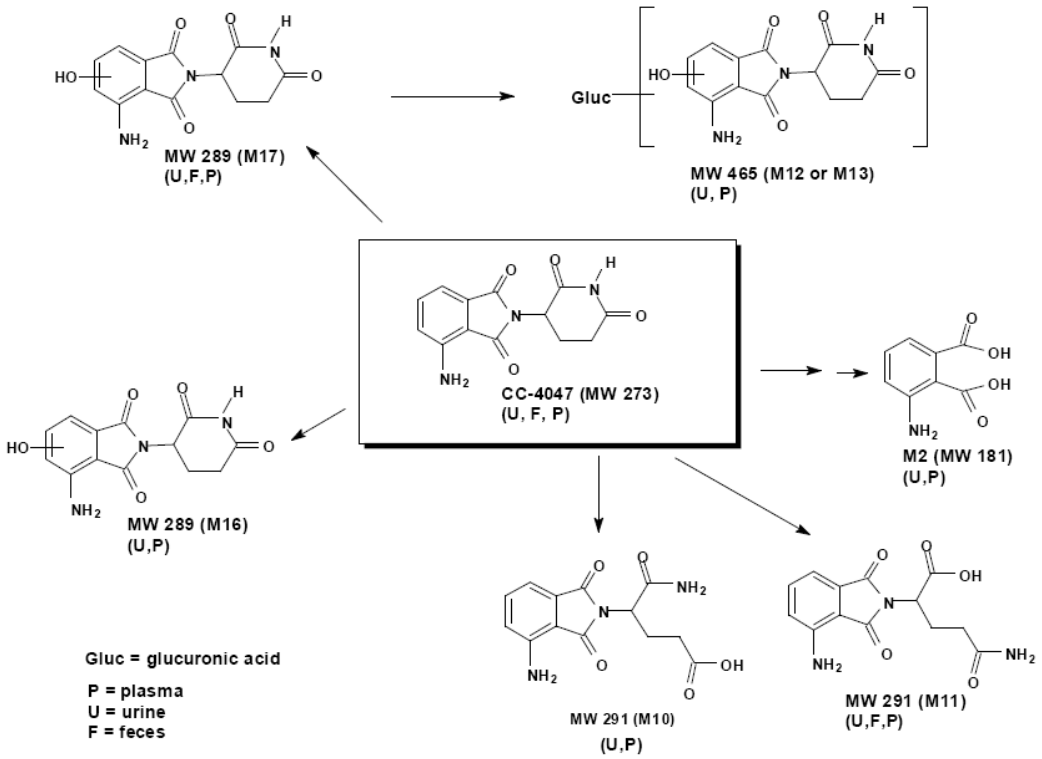


Table 21: Distribution of CC-4047 and major metabolites in plasma

(excerpted from Applicant's submission)

Metabolite	Group 1 Male					Group 2 Male					
	2 min	1 hr	4 hr	10 hr	24 hr	0.5 hr	1 hr	4 hr	10 hr	24 hr	48 hr
ID	µg/mL	µg/mL	µg/mL	µg/mL	µg/mL	µg/mL	µg/mL	µg/mL	µg/mL	µg/mL	µg/mL
M2	ND	0.011	0.013	0.002	0.001	ND	ND	ND	0.019	0.012	0.008
M10	ND	0.003	ND	ND	ND	ND	ND	ND	ND	ND	ND
M11	0.006	0.036	0.020	0.007	<0.001	0.204	0.239	0.145	0.058	0.033	0.003
M12	ND	0.040	0.028	0.002	0.001	0.102	0.043	0.042	0.023	0.012	0.002
M13	0.008	0.022	0.025	0.003	<0.001	0.204	0.069	0.083	0.026	0.012	0.002
M16	0.004	0.043	0.025	0.004	ND	0.172	0.139	0.119	0.044	0.012	ND
M17	0.024	0.029	0.009	0.003	<0.001	0.167	0.165	0.057	0.051	0.018	ND
CC-4047	0.948	0.620	0.332	0.141	0.026	4.281	6.136	4.441	1.875	0.506	0.084
Total	0.990	0.804	0.452	0.162	0.028	5.130	6.791	4.908	2.096	0.605	0.099

ND = Not detected

Table 22: Pharmacokinetic parameters of CC-4047 and major metabolites

(excerpted from Applicant's submission)

Group 1 (IV)					Group 2 (Oral)				
Metabolite ID	$t_{½,z}$ (hr)	AUC_t (hr*µg/mL)	AUC_∞ (hr*µg/mL)	$\%AUC_\infty$[2]	Metabolite ID	$t_{½,z}$ (hr)	AUC_t (hr*µg/mL)	AUC_∞ (hr*µg/mL)	$\%AUC_\infty$[2]
TRR[1]	5.14	5.97	6.19	NA	TRR	8.72	70.19	71.45	NA
CC-4047	5.49	4.81	5.01	100.00%	CC-4047	8.56	62.24	63.27	100.00%
M11	3.83	0.19	0.22	4.39%	M11	8.63	2.42	2.45	3.87%
M12	4.23	0.26	0.26	5.19%	M12	10.60	0.80	0.83	1.31%
M13	2.89	0.17	0.18	3.59%	M13	10.15	1.11	1.14	1.80%
M16	2.57	0.21	0.23	4.59%	M16	6.25	1.39	1.50	2.37%
M17	2.86	0.12	0.13	2.59%	M17	11.44	1.26	1.56	2.47%

NA: not applicable

[1]Total radioactive residue values in the pooled plasma assayed at XBL; pk parameters for TRR were determined using the values from the same intervals as used for CC-4047 and metabolites.

[2]Relative exposure of metabolites to that of CC-4047

Study title: [14C]CC-4047: P450 reaction phenotyping

Study No.:	CC-4047-DMPK-022
Study report location:	eCTD 4.2.2.4
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 8, 2010
GLP compliance:	No
QA statement:	NA
Drug, lot #, and % purity:	[^{14}C]CC-4047, Lot # 60257-10-001, Specific activity 58 mCi/mmol; Radiopurity: >98%; chemical purity: 99.6%, (b) (4)

Key Study Findings

- CC-4047 was metabolically stable when incubated for an hour with several CYPs *in vitro*. There was <10% detectable loss

Methods

Concentrations:	[^{14}C]CC-4047: 1 µM
Incubation time/conditions:	Mixture of 0.1 M potassium phosphate buffer (pH 7.4), rCYP enzymes, $MgCl_2$, [^{14}C]CC-4047 or P450-selective marker substrates (positive control) pre-incubated at 37°C for 5 minutes in a shaking water bath Reaction initiated by addition of 1 mM NADPH
Method:	Incubation mixtures (500 µL) of rCYP enzymes (Bactosomes^{TM} CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4, and 3A5), cofactors and 1 µM of [^{14}C]CC-4047. At 0, 0.5, and 1 hr, incubations were terminated by the addition of 1 mL of ice-cold 0.1% formic acid in acetonitrile containing IS ([^{13}C5]CC-4047) processed and analyzed using LCMS-MS
Measurements:	Percent of [^{14}C]CC-4047 remaining at 0.5 and 1 hour
Positive Controls:	P450-selective marker substrates at concentrations approximately equal to the K_m values previously determined by XBL
Negative Controls:	Incubation mixtures without rCYP enzymes
Species/Strain:	Human recombinant CYP enzymes
Number of samples:	Duplicate preparations

Results

The results for the positive controls suggested that the rCYP were metabolically active. The negative control showed minimal formation of M11 (CC-8017; hydrolysis of glutarimide) during the 1 hour incubation period with the mixture lacking rCYP enzymes. However, [^{14}C]CC-4047 did not show detectable loss of compound, indicating that CC-4047 was chemically stable over the 1 hour incubation time.

The hydrolytic metabolite M11 was formed in all incubation samples and it was previously shown that chemical hydrolysis rather than CYP metabolism may be the mechanism of M11 formation. The M16 metabolite was not detected (ND) as a product of any reaction mixture, and M17 was produced by CYP1A2 (1.7%), 2C19 (1.4%), 2D6 (0.7%), and 3A4 (0.5%) (Table 23). CC-4047 can be considered metabolically stable based on less than 10% detectable loss of [^{14}C]CC-4047 over an hour of incubation in the presence of human recombinant CYP450 enzymes.

Table 23: Percent of CC-4047 metabolites M11, M16 and M17 formed in incubations with human recombinant CYP450

rCYP	Percent of Parent Peak Area [a]		
	M11	M16	M17
1A2	3.4	ND	1.7
2A6	2.9	ND	ND
2B6	2.6	ND	ND
2C8	2.5	ND	ND
2C9	2.9	ND	ND
2C19	2.9	ND	1.4
2D6	4.4	ND	0.7
2E1	2.6	ND	ND
3A4	3.6	ND	0.5
3A5	5.9	ND	ND
Negative Control	5.5	ND	ND

a= Parent Peak Area was determined from incubations terminated at 0 hour

The relative contribution of each CYP450 isozyme to the hepatic metabolism of CC-4047 was estimated to be 54, 11, 4 and 30% for CYPs 1A2, 2C19, 2D6 and 3A4, respectively, based on the relative amount of each CYP isozyme in human liver.

Study title: CC-4047: Determination of relative plasma exposures of CC-4047 metabolites in rat and monkey plasma to human plasma

Study No.:	CC-4047-DMPK-042
Study report location:	eCTD 4.2.2.4
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 17, 2010
GLP compliance:	No
QA statement:	NA
Drug, lot #, and % purity:	[^{14}C]CC-4047, Lot # 60257-10-001and CC-4047 Lot # CMLW-377/09-CC2, Purity: 100.8% (for the human metabolism study) CC-4047, Lot # CMLW-174/06-CC2 and 75838-07 (for the rat toxicity study) CC-4047, Lot # CMLW-174/06-CC2 (for the monkey toxicity study)

Key Study Findings

- All five human metabolites (M11, M12, M13, M16 and M17) were detectable in rat and monkey pooled plasma samples from toxicological studies.
- The percent distribution of metabolites was comparable in monkey and human plasma samples.

- None of the metabolites accounted for >10% of total drug-related material or 10% of the exposure to parent drug in plasma in the human radiolabeled ADME study.

Methods

This study used samples from nonclinical toxicity studies to investigate the exposure of CC-4047 human metabolites in animal species used in toxicology studies. Rat and monkey plasma samples were from the repeat-dose toxicology studies, CC-4047-TOX-013 and CC-4047-TOX-006, respectively, and the human plasma samples were from the human radiolabeled metabolism study, CC-4047-CP-004 (Table 24).

Table 24: Origin of samples for determination of CC-4047 human metabolites exposure in rat and monkey used in toxicological studies

(excerpted from Applicant's submission)

	Human Plasma	Rat Plasma	Monkey Plasma
Day[a]	NA (single dose)	Day 180	Day 272
Group (Dose rate)	Group 1 (2 mg/subject)	Group 4 (1000 mg/kg/day)	Group 3 (0.1 mg/kg/day) Group 4 (1 mg/kg/day)
Gender	Male	Male and Female	Male and Female
Time-points (hr)	0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 24	0, 0.5, 2, 4, 8, 24	0, 0.5, 2, 4, 8, 24
Number of AUC-pooled samples[b]	1	2	4

[a] Day of dose for multiple dose.

[b] AUC-pooled sample was prepared across animals/subjects by sex following the procedures described by Hop et al., 1998.

NA = not applicable.

Results

All five human metabolites (M11, M12, M13, M16 and M17) were detectable in rat and monkey plasma pooled from toxicological studies. The percent distribution of the metabolites was comparable in monkey and human plasma samples and comparable to values obtained during previous human and monkey metabolism studies (Table 25).

Direct comparisons of metabolites in rat or monkey vs. human plasma were not possible due to the low levels of most of metabolites in the AUC-pooled/diluted samples. Two metabolites, M18 (CC-4067) and M19 (CC-12074; Table 26), detected as trace metabolites in human during the human metabolism study were also detectable in rat and monkey plasma. None of the metabolites accounted for >10% of total drug-related material or 10% of the exposure to parent drug in plasma in the human radiolabeled ADME study.

Table 25: Percent distribution of CC-4047 and metabolites in human, rat and monkey plasma

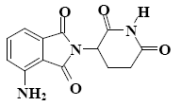
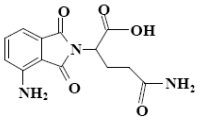
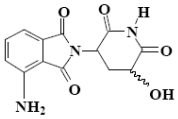
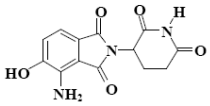
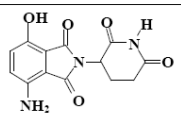
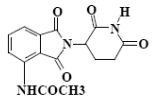
(excerpted from Applicant's submission)

Metabolite ID[f]	Human[a]	Rat		Monkey			
		Group 3[b]		Group 3[c]		Group 4[d]	
	%Dist[e]	Male %Dist[e]	Female %Dist[e]	Male %Dist[e]	Female %Dist[e]	Male %Dist[e]	Female %Dist[e]
CC-4047	77.2	87.5	94.9	77.0	82.9	91.0	92.9
M11	5.63	2.01	1.93	6.07	4.69	3.56	2.04
M12	3.92	0.85	0.76	4.81	1.28	2.10	1.20
M13	4.92	2.60	1.53	3.35	5.76	0.37	1.61
M16	6.41	6.84	0.74	1.88	0.43	0.03	0.03
M17	1.89	0.16	0.16	6.90	4.90	2.93	2.19
Sum	100	100	100	100	100	100	100

a. 2 mg/subject; b. 1000 mg/kg/day on Day 180; c. 0.1 mg/kg/day on Day 272; d. 1 mg/kg/day on Day 272; e. % distribution; f. structures of the human metabolites as shown in Table 26

Table 26: Structure of CC-4047 and some of its metabolites detectable in human, monkey and rat plasma

(excerpted from Applicant's submission)

Compound Code	Structure	Lot No	Nominal MW
CC-4047		CMLW-376/09CC2	273
CC-8017 (M11)		40761-47-B	291
CC-12074 (M19)		5115-139-B	289
CC-17368 (M17)		7585-052	289
CC-17369 (M16)		7585-053	289
CC-4067 (M18)		40303-23-A	315

Excretion

Study title: [^{14}C]CC-4047: Pharmacokinetics and mass balance following intravenous or oral administration to cynomolgus monkeys

Study No.:	CC-4047-DMPK-009
Study report location:	eCTD 4.2.2.5
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 4, 2005
GLP compliance:	FDA. Statement included and signed
QA statement:	Statement included and signed
Drug, lot #, and % purity:	[^{14}C]CC-4047, Lot # CFQ14361, Specific activity 58 mCi/mmol; Radiopurity: 99.7%; chemical purity: 99.9%, (b) (4) CC-4047, Lot # 61749-06, Purity: >99%

Key Study Findings

- CC-4047 was excreted primarily in urine following either intravenous or oral dosing, indicating substantial oral bioavailability.
- Similar CC-4047 exposure and partitioning was observed in blood and plasma for both the intravenous and oral routes of administration through 24 hours post-dose with a slightly greater exposure in blood due to the longer half-lives in blood.

Methods

Doses:	[^{14}C]CC-4047: ~1 mg/kg (IV), ~10 mg/kg (oral)
Frequency of dosing:	Single dose
Route of administration:	IV and oral gavage
Dose volume:	1 mL/kg (IV) and 5 mL/kg (oral)
Time points for blood collection:	Predose and at 0.033, 0.117, 0.25, 0.5, 1, 2, 4, 6, 10, 24, 48, 72, and 96 hours after dosing. Whole blood and plasma were analyzed.
Time points for urine collection:	Before dosing, 0-8, 8-24, and over 24-hour intervals through 168 hours after dosing.
Time points for feces collection:	Separate from urine collection; before dosing, 0-8, 8-24, and over 24-hour intervals through 168 hours after dosing.
Formulation/Vehicle:	5% dimethylacetamide:95% PEG-400 (v/v) for IV dosing 1% w/v carboxymethylcellulose for oral dosing
Species/Strain:	Cynomolgus monkeys (*Macaca fascicularis*)
Number/Sex/Group:	3 males/group
Age:	No specified
Weight:	3.1 – 5.7 kg

Results

[^{14}C]CC-4047 was rapidly absorbed after oral administration reaching the C_{max} after 1 hour (T_{max}). The mean C_{max} and AUC_{0-24} values in blood and plasma were similar. Comparable patterns were observed after intravenous administration showing no preferential partitioning of CC-4047 between blood and plasma after either route of administration (Table 27). Half-lives were slighter longer in blood than in plasma.

Table 27: Pharmacokinetics of radioactive CC-4047 in blood and plasma after intravenous or oral administration of CC-4047 to male cynomolgus monkeys

(excerpted from Applicant's submission)

Group/Route	Blood		Plasma	
Parameter (units)	Mean[a]	SD	Mean[a]	SD
Group 1 - Intravenous				
C_{max} (ng equiv/g)	1120	36.1	1080	90.7
t_{max} (h)	0	NA	0	NA
t_{last} (h)	24	NA	24	NA
AUC_{0-24} (ng equiv·h/g)	5920	1370	5720	1390
AUC_{last} (ng equiv·h/g)	6580	2510	6010	1890
AUC_{inf} (ng equiv·h/g)	7330	3440	6120	1910
$t_{1/2}$ (h)	32.7[b]	47.1	5.9	2.2
Group 2 – Oral				
C_{max} (ng equiv/g)	6790	221	7510	150
t_{max} (h)	1	NA	1	NA
t_{last} (h)	72	NA	48	NA
AUC_{0-24} (ng equiv·h/g)	67900	5450	66600	3900
AUC_{last} (ng equiv·h/g)	86500	14200	73800	9920
AUC_{inf} (ng equiv·h/g)	91700	18000	76500	8430
$t_{1/2}$ (h)	24.5	19.5	8.2	1.6

SD Standard deviation.
NA Not applicable.
a: Median for t_{max} and t_{last}; n=3.
b: $t_{½}$ values for individual animals were 5.5, 5.6, and 87.1 h.

[^{14}C]CC-4047 was excreted primarily in urine following either intravenous (71.6%) or oral dosing (72.9%, Table 28). [^{14}C]CC-4047 was also excreted in feces after intravenous (11.6%) or oral (13.1%) dosing. These similar excretion profiles in urine and feces following IV or oral administration suggested that [^{14}C]CC-4047 radio equivalents circulated in a similar pattern following either route of administration (Figure 23). The high urine excretion after oral dosing also implied substantial oral bioavailability.

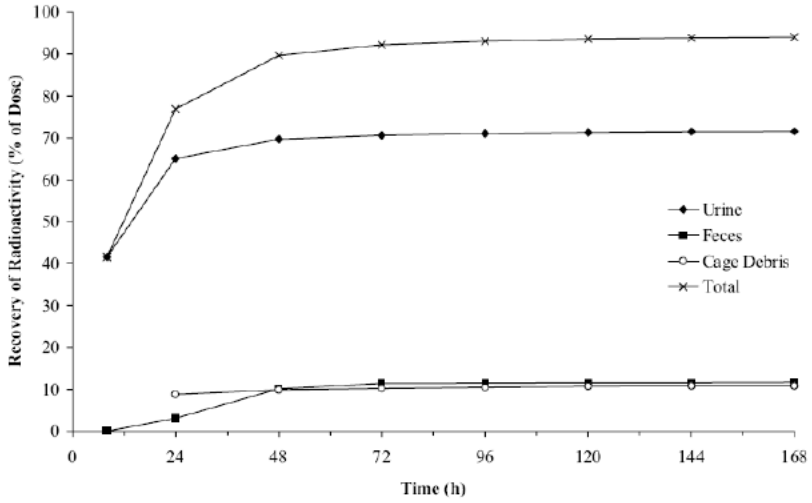
Table 28: Total mean recoveries of radioactivity at 168 hours after IV or oral administration of CC-4047 to male cynomolgus monkeys

Sample	Mean percent excreted (% of the administered dose)	
	IV	Oral
Urine	71.6	72.9
Feces	11.6	13.1
Cage debris/rinse	10.8	9.0
Cage wash	0.3	0.3
Cage wipe	0.1	0.2
Total recovery	94.4	95.5

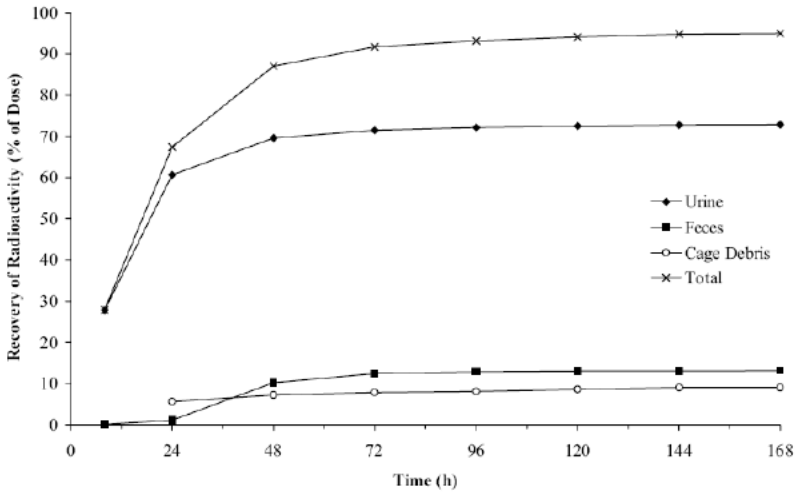
Figure 23: Cumulative mean recoveries of radioactivity in urine, feces and cage rinse samples after intravenous or oral administration of CC-4047 to male cynomolgus monkeys

(excerpted from Applicant's submission)

Intravenous administration (1 mg/kg)



Oral administration (10 mg/kg)



Pharmacokinetic Drug Interactions

Study title: *In vitro* evaluation of CC-4047 as an inducer of cytochrome P450 expression in cultured human hepatocytes

Study No.:	CC-4047-DMPK-023
Study report location:	eCTD 4.2.2.6
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 14, 2006
GLP compliance:	FDA, OECD, MHLW. Statement included and signed
QA statement:	Statement included and signed
Drug, lot #, and % purity:	CC-4047, Lot # 61749-06, Purity: 99.9%

Key Study Findings

- There were no effects of CC-4047 on activity of CYP1A2, 2B6, 2C9, 2C19, and 3A4/5 in hepatocytes exposed to concentrations up to 3 µM twice daily for 3 days.

Methods

Concentrations:	CC-4047: 0.3, 1, 3 µM
Incubation time/conditions:	3 days in a humidified culture chamber (37 ± 1°C, at 95% relative humidity, 95/5% air/CO_2)
Method:	Microsomes prepared from treated human hepatocytes
Measurements:	Activity towards enzyme-specific probe substrates: CYP1A2 (Phenacetin, O-dealkylation), CYP2B6 (Bupropion, hydroxylation), CYP2C9 (Diclofenac, 4´-hydroxylation), CYP2C19 (S-mephenytoin, 4´-hydroxylation) and CYP3A4/5 (Testosterone, 6β-hydroxylation)
Positive Control Inducers:	Omeprazole (100 µM), Phenobarbital (750 µM) Rifampin (10 µM)
Species/Strain:	Cultured human hepatocytes / microsomes
Number of samples:	3 preparations from 3 separate human livers

Results

CC-4047 at concentrations up to 3 µM twice daily for 3 days caused no toxicity to cultured human hepatocytes. Microscopic evaluation of cell morphology during the 3-day culture period showed that cells remained cuboidal with intact cell membranes and granular cytoplasm and maintained a confluent monolayer with few intercellular spaces. Prototype CYP450 inducers (positive controls) caused the anticipated increases in CYP

activity (Table 29). CC-4047 at concentrations up to 3 µM had little or no effects on CYP activity in cultured human hepatocytes.

Table 29: Cytochrome P450 enzyme activity measured in microsomes prepared using cultured human hepatocytes after 3-Day exposure to CC-4047 or prototype inducers

(excerpted from Applicant's submission)

Treatment	Concentration	Enzymatic activity (pmol/mg microsomal protein/min) [a]				
		Phenacetin *O*-dealkylation (CYP1A2)	Bupropion hydroxylation (CYP2B6)	Diclofenac 4´-hydroxylation (CYP2C9)	*S*-Mephenytoin 4´-hydroxylation (CYP2C19)	Testosterone 6β-hydroxylation (CYP3A4/5)
Dimethyl sulfoxide	0.1% (v/v)	44.8 ± 1.1	25.7 ± 7.5	1200 ± 150	5.82 ± 1.53	3360 ± 2040
CC-4047	0.3 µM	48.0 ± 2.0	26.4 ± 7.7	1370 ± 110	6.56 ± 1.15	3870 ± 2180
CC-4047	1 µM	35.9 ± 8.1	23.0 ± 11.1	1250 ± 250	5.61 ± 1.92	3480 ± 2390
CC-4047	3 µM	43.5 ± 2.8	23.9 ± 6.9	1350 ± 90	6.17 ± 0.73	4240 ± 2500
Omeprazole	100 µM	1300 ± 700	143 ± 114	1920 ± 420	8.76 ± 4.34	5110 ± 2390
Phenobarbital	750 µM	100 ± 32	169 ± 149	1790 ± 750	12.9 ± 9.5	13800 ± 6400
Rifampin	10 µM	83.5 ± 25.2	140 ± 93	2850 ± 790	67.1 ± 42.5	16300 ± 4200

a Values are the mean ± standard deviation of three determinations (human hepatocyte preparations H719, H723 and H724).

Study title: ***In vitro* evaluation of CC-4047 as an inhibitor of human cytochrome P450 enzymes**

Study No.:	CC-4047-DMPK-024
Study report location:	eCTD 4.2.2.6
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 24, 2006
GLP compliance:	Yes. US and OECD. Statement included and signed
QA statement:	Statement included and signed
Drug, lot #, and % purity:	CC-4047, Lot # 61749-06, Purity: 99.9%

Key Study Findings

- There was little or no evidence that CC-4047 had the potential to cause direct or time-dependent inhibition of CYP450 activity.

Methods

Concentrations:	CC-4047: 0.1, 0.3, 1, 3, 10, 20, 30 µM
Incubation time/conditions:	Approximately 5 minutes at 37°C
Method:	Microsomes prepared from a pool of sixteen individuals, mixed age and gender
Measurements:	Direct and time-dependent inhibition measuring activity towards enzyme-specific probe substrates with concomitant or pre-CC-4047

incubation:
CYP1A2 (Phenacetin, O-dealkylation)
CYP2A6 (Coumarin, 7-hydroxylation)
CYP2B6 (Bupropion, hydroxylation)
CYP2C8 (Paclitaxel, 6α-hydroxylation)
CYP2C9 (Diclofenac, 4´-hydroxylation)
CYP2C19 (S-mephenytoin, 4´-hydroxylation)
CYP2D6 (Dextromethorphan, O-demethylation)
CYP2E1 (Chlorzoxazone, 6-hydroxylation)
CYP3A4/5 (Testosterone, 6β-hydroxylation)

Species/Strain: Human microsomes
Number of samples: Duplicate determinations using pooled human microsomes

Results

Data suggested that CC-4047 has little or no potential to cause either direct inhibition of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4/5 or time-dependent inhibition of CYP1A2, CYP2C9, CYP2C19, CYP2D6 or CYP3A4/5, as inhibition did not increase substantially upon pre-incubation (Table 30).

Table 30: Summary of determinations evaluating direct or time-dependent inhibition of CYP450 by CC-4047

(excerpted from Applicant's submission)

Enzyme	CYP Reaction	Direct inhibition		Time-dependent inhibition		
		Zero-minute pre-incubation		30-minute pre-incubation		Potential for time-dependent inhibition [b]
		IC_{50} (μM)	Maximum inhibition at 30 μM (%) [a]	IC_{50} (μM)	Maximum inhibition at 30 μM (%) [a]	
CYP1A2	Phenacetin *O*-deethylation	>30	NA	>30	NA	Little or no
CYP2A6	Coumarin 7-hydroxylation	>30	NA	ND	ND	ND
CYP2B6	Bupropion hydroxylation	>30	3.7	ND	ND	ND
CYP2C8	Paclitaxel 6α-hydroxylation	>30	NA	ND	ND	ND
CYP2C9	Diclofenac 4´-hydroxylation	>30	NA	>30	NA	Little or no
CYP2C19	*S*-Mephenytoin 4´-hydroxylation	>30	NA	>30	NA	Little or no
CYP2D6	Dextromethorphan *O*-demethylation	>30	NA	>30	NA	Little or no
CYP2E1	Chlorzoxazone 6-hydroxylation	>30	NA	ND	ND	ND
CYP3A4/5	Testosterone 6β-hydroxylation	>30	6.9	>30	12	Little or no
CYP3A4/5	Midazolam 1´-hydroxylation	>30	NA	>30	2.3	Little or no

Notes Average data (i.e., percent of control activity) obtained from duplicate samples for each test article concentration were used to calculate IC_{50} values. IC_{50} values were calculated with XLfit.

[a] Maximum inhibition (%) is calculated with the following formula and data for the highest concentration of test article for which usable data were collected (results are rounded to two significant figures): Maximum inhibition (%) = 100% – Percent solvent control.

[b] Time-dependent inhibition was determined by comparison of IC_{50} values with and without pre-incubation, by comparison of the maximum inhibition (%) with and without pre-incubation and by visual inspection of the IC_{50} plot.

ND Not determined. Time-dependent inhibition was not determined for these enzymes.

NA Not applicable. Inhibition was not observed at the highest concentration of CC-4047 evaluated (30 μM).

Study title: *In vitro* assessment of CC-4047 as a substrate or inhibitor of P-glycoprotein

Study No.:	CC-4047-DMPK-037
Study report location:	eCTD 4.2.2.6
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 18, 2009
GLP compliance:	No
QA statement:	NA
Drug, lot #, and % purity:	CC-4047, Lot # CMLW-376/09/CC2, Purity: 100.8%

Key Study Findings

- CC-4047 was a substrate for P-gp in MDCK-MDR1 transfected and wild type cells.
- CC-4047 did not inhibit P-gp efflux in MDCK-MDR1 transfected and wild type cells.

Methods

Concentrations:	CC-4047: 0.03, 0.1, 0.3, 1, 3, 10 µM
Incubation time/conditions:	37°C with 95% relative humidity/5% air/CO_2
Method:	24-well membranes (pore size 0.4 µm, area 0.33 cm^2) with confluent cell layers to measure transport from apical to basolateral and vice versa direction
Measurements:	Apparent permeability in both transfected and wild type cells for P-glycoprotein substrate potential Apparent permeability of digoxin P-gp transport after preincubation with CC-4047 for inhibition of P-glycoprotein transport
Positive controls:	Known P-gp substrate (^{3}H-digoxin) Low permeability (^{14}C-mannitol) High permeability (^{14}C-caffeine) P-gp inhibitors, verapamil (250 µM) and ketoconazole (25 µM)
Species/Strain:	MDCK-MDR1 transfected and MDCK-WT cells
Number of samples:	Triplicate determinations using 24-well membrane plated cells

Results

CC-4047 exhibited higher P_{app} (cm/s) in the basolateral-to-apical direction ($P_{B\rightarrow A}$) in both the transfected and wild type (WT) cells suggesting that CC-4047 is a substrate for P-gp transport (Figure 24). The resulting net efflux ratios ($R_{E\text{-}MDR1}/R_{E\text{-}WT}$) were 2.65,

4.72, and 5.36 at test concentrations of 1, 5, and 10 µM CC-4047. The apparent permeabilities ($P_{A\rightarrow B}$ and $P_{B\rightarrow A}$) of marker compounds mannitol, caffeine, and digoxin with MDCK-MDR1 and MDCK-WT cells were within expected ranges.

Inhibition of the P-gp in both cell types using verapamil (250 µM) and ketoconazole (25 µM) reduced the CC-4047 efflux ratios by ~90% providing more evidence for the potential of CC-4047 as a substrate for P-gp transport.

Inhibition studies confirmed that CC-4047 at concentrations up to 10 µM did not inhibit efflux of the known P-gp substrate ^{3}H-digoxin (Figure 25). In contrast, verapamil and ketoconazole almost abolish the efflux of digoxin in MDCK-MDR1 monolayers.

Figure 24: Apparent permeability of CC-4047 in MDCK-MDR1 (top panel), MDCK-WT (mid panel) and in MDCK-MDR1 (bottom panel) after inhibition of P-gp

(excerpted from Applicant's submission)

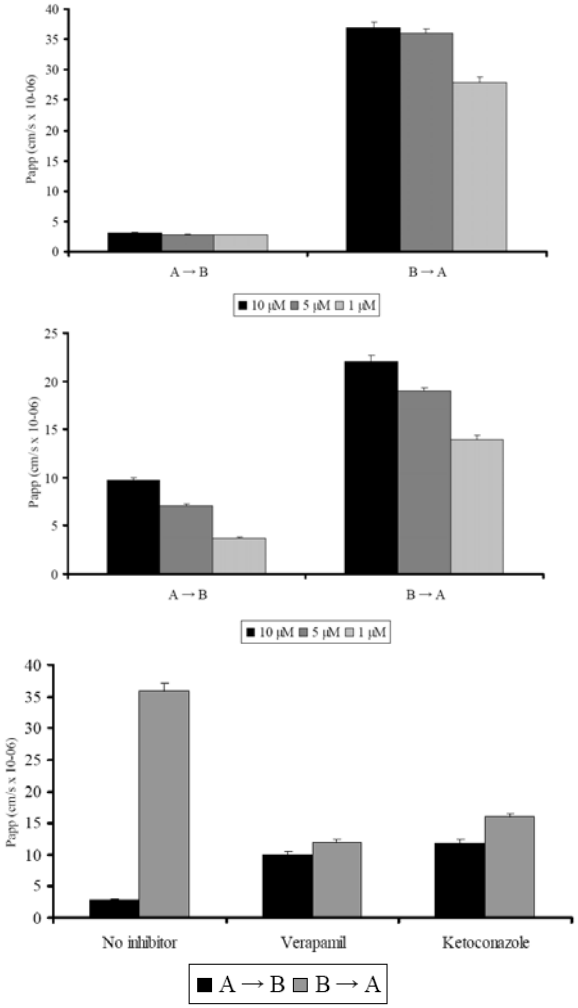
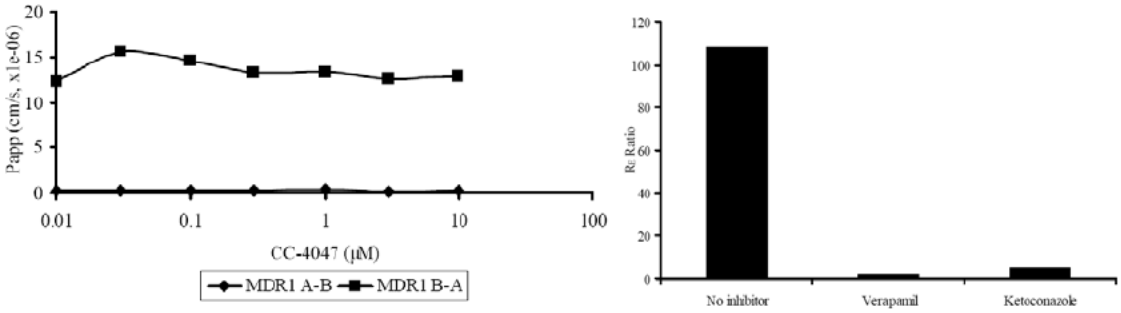


Figure 25: Inhibition of P-gp preventing the efflux of ^{3}H-digoxin in MDCK-MDR1 (left panel). CC-4047 did not have an effect on P-gp efflux (right panel).

(excerpted from Applicant's submission)



Other Pharmacokinetic Studies

Study title: *In vitro* stability and interconversion of CC-4047 enantiomers in PBS and monkey/human plasma

Study No.:	CC-4047-DMPK-030
Study report location:	eCTD 4.2.2.7
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 21, 2006
GLP compliance:	No
QA statement:	NA
Drug, lot #, and % purity:	CC-5083 (S-CC-4047), Lot # 5373-110-C, Purity: 98.5% CC-6016 (R-CC-4047), Lot # 5423-3-B, Purity: 99.1%

Key Findings

- Interconversion of CC-4047 enantiomers was observed in PBS, monkey and human plasma.
- CC-5083 and CC-6016 enantiomers presented similar patterns in degradation or racemization in PBS or monkey and human plasma.

Methods

Concentrations:	CC-5083 and CC-6016: 200 ng/mL
Incubation time/conditions:	37°C with 95% relative humidity/5% air/CO_2
Method:	Spiked 0.2 mL of PBS, monkey or human plasma incubated at 37 ºC for 0, 0.5, 1, 2, 4, 8 and 24 hours. Samples extracted and processed

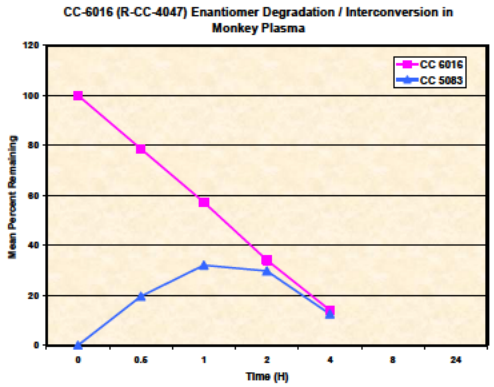
	for chiral LCMS-MS quantitative analysis
Measurements:	Positive ions were detected in the multiple reaction monitoring (MRM) mode with precursor→product ion pairs of m/z 274.1→83.9 for CC-6016 and CC-5083 and m/z 259.1→186.1 for the IS (Thalidomide).
Species/Strain:	Monkey and human plasma unknown origin
Number of samples:	Triplicate determinations

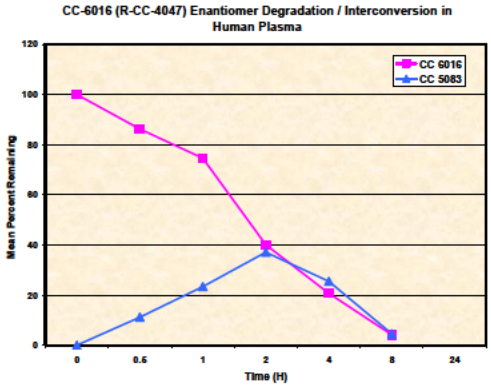
Results

Gradual degradation of each enantiomer occurred with similar pattern in monkey and human plasma or PBS accounting for more than 80% degradation by 4, 8, or 24 hours after incubation in monkey and human plasma or PBS (Figure 26 and Figure 27).

Interconversion from R to S or S to R occurred with a similar pattern in all three matrices.

Figure 26: CC-6016 R-enantiomer degradation/interconversion to CC-5083 S-enantiomer in monkey and human plasma and PBS





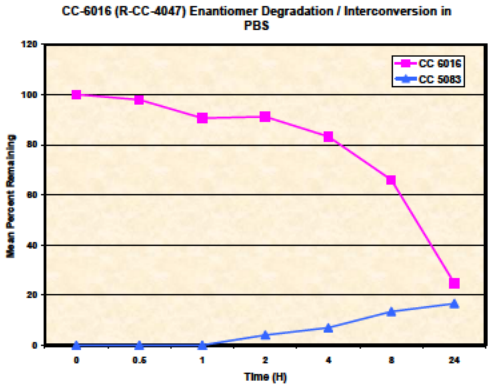
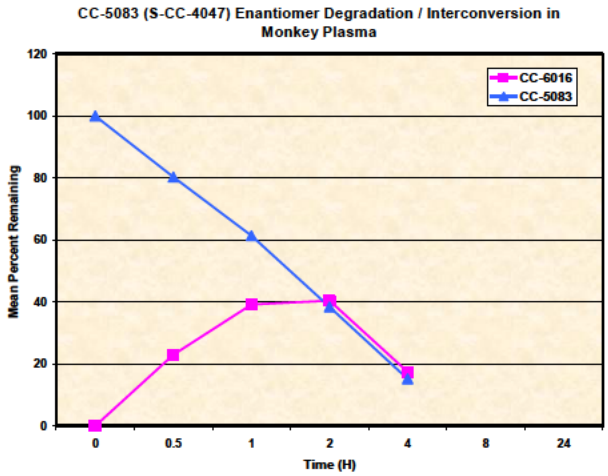
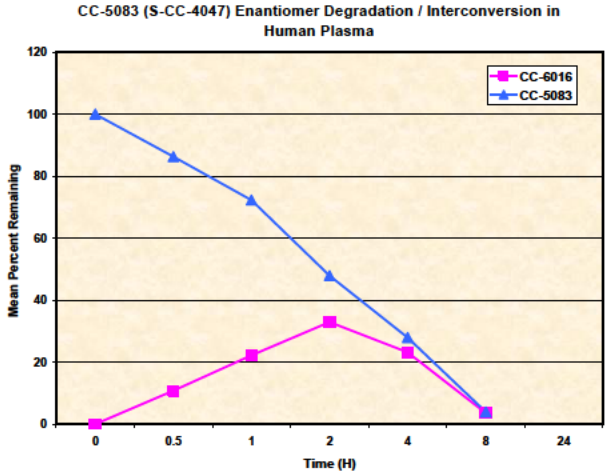
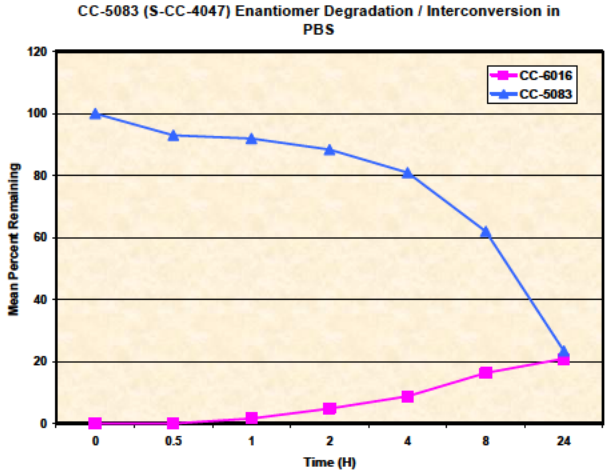


Figure 27: CC-5083 S-Enantiomer Degradation/Interconversion to CC-6016 R-Enantiomer in Monkey and Humans Plasma and PBS







6 General Toxicology

6.1 Single-Dose Toxicity

The single-dose toxicity studies were not reviewed.

6.2 Repeat-Dose Toxicity

Study title: CC-4047: 7-day oral (gavage) range-finding toxicity study in the rat

Study no.:	1398/114
Study report location:	eCTD 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 6, 1998
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CC-4047, lot # 6, batch # 40753-10, Purity: 99.6%

Study was reviewed under IND 66188 by Dr. M. Anwar Goheer, in the Division of Oncology Drug Products. The review was slightly modified to fit this NDA review:

Key Study Findings

- CC-4047 up to 5000 mg/kg/day for 7 days was well tolerated by rats.

Methods

Doses:	0, 100, 300, or 1000 mg/kg/day
Frequency of dosing:	Daily for 7 days
Route of administration:	Oral (gavage)
Dose volume:	10 mL/kg
Formulation/Vehicle:	Carboxymethyl cellulose (1%, w/v)
Species/Strain:	Crl:CDBR (SD) rats
Number/Sex/Group:	5/sex/group
Age:	6-7 weeks
Weight:	Males: 193-289 g Females: 128-170 g
Satellite groups:	Toxicokinetics: 5 males/group
Unique study design:	None
Deviation from study protocol:	Due to a lack of any overt toxicity after 7 days, additional males (5/group for main study and 4/group for toxicokinetics) were dosed for 7 days at 2000, 3000, or 5000 mg/kg/day. The 5000 mg/kg/day dose was administered at a dose

volume of 20 mL/kg.

Observations and times

Clinical signs:	Daily
Body weights:	Days 0, 3, 7, and before necropsy
Food consumption:	Daily
Hematology:	At necropsy
Clinical chemistry:	At necropsy
Gross pathology:	At necropsy
Toxicokinetics:	Day 1 at 1, 2, 4, 8, and 24 hours after dosing

Results

Mortality:	None
Clinical signs:	No test article-related clinical signs except yellow feces on days 6 and 7.
Body weights:	No effect
Food consumption:	No difference between the treatment groups
Hematology:	No difference
Clinical chemistry:	No difference between groups
Organ weights:	No test article-related trends were apparent
Gross pathology:	No test article-related gross findings
Toxicokinetics:	

Dose (mg/kg/day)	Male		
	AUC $_{0\text{-}24\ h}$ (ng.h/mL)	C_{max} (ng/mL)	T_{max} (h)
2000	104423	7163	2
3000	80620	7592	2
5000	87563	8047	2

There was no dose-related increase in exposure indicating that the absorption process may have become saturated with test article.

Study title: CC-4047: 28 day oral (gavage administration) toxicity study in the rat

Study no.:	1398/115
Study report location:	eCTD 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 30, 1998

GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CC-4047, batch # 40753-10 and 40753-09, Purity: 99.6%

Study was reviewed under IND 66188 by Dr. M. Anwar Goheer, in the Division of Oncology Drug Products. The review was slightly modified to fit this NDA review:

Key Study Findings

- The oral (gavage) administration of the test article (CC-4047) at 300, 800 or 2000 mg/kg/day for 28 days did not produce any toxicity in the rat.

Methods

Doses:	0, 300, 800, or 2000 mg/kg/day
Frequency of dosing:	Daily for 28 days
Route of administration:	Oral (gavage)
Dose volume:	10 mL/kg
Formulation/Vehicle:	Carboxymethyl cellulose (1%, w/v)
Species/Strain:	Crl:CDBR (SD) rats
Number/Sex/Group:	10/sex/group
Age:	6 weeks
Weight:	Males: 181-210 g Females: 152-177 g
Satellite groups:	Toxicokinetics: 12/sex/group (300, 800, or 2000 mg/kg/day)

Observations and times

Clinical signs:	Daily
Body weights:	Weekly
Food consumption:	weekly
Ophthalmoscopy:	Weeks 0 and 4
Hematology:	Week 4
Clinical chemistry:	Week 4
Urinalysis:	During week 4
Gross pathology:	At the end of study
Organs weighed:	See histopathology inventory at the end of this section
Histopathology:	See histopathology inventory at the end of this section
Toxicokinetics:	Days 1 and 28 at 0, 15, 30 minutes, 1, 2, 4, 8, and 16 hours after dosing.

Results

Mortality:	Group 4 – 1 female died on day 15, unrelated to treatment
Clinical signs:	No test article-related clinical findings.
Body weights:	No significant differences between test and control animals.
Food consumption:	No test article-related effect

Ophthalmoscopy: No treatment-related changes
Hematology: No effect of treatment during week 4
Clinical chemistry: No test article-related effect on clinical chemistry parameters.
Urinalysis: No effect
Organ weights: There was no effect of the test-article on organ weights.
Gross pathology: No macroscopic evidence of test article toxicity.
Histopathology: No microscopic findings suggestive of systemic toxicity.
Toxicokinetics: There was no accumulation or induction of metabolism with multiple dosing. AUC and C_{max} values were not proportional to dose.

Table 31: Summary of toxicokinetic parameters of CC-4047 in rat following oral administration of 300 mg/kg for 28 days

(excerpted from Applicant's submission)

Parameter	Male		Female	
	Day 1	Day 28	Day 1	Day 28
C_{max}(ng/mL)	4496.4	3673.8	7246.1	5461.1
T_{max} (h)	4.0	2.0	1.0	2.0
$AUC_{(0\text{-}16\ h)}$ (ng.h/mL)	50021.6	30920.2	69077.6	61305.0

Table 32: Summary of toxicokinetic parameters of CC-4047 in rat following oral administration of 800 mg/kg for 28 days

(excerpted from Applicant's submission)

Parameter	Male		Female	
	Day 1	Day 28	Day 1	Day 28
C_{max} (ng/mL)	6964.2	4825.1	7911.5	11045.20
T_{max} (h)	4.0	4.0	4.0	2.0
$AUC_{(0\text{-}16\ h)}$ (ng.h/mL)	69160.0	35755.4	87182.9	92903.3

Table 33: Summary of toxicokinetic parameters of CC-4047 in rat following oral administration of 2000 mg/kg for 28 days

(excerpted from Applicant's submission)

Parameter	Male		Female	
	Day 1	Day 28	Day 1	Day 28
C_{max} (ng/mL)	9024.2	7347.7	11328.3	13564.7
T_{max} (h)	4.0	4.0	4.0	2.0
$AUC_{(0\text{-}16\ h)}$ (ng.h/mL)	93163.7	62096.5	132777.0	130315.0

Study title: CC-4047: A 90-day oral toxicity and toxicokinetics study in Sprague-Dawley rats

Study no.:	CC-4047-TOX-001
Study report location:	eCTD 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 5, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CC-4047, lot # 61749-06, Purity: 99.39%

Study was briefly reviewed under IND (b) (4) by (b) (4) The review was slightly modified to fit this NDA review:

Oral gavage doses of 100, 500, or 1500 mg/kg CC-4047 were administered to 10 female and 10 male rats per dose group. Toxicokinetics showed small increases of AUC from Day 1 to Day 29 to Day 90 with higher increases in females. C_{max} did not change significantly either, although a slight accumulation was observed in females.

Effects: Reduced body weights in males of 1500 mg/kg from Days 63 to 90. No other findings in clinical parameters, in macro or microscopic evaluation.

Study title: A 6-month toxicity study of CC-4047 administered by oral gavage to rats with a 1-month recovery period

Study no.:	CC-4047-TOX-013
Study report location:	eCTD 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 5, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CC-4047, lot # CMLW-174/06-CC2, Purity: 100.4% and lot # 75838-07, Purity: 99.8%

Key Study Findings

- Oral administration of CC-4047 at 50, 250 or 1000 mg/kg/day for 180 days did not produce any clear toxicity in the rat.

Methods

Doses:	0, 50, 250, or 1000 mg/kg/day
Frequency of dosing:	Once daily for 180 days; 28-day recovery period Males dosed on Days 1-180 and females were dosed Days 1-181
Route of administration:	Oral (gavage)
Dose volume:	10 mL/kg
Formulation/Vehicle:	1% carboxymethylcellulose in reverse osmosis deionized water
Species/Strain:	Sprague-Dawley (Crl:CD(SD)) rat
Number/Sex/Group:	Main study: 15/sex/group Recovery: 5/sex/group
Age:	8 weeks at time of randomization
Weight:	Males: 228-260 g at time of randomization Females: 168-205 g at time of randomization
Satellite groups:	Toxicokinetics: 3/sex/group (control group) and 9/sex/group (50, 250, and 1000 mg/kg/day CC-4047 groups)

Table 34: Experimental design for 6-month rat study

(excerpted from Applicant's submission)

Group No.	No. of Animals				Dose Material	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Conc. (mg/mL)
	Toxicity (Recovery)		Toxicokinetic					
	Male	Female	Male	Female				
1	15 (5)	15 (5)	3	3	1% CMC	0	10	0
2	15 (5)	15 (5)	9	9	CC-4047	50	10	5
3	15 (5)	15 (5)	9	9	CC-4047	250	10	25
4	15 (5)	15 (5)	9	9	CC-4047	1000	10	100

Observations and times:

Mortality:	Twice daily
Clinical signs:	Detailed observations on Day -3, twice daily during the treatment period (Days 1-180/181), and weekly during the recovery period (Days 187,194, 201, and 208), and on day of scheduled necropsy
Body weights:	Day -3, weekly during the treatment and recovery periods, and prior to necropsy
Food consumption:	Weekly during the treatment and recovery periods
ECG:	Not conducted
Ophthalmoscopy:	Day -4, Day 180, and Day 208 (recovery)
Hematology:	Days 91 and 181 (males), Days 92 and 182 (females), Day 209 (recovery)
Clinical chemistry:	Days 91 and 181 (males), Days 92 and 182 (females), Day 209 (recovery)
Coagulation:	Days 91 and 181 (males), Days 92 and 182 (females), Day 209 (recovery)
Urinalysis:	Days 91 and 181 (males), Days 92 and 182 (females), Day 209 (recovery)
Gross pathology:	At necropsy*
Organ weights:	At necropsy*
Histopathology:	At necropsy*
Toxicokinetics:	Days 1, 92, and 180 at 0 (prior to dosing), 0.5, 2, 4, 8, and 24 hours after dosing for 50, 250, and 1000 mg/kg/day groups and prior to dosing in the control (0 mg/kg/day) group

* Necropsy on Day 181 for males and on Day 182 for females for main study and on Day 209 for recovery animals

Results

Mortality

Two control males, two females at 50 mg/kg/day, two females at 250 mg/kg/day and 4 females at 1000 mg/kg/day were found dead or euthanized prior to the end of the study for various reasons. None of the early deaths were attributed to the test article by the Applicant because they occurred shortly after blood collection or were the result of injury or incidental causes with no relationship to test article administration. The early deaths are listed in the table below.

Table 35: Summary of mortality for 6-month rat study

(excerpted from Applicant's submission)

Group No.	Dose Level (mg/kg/day)	Animal No.	Sex	Study Day	Cause of Death
1	0	5889	male	134	Moribund Euthanasia (pyelonephritis observed histopathologically)
		5892	male	80	Found Dead (pyelonephritis observed histopathologically)
2	50	5975	female	92	Accidental Death (post-blood collection)
		5982	female	77	Moribund Euthanasia (fractured leg)
3	250	5995	female	92	Found Dead (shortly after blood collection)
		6012	female	90	Moribund Euthanasia (impaired mobility dragging hindlimbs/urinary bladder hemorrhage observed histopathologically)
4	1000	6017	female	28	Moribund Euthanasia (intubation error confirmed at necropsy)
		6018	female	92	Found Dead (shortly after blood collection)
		6032	female	92	Accidental Death (post-blood collection)
		6015	female	141	Moribund Euthanasia (fractured leg)

Clinical Signs

There were no clear test article-related clinical signs observed during the study. Hairloss and scabbing were frequent observations observed in all groups; the incidences of these observations were higher in males treated with CC-4047, but not females. Higher incidences of urine stain were also observed in males treated with CC-4047. Malaligned, broken, and/or missing teeth were observed in all groups and the incidences were higher in CC-4047-treated animals.

Table 36: Clinical signs observed during the treatment period

Clinical signs	No. of animals affected (No. of Observations)							
	Males				**Females**			
Dose (mg/kg/day)	0	50	250	1000	0	50	250	1000
Number of animals examined	20	20	20	20	20	20	20	20
Scabs	1 (8)	3 (15)	2 (9)	5 (91)	13 (3)	-	2 (12)	2 (13)
Hairloss	6 (405)	9 (711)	8 (934)	10 (1179)	8 (822)	10 (1233)	10 (792)	6 (594)
Urine stain	1 (2)	2 (18)	3 (13)	3 (131)	-	1 (1)	-	3 (4)
Malalignment	-	1 (139)	3 (458)	3 (180)	1 (4)	2 (3)	1 (8)	3 (167)
Incisor(s) broken	2 (15)	2 (8)	3 (114)	3 (59)	1 (1)	3 (15)	5 (38)	4 (38)
Incisor(s) missing	1 (5)	-	2 (185)	2 (8)	-	1 (3)	2 (97)	3 (208)

- = Clinical sign not observed in this group

Body Weights

Unremarkable

Food Consumption

Unremarkable

Ophthalmoscopy

Unremarkable

ECG

Not conducted

Hematology

Although there were statistically significant changes in several parameters including erythrocytes, hemoglobin, reticulocytes, platelets, lymphocytes, prothrombin time, and fibrinogen, there were no clear dose-response relationships, findings consistent across time points, or findings observed in both males and females at the same doses and time points. Additionally, most of the differences were relatively small and the values were within the range for historical control values provided by the Applicant. Therefore, no clear test article-related changes in hematology or coagulation parameters were observed in the study. Below are the larger changes observed:

- Fibrinogen was increased by 10-16% in females treated with CC-4047 on Day 92, however, similar increases were not observed on Day 182 at the end of treatment or in males.
- Platelets were decreased by 11% in females treated with 250 and 1000 mg/kg/day on Day 182, but the finding was not observed on Day 92 or in males.
- Lymphocytes were decreased by 20% and 17% in males treated with 250 and 1000 mg/kg/day respectively on Day 91, but the finding was not observed on Day 181 or in females.

Clinical Chemistry

There were no clear test article-related changes in clinical chemistry. There were statistically significant changes in several parameters, however, the values were all within the range for historical control values provided by the Applicant.

- Decreases in total protein, albumin, and globulin were observed in females treated with CC-4047 at all doses on Days 92 and 182, but the decreases were generally not dose dependent. Globulin only was significantly decreased (7%) in males treated with 250 mg/kg/day on Day 181; this finding was not observed at 1000 mg/kg/day in males.
- Phosphorus was increased in females treated with CC-4047 at all doses on Day 92, but this finding was not observed at Day 182 or in males. Phosphorus was actually decreased (10%) in males at 250 mg/kg/day on Day 91.
- Potassium was also increased in females at all doses on Day 92 and at 50 and 250 mg/kg/day on Day 182; this finding was not observed in males.

The clinical chemistry changes in females are shown in the table below.

Table 37: Clinical chemistry changes in females

Parameter	Mean		Percentage deviation from Control					
	Control 0 mg/kg/day		50 mg/kg/day		250 mg/kg/day		1000 mg/kg/day	
	Day 92	Day 182	Day 92	Day 182	Day 92	Day 182	Day 92	Day 182
Total protein	7.19	6.64	↓8*	↓11*	↓10*	↓12*	↓10*	↓11*
Albumin	3.81	3.56	↓10*	↓13*	↓12*	↓15*	↓13*	↓12*
Globulin	3.38	3.07	↓6*	↓8	↓8*	↓9*	↓7*	↓10*
Phosphorus	4.63	5.53	↑25*	-	↑27*	-	↑21*	-
Potassium	4.15	4.08	↑8*	↑13*	↑6*	↑11*	↑8*	-

↑= increase ↓=decrease - = no test-article related changes
Significantly different from Control, * $p<0.05$

Urinalysis

- Total urine volume was significantly decreased by 30% in males treated with 1000 mg/kg/day on Day 91; this decrease was not observed on Day 181 or in females.
- There was an increased incidence of hazy urine in females treated with CC-4047 on Days 92 and 182. This finding was not observed in males.

Table 38: Appearance of urine in females

		No. of animals affected			
		Females			
Dose (mg/kg/day)		0	50	250	1000
Day 92	Clear	20	4	4	2
	Hazy	0	15	15	16
	Cloudy	0	0	0	1
Day 182	Clear	14	10	6	9
	Hazy	1	0	7	2
	Cloudy	0	3	0	0

- There was an increased incidence of nitrites in urine for females treated with CC-4047 on Day 92; this finding was not observed on Day 182 or in males.

Table 39: Nitrites in urine

Dose (mg/kg/day)		No. of animals affected							
		Males				Females			
		0	50	250	1000	0	50	250	1000
Day 91/92	Negative	16	19	20	19	20	13	12	9
	Positive	3	1	0	1	0	6	7	10
Day 181/182	Negative	13	14	15	15	15	13	13	11
	Positive	0	1	0	0	0	0	0	0

Gross Pathology

Abnormal contents consisting of yellow-colored ingesta, digesta, or fecal material were observed in various organs of the gastrointestinal tract (stomach and small and large intestines) of CC-4047-treated animals. These findings are consistent with oral administration of CC-4047, which had a yellow color. There appears to be no toxicological significance of the findings.

Table 40: Gross pathology for 6-month rat study

Treatment-Related Macroscopic Findings		No. of animals affected							
		Males				Females			
Dose (mg/kg/day)		0	50	250	1000	0	50	250	1000
Number of animals examined		2*/13/**5**	0/15/**5**	0/15/**5**	0/15/**5**	0/15/**5**	2*/13/**5**	2*/13/**5**	4*/11/**5**
Cecum	Abnormal content	-	0/1/**0**	0/7/**0**	0/14/**0**	-	-	0/13/**0**	1*/9/**0**
Colon	Contents	1*/0/**0**	-	0/1/**0**	0/7/**0**	-	-	-	0/3/**0**
Ileum	Content	-	-	0/1/**0**	0/3/**0**	-	-	-	1*/1/**0**
Jejunum	Content	-	-	-	0/1/**0**	-	-	-	-
Rectum	Contents	1*/0/**0**	-	-	0/2/**0**	-	-	-	0/2/**0**
Stomach	Abnormal content	-	-	0/1/**0**	0/4/**0**	-	-	-	1*/1/**0**

Number of animals examined and affected: Early deaths*/ Terminal necropsy / **Recovery necropsy**
- = no test-article related changes

Organ Weights

Unremarkable

Histopathology

Adequate Battery: Yes

Peer Review: Yes; a peer review of selected histopathologic findings was conducted

Histological Findings

Microscopic examinations were conducted for all tissues and organs from the control (0 mg/kg/day) and high dose (1000 mg/kg/day) groups, the gross lesions from all animals in all groups, and all tissues from early death animals in the low and mid dose (50 and 250 mg/kg/day) groups.

There were no test article-related histopathology findings.

Toxicokinetics

The toxicokinetics of CC-4047 (50, 250, and 1000 mg/kg/day) were evaluated Days 1, 92, and 180 with samples collected prior to dosing and at 0.5, 2, 4, 8, and 24 hours after dosing. Day 1 and Day 180 plasma samples were analyzed for CC-4047 (racemate) and Day 92 plasma samples were analyzed for CC-4047 enantiomers CC-5083 and CC-6016.

- CC-4047 (racemate) was absorbed after oral administration to rats with time of peak plasma concentrations (T_{max}) of 2-4 hours
- Systemic exposures (mean AUC_{24h}) for CC-4047 increased in a less than dose-proportional manner from 50 to 1000 mg/kg/day.
- Exposure (AUC_t) was 1.4-2.4 fold higher in females than male

Table 41: Summary of mean toxicokinetic parameters for CC-4047 and its enantiomers CC-5083 and CC-6016 in male and female rats after daily oral administration of CC-4047

(excerpted from Applicant's submission)

Dosing Day	Dose Level (mg/kg/day)	Analyte	Gender	C_{max} (ng/mL)	T_{max} (hr)	AUC_t (hr • ng/mL)	Dose-Normalized AUC_t[a]	Ratio of AUC_t to Lowest Dose AUC[b]	Rc[c]
Day 1	50	CC-4047	M	1457	4.0	21710	434.2	1.0	NA
	(Group2)		F	2043	4.0	30260	605.2	1.0	NA
	250		M	2045	4.0	30770	123.1	1.4	NA
	(Group3)		F	2949	4.0	47440	189.8	1.6	NA
	1000		M	2887	2.0	38490	38.49	1.8	NA
	(Group4)		F	4078	4.0	63050	63.05	2.1	NA
Day 92	50	CC-5083	M	472.6	2.0	6172	-	-	-
	(Group2)		F	990.7	4.0	10870	-	-	-
	250		M	691.7	4.0	7822	-	-	-
	(Group3)		F	1534	4.0	20800	-	-	-
	1000		M	1120	2.0	11410	-	-	-
	(Group4)		F	1913	4.0	25280	-	-	-
	50	CC-6016	M	850.9	2.0	13100	-	-	-
	(Group2)		F	1660	4.0	19850	-	-	-
	250		M	1487	4.0	18070	-	-	-
	(Group3)		F	2679	4.0	36770	-	-	-
	1000		M	2049	2.0	25160	-	-	-
	(Group4)		F	3427	4.0	44670	-	-	-
	50	CC-5083+ CC-6016	M	1324	2.0	19270	385.4	1.0	0.89
	(Group2)		F	2651	4.0	30730	614.6	1.0	1.02
	250		M	2178	4.0	25890	103.6	1.3	0.84
	(Group3)		F	4213	4.0	57570	230.3	1.9	1.21
	1000		M	3169	2.0	36570	36.57	1.9	0.95
	(Group4)		F	5340	4.0	69950	69.95	2.3	1.11
	50	S/R AUC_t Ratio	M	-	-	47.11	-	-	-
	(Group2)		F	-	-	54.76	-	-	-
	250		M	-	-	43.29	-	-	-
	(Group3)		F	-	-	56.57	-	-	-
	1000		M	-	-	45.35	-	-	-
	(Group4)		F	-	-	56.59	-	-	-
Day 180	50	CC-4047	M	1566	2.0	21440	428.8	1.0	0.99
	(Group2)		F	3377	4.0	40420	808.4	1.0	1.34
	250		M	2277	4.0	31120	124.5	1.5	1.01
	(Group3)		F	4322	4.0	70170	280.7	1.7	1.48
	1000		M	3813	2.0	42530	42.53	2.0	1.10
	(Group4)		F	6776	2.0	98010	98.01	2.4	1.55

C_{max} and AUC_t are presented to 4 significant figures

NA = not applicable

[a] Dose-normalized AUC is calculated as AUC_t ÷ Dose, expressed as (ng • hr/mL)/(mg/kg)

[b] AUC ratio between the dose and the lowest dose

[c] Rc = AUC_t(Day 92 or 180)/AUC_t(Day 1)

S/R AUC_t Ratio = AUC_t(CC-5083)/AUC_t(CC-6016)*100%

Study title: CC-4047: Maximum tolerated dose (MTD) followed by a fixed dose oral (gavage) toxicity study in the monkey

Study no.:	1398/116
Study report location:	eCTD 4.2.3.2
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	May 7, 1998
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CC-4047, batch # 40753-10, Purity: 99.6%

Study was reviewed under IND 66188 by Dr. M. Anwar Goheer, in the Division of Oncology Drug Products. The review was slightly modified to fit this NDA review:

Key Study Findings

- Repeat dosing at 50 mg/kg/day for 7 days was well tolerated. Doses to 1200 mg/kg/day for 14 days were not lethal. Statistical analyses were not done due to small group size (2 animals/sex/group).

Methods

Doses:	0, 50, 100, 300, 600, and 1200 mg/kg/day, see tables below
Frequency of dosing:	See tables below for schedule
Route of administration:	Oral (gavage)
Dose volume:	4 mL/kg
Formulation/Vehicle:	Carboxymethyl cellulose (1%, w/v)
Species/Strain:	Cynomolgus monkeys
Number/Sex/Group:	2/sex/group
Age:	Not provided
Weight:	1.8-2.8 kg
Satellite groups:	None

The objective of this study was to determine the MTD for oral administration of CC-4047 in the cynomolgus monkey. After completion of the MTD phase study, the animals were allocated to Fixed Dose Phase 1 (1200 mg/kg/day for 14 days), Phase 2 (300 mg/kg/day for 14 days) and Phase 3 (50 mg/kg/day for 7 days).

MTD Phase

Group #	Group description	Dose level (mg/kg/day)	Day of study	Animals/group	
				Male	Female
1	Control	0	1-14	2	2
2	Test	100	1-4	2	2
		300	5-7		
		600	8-11		

Group #	Group description	Dose level (mg/kg/day)	Day of study	Animals/group	
				Male	Female
		1200	12-14		

Fixed Dose Phase

Group #	Group description	Fixed dose phase	Dose level (mg/kg/day)	Duration of dosing (days)	Animals/group	
					Male	Female
1	Test	1	1200	14	2	2
3	Test	2	300	14	2	2
4	Control	3	0	7	2	2
5	Test	3	50	7	2	2

Observations and times

Clinical signs: Daily
Body weights: Once or twice weekly
Food consumption: Daily
Hematology: Pre and at the end of treatment
Clinical chemistry: Days 3, 6, 10, and 13
Gross pathology: At necropsy
Organs weighed: See histopathology inventory at the end of this section
Histopathology: See histopathology inventory at the end of this section
Toxicokinetics: Fixed dose animals

Phase (dose)	Day(s)
1	14
2 (300 mg/kg/day)	1 & 14
3 (50 mg/kg/day)	1 & 7

Samples were drawn at 0, 1, 2, 4, 8 and 24 hours after dosing.

Results

Mortality: None
Clinical signs: 300 mg/kg/day – Yellow discolored urine and feces (all animals), hunched posture and piloerection (1 animal)
Body weights: 50 mg/kg/day – comparable to controls
300 & 1200 mg/kg/day - Decreased
Food consumption: No effect
Hematology: 300 and 1200 mg/kg/day - Red (Hb, RBC and PCV) and white blood cell counts were reduced compared to control animals.
Clinical chemistry: Not affected
Organ weights: No difference except slightly increased spleen weights at all doses.
Gross pathology: Enlarged spleen, toxicological significance uncertain due to lack of histopathological examination.
Histopathology: Not submitted

Toxicokinetics:

Dose level (mg/kg/day)	Sex	C_{max} (ng/mL)		T_{max} (h)		$AUC_{0-24\ h}$ (ng.h/mL)	
		Day 1	Day 7or 14	Day 1	Day 7 or 14	Day 1	Day 7 or 14
300	M	2560	7376	3.0	3.0	33048	113787
	F	2649	10219	3.0	1.5	35282	173278
50	M	1648	6772	6.0	2.5	33830	93366
	F	2327	5813	5.0	1.5	39791	84595

At 300 mg/kg/day, C_{max} and $AUC_{0-24\ h}$ increased up to six folds from day 1 to day 14 for both males and females. C_{max} and $AUC_{0-24\ h}$ at 50 mg/kg/day also increased two to four folds from day 1 to day 7 for both sexes.

Study title: CC-4047: 28-day oral (gavage administration) toxicity study in the monkey

Study no.:	1398/117
Study report location:	eCTD 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 23, 1998
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CC-4047, batch # 40753-10, Purity: 99.6%

Study was reviewed under IND 66188 by Dr. M. Anwar Goheer, in the Division of Oncology Drug Products. The review was slightly modified to fit this NDA review:

Key Study Findings

- Daily administration of CC-4047 at 30, 100 or 300 mg/kg/day to monkeys caused hemopoietic and lymphoid systems toxicity leading to early mortalities. The study was terminated after 18 days.

Methods

Doses:	0, 30, 100, or 300 mg/kg/day
Frequency of dosing:	Daily
Route of administration:	Oral (gavage)
Dose volume:	4 mL/kg
Formulation/Vehicle:	Carboxymethyl cellulose (1%, w/v)
Species/Strain:	Cynomolgus monkeys
Number/Sex/Group:	3/sex/group
Age:	Not mentioned
Weight:	1.65-2.5 kg

Satellite groups: None
Deviation from study protocol: The study was originally planned for 28 days but was terminated after 18 days due to deterioration in the condition of the animals.

Table 42: Study design for 28-day monkey study

Group #	Dose level (mg/kg/day)	Group description	Animals/group	
			Males	Females
1	0	Control	1-3	13-15
2	30	Low	4-6	16-18
3	100	Intermediate	7-9	19-21
4	300	High	10-12	22-24

Observations and times

Clinical signs: Daily
Body weights: Weekly
Food consumption: Daily
Ophthalmoscopy: Pretreatment only
EKG: Pretreatment only
Hematology: Days 0, 8, 14 and necropsy
Clinical chemistry: Before treatment and necropsy
Gross pathology: At necropsy
Organs weighed: See histopathology inventory at the end of this section
Histopathology: See histopathology inventory at the end of this section
Toxicokinetics: At 0 (pre-dose), 15, 30 minutes, 1, 2, 4, 8, and 16 hours after dosing on days 1 and 14.

Results

Mortality

Table 43: Mortality for 28-day monkey study

Animal #	Sex	Group #	Day found dead or necropsy
18	F	2	18
20	F	3	16
8	M	3	18
24	F	4	15
11	M	4	18
23	F	4	18

Other animals were sent to necropsy on Day 18 due to deterioration in their condition.

Clinical Signs

Soft/loose feces, yellow discolored urine, hunched posture, piloerection, subdued behavior, tremors, facial and limb swelling, bleeding from the gums, and sunken eyes were noted in most treated animals.

Body Weights

The following tables (excerpted from Applicant's submission) show individual body weight gains and percentage change in body weight from Week -1 to necropsy.

Table 44: Body weight changes in male and female monkeys

(excerpted from Applicant's submission)

Group Sex	Animal number	Body weight gain (g) #	Percentage change (%) #
1M	1	100	+4
	2	0	0
	3	50	+3
2M	4	-650	-30
	5	0	0
	6	-50	-2
3M	7	-50	-3
	8	-100	-5
	9	-100	-4
4M	10	-200	-10
	11	0	0
	12	-300	-14

from Week-1 to necropsy body weight

Group Sex	Animal number	Body weight gain (g) #	Percentage change (%) #
1F	13	+100	+5
	14	+100	+4
	15	0	0
2F	16	-150	-7
	17	-50	-2
	18	-350	-17
3F	19	0	0
	20	-500	-21
	21	0	0
4F	22	-50	-3
	23	0	0
	24	-200	-9

from Week-1 to necropsy body weight

Food consumption

Food consumption was intermittently reduced for the majority of treated animals.

Ophthalmoscopy

Week 4 examination was not done.

Electrocardiography

Week 4 examination was not performed.

Hematology

Table 45: Hematology value different from control (%)

Parameter	Day	Group 2 30 mg/kg/day		Group 3 100 mg/kg/day		Group 4 300 mg/kg/day	
		Males	Females	Males	Females	Males	Females
Platelets	14	19 ↓	50 ↓	63 ↓	35 ↓	50 ↓	75 ↓
WBC	14	48 ↓	49 ↓	84 ↓	34 ↓	77 ↓	72 ↓

Clinical chemistry

Lactate dehydrogenase, total bilirubin, urea, creatinine, total cholesterol and triglycerides were increased. Calcium, glucose and total protein levels were reduced. There were lot of differences in values and no dose response.

Urinalysis

Week 4 urinalysis examination was not performed.

Organ weights

Increased liver, spleen and adrenal weights were observed.

Gross pathology

Small thymus observed in a number of animals.

Histopathology

Table 46: Incidence of selected histopathology findings in bone marrow

(excerpted from Applicant's submission)

		Group and sex							
Tissue and finding		1M	2M	3M	4M	1F	2F	3F	4F
Femoral bone marrow	Number examined	3	3	2	2	3	2	2	1
Marrow atrophy	Incidence	0	0	0	2	0	0	0	0
Altered haemopoiesis		0	0	0	0	0	1	0	0
Marrow hyperplasia		0	0	2	0	0	0	2	1
Sternal bone marrow	Number examined	3	3	2	2	3	2	2	1
Altered haemopoiesis	Incidence	0	0	0	2	0	1	0	0
Marrow hyperplasia		0	0	2	0	0	0	2	1

Table 47: Incidence of selected histopathology findings in lymphoid organs

(excerpted from Applicant's submission)

		Group and sex							
Tissue and finding		1M	2M	3M	4M	1F	2F	3F	4F
Mesenteric lymph node	Number examined	3	3	2	2	3	2	2	1
Lymphoid atrophy	Incidence	0	0	1	2	0	2	2	1
Lymphadenitis		0	1	0	0	0	0	0	0
Mandibular lymph node	Number examined	3	3	2	2	3	2	2	1
Lymphoid atrophy	Incidence	0	1	2	2	0	2	1	1
Lymphadenitis		0	1	0	0	0	2	1	0
Fibrosis		0	0	0	1	0	0	0	0
Spleen	Number examined	3	3	2	2	3	2	2	1
Lymphoid atrophy	Incidence	0	0	2	2	0	2	2	1
Thymus	Number examined	3	3	2	2	3	2	2	1
Atrophy	Incidence	0	3	2	2	0	2	2	1
Ileum	Number examined	3	3	2	2	3	2	2	1
Lymphoid atrophy	Incidence	0	3	1	2	0	1	1	1

Toxicokinetics

The C_{max} and AUC values on day 14 were higher than those on day 1 indicating an accumulation of CC-4047 with time. The T_{max} values were variable in both sexes.

Table 48: Summary of toxicokinetics for 28-day monkey study

Parameter	Dose (mg/kg/day)	Male		Female	
		Day 1	Day 14	Day 1	Day 14
C_{max} (ng/mL)	30	2038	4469	3023	4944
	100	2267	3763	3052	8396
	300	4406	11384	3440	16275
T_{max} (h)	30	4.0	1.7	2.7	1.7
	100	2.7	2.0	4.7	2.0
	300	2.7	1.7	3.3	3.0
AUC $_{0\text{-}16\ h}$ (ng.h/mL)	30	22784	48771	27511	51858
	100	26092	41822	38233	96600
	300	47043	143388	39571	127655

Study title: CC-4047 & CC-5013: 28-day oral (gavage administration) toxicity study in the monkey

Study no.:	1398/126
Study report location:	eCTD 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 2, 1998
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CC-4047, batch # 40753-09, Purity: Not stated CC-5013, batch #40778-10-4, Purity: 95%

Study was reviewed under IND 66188 by Dr. M. Anwar Goheer, in the Division of Oncology Drug Products. The review was slightly modified to fit this NDA review:

Key Study Findings

- CC-4047 at 2 mg/kg/day induced mortality (1/3) and reduced white blood cell count.
- Toxicokinetics showed an accumulation of CC-4047 in primates over the period of the study. The AUC $_{0\text{-}24\ h}$ and C_{max} values for CC-5013 did not change significantly between days 1 and 28.

Methods

Doses:	CC-4047: 0, 0.2, or 2.0 mg/kg/day CC-5013: 2.0 mg/kg/day
Frequency of dosing:	Daily
Route of administration:	Oral (gavage)
Dose volume:	4 mL/kg
Formulation/Vehicle:	Carboxymethyl cellulose (1%, w/v)
Species/Strain:	Cynomolgus monkeys
Number/Sex/Group:	3/sex/group
Age:	15-25 months
Weight:	1.6-2.9 kg
Satellite groups:	None

Observations and times

Clinical signs:	Daily
Body weights:	Weekly
Food consumption:	Weekly
Ophthalmoscopy:	Pretreatment and Week 4
EKG:	Pretreatment and Week 4
Hematology:	Before treatment and on Days 3, 7, 10, 14, 17, 21, 24, and 28
Clinical chemistry:	Before treatment and on Days 3, 7, 10, 14, 17, 21, 24, and 28
Urinalysis:	Pretreatment and in Week 4
Gross pathology:	Day 29
Organs weighed:	See histopathology inventory at the end of this section
Histopathology:	See histopathology inventory at the end of this section
Toxicokinetics:	Predose, 30 min., 1, 2, 4, 8, and 24 hours after dosing on Days 1, 14 and 28.

Results:

Mortality:	Moribund sacrifice: 1 male in 2.0 mg/kg/day CC-4047 group on Day 26 [facial swelling with ulceration and necrosis on the inside of the upper lip, no microscopic lesions but abscess was present].
Clinical Signs:	Loose feces: 1 male & 1 female in 2 mg/kg CC-4047 group
Body weights:	No effect in any group
Food consumption:	Not affected
Ophthalmoscopy:	Unaffected
Electrocardiography:	No effect on heart rate
Hematology:	White cell count was reduced (~50%) at 2.0 mg/kg/day CC-4047 (group 3) at Day 21-28 in both sexes.
Clinical chemistry:	No treatment related effect

Urinalysis: No effect
Organ weights: No effect
Gross pathology: No macroscopic findings
Histopathology: No microscopic findings except papillary mineralization in the kidneys of 2 mg/kg/day CC-4047 treated animals as shown below.

Table 49: Histopathology findings

Group	Number of animals with finding							
	1 M	2 M	3 M	4 M	1 F	2 F	3 F	4 F
Number examined	3	3	3	3	3	3	3	3
Papillary mineralization			1	1			1	
Focal nephropathy	3	2	2	3	2		1	1

Toxicokinetics:

Only CC-4047 was accumulated in the body.

Table 50: Toxicokinetic parameters of CC-4047 at 0.2 mg/kg/day

Parameter	Male		Female	
	Day 1	Day 28	Day 1	Day 28
C_{max} (ng/mL)	79	113	125	83
T_{max} (h)	1.7	16.0	1.7	8.0
AUC $_{0\text{-}24\ h}$ (ng.h/mL)	195	2,258	734	1,493

Table 51: Toxicokinetic parameters of CC-4047 at 2.0 mg/kg/day

Parameter	Male		Female	
	Day 1	Day 28	Day 1	Day 28
C_{max} (ng/mL)	356	735	385	651
T_{max} (h)	2.7	4.5	0.8	1.3
AUC $_{0\text{-}24\ h}$ (ng.h/mL)	4,882	8,116	5,134	6,133

Table 52: Toxicokinetic parameters of CC-5013 at 2.0 mg/kg/day

Parameter	Male		Female	
	Day 1	Day 28	Day 1	Day 28
C_{max} (ng/mL)	1,590	1,5040	1,692	1,552
T_{max} (h)	0.5	0.8	0.5	0.5
AUC $_{0\text{-}24\ h}$ (ng.h/mL)	3,138	2,865	2,778	2,765

Study title: CC-4047: A 13-week oral toxicity study in Cynomolgus monkeys

Study no.:	CC-4047-TOX-002
Study report location:	eCTD 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 11, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CC-4047, lot #61749-06, Purity: 99.39%

Study was reviewed under IND (b) (4) by (b) (4) The review was slightly modified to fit this NDA review:

CC-4047 was administered by nasogastric intubation once daily for 13 weeks at 0.05, 0.2, 2, and 10 mg/kg/day. The dosing formulations prepared for this study were within -1.8% and +12.8% of their targeted concentrations. Control groups were composed of 5 males and 5 females. Other groups consisted of 3 male and 3 female monkeys. Toxicokinetics show that systemic exposure (C_{max} and AUC) was proportional to the dose from 0.05 to 10 mg/kg/day through Week 4, and then up to 2 mg/kg/day through Week 13. No sex differences were observed. Dosing in the 10 mg/kg group was discontinued at the end of Week 5 due to adverse clinical signs and noteworthy changes in hematology parameters in several animals. A necropsy was conducted Week 6 for 3 animals/sex for the 10 mg/kg group and the other 2 animals/sex were used to assess recovery and were necropsied with the other groups Week 13/14.

Table 53: Study design for 13-week monkey study

(excerpted from Applicant's submission)

Group No.	Number of Males/Females	Dose Level (mg/kg)	Number Necropsied: Week 6	Week 13/14
1	5/5	0 (control)	-	5/5
2	3/3	0.05	-	3/3
3	3/3	0.2	-	3/3
4	3/3	2	-	3/3
5	5/5	10	3/3	2/2

Results

There were no significant changes in the 0.05 and 0.2 mg/kg/day groups.

Clinical signs

At 2 and 10 mg/kg/day: watery stool with more severe incidence in the 10 mg/kg/day group. This sign was also observed with lower incidence in control group (3/10), 0.05 mg/kg/day group (5/6), and 0.2 mg/kg/day group (1/6). Sunken eyes were noted in 1 female at 2 mg/kg/day, and 3 females at 10 mg/kg/day, and this effect resolved within days.

Body weight

In the 10 mg/kg/day group, 15 to 31% body weight loss was noted in 4/10 monkeys, although it was not considered statistically significant. This decrease was associated with low food consumption and watery stool.

ECG

ECG at pre-study, and during Week 13, at 2-4 hours following test article administration to coincide with T_{max}, were considered qualitatively and quantitatively normal with no occurrence of arrhythmias and no QTc changes.

Ophthalmoscopy

One monkey treated with 2 mg/kg/day had subcapsular opacities in the right eye on Week 12-13, which was not considered drug related by the Applicant.

Clinical chemistry

Decreased albumin and total protein concentrations were noted in all animals of 10 mg/kg/day group on Week 5, and in 3/6 animals of 2 mg/kg/day group on Weeks 5, 9, and 13. This finding was attributed to inflammation resulting from CC-4047 treatment. Sporadic decreases of glucose concentration were noted in animals that exhibited weight loss in the 10 mg/kg/day group (6/10). There was an increase in ALP levels in 4/10 animals of 10 mg/kg/day group by Week 5.

Hematology

Eight of 10 animals of the 10 mg/kg/day group had clinical pathology findings suggestive of systemic inflammation including band neutrophils, Dohle bodies in neutrophils, increase in fibrinogen concentration, and an increase or decrease of neutrophil counts. Band cell neutrophils were also observed in 5/6 animals dosed at 2 mg/kg/day by week 9, but were present in only 2 animals of this group on Week 13. Low RBC and low Hb and Hct were noted in 4/10 monkeys in the 10 mg/kg/day group. The decreases in erythrocytic mass were associated with slight to moderate reticulocytosis in 6/10 animals.

Flow cytometry analyses results

At 2 and 10 mg/kg/day, decrease in B-lymphocytes (CD20+), NK cells (CD3-/CD16+), monocytes in Weeks 6-9. Low B-lymphocyte and monocyte counts persisted through Week 13. At 10 mg/kg/day, there were decreases in T-lymphocytes and T-helper lymphocytes.

Necropsy and Microscopic findings

Decreased thymus size was noted in 2/10 control animals, in 2/6 animals dosed with 0.05 mg/kg/day, in 1/6 animal dosed with 0.2 mg/kg/day, and 6/6 animals dosed with 2 mg/kg/day CC-4047. This decrease was attributed by the sponsor to maturation.

A significant decrease in thymus weights was observed in the 2 and 10 mg/kg/day groups. A 30% increase in the size of the liver was noted in the 2 mg/kg/day group; however, there were no associated liver function changes (ALP, AST).

Lesions were observed in bone marrow, spleen and thymus in the 2 and 10 mg/kg/day groups.

1. Lesions in bone marrow were associated with hypercellularity, myeloid series immaturity, and decreased megakaryocytes.

2. Lesions in spleen included either lymphoid depletion or lymphoid hyperplasia.

3. Lesions in thymus were found in all groups with increased incidence and severity in the highest dosed groups: 2/10 in controls, 2/6 at 0.05 mg/kg/day, 1/6 at 0.2 mg/kg/day, 6/6 at 2 mg/kg/day, and 5/6 (6-week sacrifice) at 10 mg/kg/day.

Spleen and marrow lesions persisted but thymus lesions were not found in the 10 mg/kg/day animals allowed to recover for 8 weeks. Thus, the thymus lesions observed at 0.05 and 0.2 mg/kg/day may likely resolve after the same recovery period.

Study title: A 9-month oral toxicity study of CC-4047 administered by nasogastric gavage to cynomolgus monkeys, with an 8-week recovery period

Study no.:	CC-4047-TOX-006
Study report location:	eCTD 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 8, 2008
GLP compliance:	Yes
QA statement:	Yes

Drug, lot #, and % purity: CC-4047, lot # CMLW-174/06-CC2, Purity: 100.4%

Key Study Findings

- Treatment with the highest dose (1.0 mg/kg/day) produced morbidity resulting in early euthanization of 3 males and 3 females. The principle causes of morbidity were chronic inflammation of the large intestine with or without villous atrophy of the small intestine, staphylococcus infection, and acute myeloid leukemia.
- At 1.0 mg/kg/day, a decrease in peripheral lymphocytes correlated with lymphoid hypocellularity of the bone marrow, and lymphoid depletion of various lymphoid tissues. Lymphoid depletion of the spleen was also observed at 0.05 and 0.1 mg/kg/day.
- Chronic inflammation of the large intestine and irreversible proliferation of intrahepatic bile ducts were observed in surviving monkeys at 1.0 mg/kg/day.

Methods

Doses:	0, 0.05, 0.1 or 1.0 mg/kg/day
Frequency of dosing:	Once daily for 39 weeks; 8-week recovery period
Route of administration:	Nasogastric gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	1% w/v sodium carboxymethylcellulose sodium salt in deionized water and 100 mM acetate buffer
Species/Strain:	Cynomolgus monkeys
Number/Sex/Group:	Main study: 4/sex/group Recovery: 2/sex/group
Age:	Males: 2.4-3.9 years Females: 2.6-4 years
Weight:	2.1-2.7 kg on Day -1
Satellite groups:	None
Deviation from study protocol:	Due to 2 early deaths in the 1.0 mg/kg/day group, the study design was revised to add 2 monkeys (1/sex) to the 1.0 mg/kg/day group

Table 54: Experimental design for 9-month monkey study

(excerpted from Applicant's submission)

Group No.	No. of Males/ Females	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Solution Concentration (mg/mL)	No. Necropsied	
					Terminal Day 274	Recovery Day 330
1	6/6	0 (vehicle control)	5.0	0.00	4/4	2/2
2	6/6	0.05	5.0	0.01	4/4	2/2
3	6/6	0.10	5.0	0.02	4/4	2/2
4	7/7 [a]	1.00	5.0	0.20	2[b]/2[c]	2/1[d]

[a] One male and 1 female were added to Group 4 due to 2 early deaths in that group; they were assigned to Set B.
[b] Animal 4103 was euthanized on Day 44 and was replaced by Animal 4203, which was euthanized on Day 139. Animal 4205 was euthanized on Day 195.
[c] Animal 4601 was found dead on Day 40 and was replaced by Animal 4701, which was euthanized on Day 139.
[d] Animal 4603 was euthanized on Day 195 and Animal 4604 was euthanized on Day 253.

Observations and times:

Mortality:	Twice daily
Clinical signs:	Cage side observations: Daily from Day -7 to Day 330 Post-dose observations, ~2 hours after dosing
Body weights:	Twice prior to first dose and weekly starting on Day 7
Food consumption:	Daily
ECG:	Weeks 12, 24, and 39 (2-3 hours after dosing), Week 47 (recovery)
Ophthalmoscopy:	Prestudy, Weeks 18 and 39, Week 44 (recovery)
Hematology:	Week -1, near the end of Weeks 2, 4, 8, 12, 21, 27, 31, 35, and 39, Week 47 (recovery)
Clinical chemistry:	Weeks -1, near the end of Weeks 12, 21, 27, 31, 35, and 39, Week 47 (recovery)
Coagulation:	Weeks -1, near the end of Weeks 12, 21, 27, 31, 35, and 39, Week 47 (recovery)
Urinalysis:	Week prior to initiation of dosing, near the end of Weeks 12, 21, and 39, Week 47 (recovery)
Gross pathology:	At necropsy*
Organ weights:	At necropsy*
Histopathology:	At necropsy*
Toxicokinetics:	Days 1 and 28, and Weeks 28 and 39 at 0 (prior to dosing), 0.5, 2, 4, 8, and 24 hours after dosing

* Necropsy on Day 274 for main study and on Day 330 for recovery animals

At Week 35 (Day 245), Animal 4604 treated with 1.0 mg/kg/day was identified as having an exceptionally high WBC count consisting primarily of atypical cells. Prior to euthanasia and necropsy on Day 253, numerous additional procedures and sample collections were conducted to assess the animal's condition including the following:

- A complete physical evaluation of the sedated animal with ultrasound imaging and radiographs
- Blood collection on Day 250 for analysis via flow cytometry to further characterize the cell types
- A bone marrow biopsy sample was collected
- A bone marrow aspirate was collected for smear preparation and evaluation
- A bone marrow aspirate was collected for cytogenetic analysis
- Clinical pathology samples including serum chemistry, hematology, and coagulation were collected at necropsy
- Blood was collected (via vena cava) and separated into plasma and whole blood and archived
- Representative sections of liver, spleen, thymus, skin, and lymph nodes were collected, snap frozen and archived
- Three sets of bone marrow smears were prepared from the 6^{th} rib and 7^{th} rib and were archived

- Bone marrow was collected from both femurs and humerous; the bone marrow was processed to RNA extracts and cell pellets; additional bone marrow was collected from radius and pelvis and prepared as both fresh-frozen OCT and FFPE
- Additional OCT and FFPE samples of pancreatic and sublumbar lymph nodes were collected at necropsy
- Photographs were collected at necropsy
- Serum and EDTA blood samples were collected at necropsy and were sent for viral testing for multiple viruses

Results

Mortality

The monkeys who were found dead or euthanized early in the study are listed along with the cause of death or morbidity in the table below.

Table 55: Early deaths in 9-month monkey study

(excerpted from Applicant's submission)

Animal Number / Gender	Day of Death	Death Code	Principal Cause of Death or Morbidity
4601/F[a]	40	D	Pulmonary edema likely secondary to vomiting and aspiration
4103/M	44	E	Staphylococcus infection
4203/M	139	E	Chronic inflammation of large intestine and villous atrophy of the small intestine.
4701/F	139	E	Chronic inflammation of large intestine and villous atrophy of the small intestine.
4205/M	195	E	Chronic inflammation of large intestine
4603/F	195	E	Chronic inflammation of large intestine and villous atrophy of the small intestine.
4604/F	253	E	Acute myeloid leukemia-like findings

M = male; F = female; D = died; E = euthanized
[a] = Death was unrelated to test article administration.

Test article-related morbidity was observed in the 1.0 mg/kg/day group, which resulted in 3 males and 3 females being euthanized.

- Four of the monkeys (2 males and 2 females) had chronic inflammation of the large intestine and 3 of these also had villous atrophy of the small intestine. Lymphoid depletion of various lymphoid tissues and thymus was also observed in histopathology and correlated to the decrease in peripheral lymphocytes and bone marrow lymphoid depletion. Clinical signs observed in these animals included watery feces, inappetence, dehydration, hunched posture, and decreased body weight gain compared to controls.

- One male (Animal # 4103) had disuse of both hind limbs on Study Day 43 and was euthanized on Day 44. The animal actually died after receiving sedation prior to euthanasia. Histopathology findings indictated that the monkey had an acute *Staphylococcus aureus* infection involving the tissue surrounding the thoracic and lumbar vertebrae, marrow cavity and meninges of the spinal cord and brain, with hematogenous spread to the lungs and myocardium. Hematology and clinical chemistry changes were consistent with findings of inflammation and infection.
- One female (Animal # 4604) had an exceptionally high WBC count (114,600 cells/µL) consisting primarily of atypical cells at Week 35 (Day 245). The animal received its last dose of drug on Study Day 251 and was euthanized on Study Day 253. As mentioned in the methods, additional procedures and sample collections were conducted to assess the animal's condition. It was determined that the animal had test-article related findings consistent with acute myeloid leukemia (AML). Based on the rarity of this type of neoplasm in nonhuman primates, the known association of AML and immunosuppression in humans, and the demonstrated immunotoxicity in the study, the neoplasm in this animal is attributed to the immunosuppressive effects of the drug. A summary of the significant findings for Animal # 4604 is in the table below.

Table 56: Summary of significant findings for Animal # 4604

(excerpted from Applicant's submission)

Clinical Pathology/Blood Smear Evaluation

- ↑ WBC Count
- Marked ↑ pleomorphic blast and atypical cells
- Abnormal granulocyte and monocyte morphology
- ↓ Platelets, RBC mass, calcium, albumin and globulin

Flow Cytometry

- Large population of CD 34+ blast cells
- ↑ CD3- CD 14+ monocytes

Immunohistochemical Stains (granulocytes/monocytes) *

- All stains moderately positive for large numbers of multilobulated and undifferentiated cells

* Periodic Acid Schiff, Sudan Black B, Peroxidase, Chloroacetate esterase, Leukocyte peroxidase, and Alpha naphthyl acetate esterase

Bone Marrow Evaluation

- Marked ↑ pleomorphic blast and atypical cells
- Erythroid and Myeloid hypocellularity
- Marked ↓ mature granulocytes
- Moderate ↓ megakaryocytes
- ↓ Lymphocyte percentage

Cytogenetics Evaluation

- Normal karyotype identified
- No chromosomal abnormalities

Gross Necropsy Findings

- Accentuated follicular pattern in the liver
- enlarged lymph nodes
- enlarged spleen

Histopathological Findings

- Leukemic infiltrates in various tissues
- Lymphoid depletion of the germinal centers in the spleen
- Chronic inflammation of the colon

↑= Elevated/increased; ↓= Decreased

One female (Animal # 4601) in the 1.0 mg/kg/day group was found dead on Day 40. This death was not considered test-article-related by the Applicant. The animal had been dosed via nasal gavage between 10:10 and 10:52 o'clock, was given dietary enrichments at 14:26 o'clock and was found dead in its cage at 15:30 o'clock. There were no notable clinical signs prior to Day 40 and no abnormal findings at the 2-hr post-dose observation on Day 40. At the time of death, bloody froth was noted at the nose and mouth and in the cage pan. Vomit was observed in the cage. The stomach was distended with fluid, mucus, and ingesta. Frothy fluid observed in the lungs, trachea, and nostrils was consistent with pulmonary edema. Histopathology findings included congestion and hemorrhage in the lungs with fibrin thrombi in the alveolar capillaries. It is possible that aspiration of gastric contents caused alveolar damage and subsequent edema and thrombus formation, but there was no foreign material evident. Pulmonary edema was considered to be the cause of death. A conclusive diagnosis of vomiting and aspiration could not be made without observation of food material in the lung, however, this was considered a likely scenario. The reason for the possible vomiting and pulmonary edema is unclear.

Clinical Signs

- Clinical signs observed in the animals euthanized early are mentioned in the mortality section above.
- In animals surviving until scheduled necropsy, watery feces were observed in the 0.1 and 1.0 mg/kg/day groups. The test article-related finding was dose-dependent and reversible during the recovery period.

Body Weights

- There was no effect on body weight gain in animals surviving to scheduled necropsy.
- Decreased body weight gain and an actual reduction in body weight was observed in the 2 males (Animal # 4203 and 4205) euthanized early with chronic inflammation of the large intestine.

Food Consumption

- Reduced food consumption was listed as a clinical sign and was observed frequently in 1 control female and 3 animals (1 male and 2 females) dosed at 1 mg/kg/day.

Ophthalmoscopy

- Crescent-shaped opacity noted on the lateral aspect of the optic discs of both eyes was observed in one male (Animal #4101) treated with 1.0 mg/kg/day at Weeks 18 and 39. There were no correlating histopathology findings.

ECG

There was no quantitative analysis of the data, and only a qualitative assessment of the electrocardiograms was conducted. A signed electrocardiography report provided in the submission stated that all electrocardiograms evaluated in this study were qualitatively

considered normal for cynomolgus monkeys and that no test article related abnormalities in rhythm were found.

Blood pressure and heart rate

There were no effects of CC-4047 on systolic, diastolic, or mean arterial blood pressure. Heart rate was decreased in animals treated with 1.0 mg/kg/day CC-4047.

Table 57: Heart rate in control and 1.0 mg/kg/day groups

Sex	Mean heart rate (beats/minute)									
	Control (0 mg/kg/day)					1.0 mg/kg/day				
	Pre	Week 12	Week 24	Week 39	Week 47 (recovery)	Pre	Week 12	Week 24	Week 39	Week 47 (recovery)
Males	225.8	220.0	229.5	242.0	227.5	232.4	209.3	205.0	205.0	245.0
Females	228.3	224.0	233.0	239.2	230.0	233.0	210.5	213.6	207.3*	222.0

*p ≤ 0.05

Hematology and Coagulation

Hematology changes included increases in red cell distribution width and reticulocytes and decreases in WBC count and lymphocytes in the 1.0 mg/kg/day group during the treatment period. Coagulation changes included increases in fibrinogen in the 1.0 mg/kg/day group during the treatment period. These changes were not observed following the recovery period.

As mentioned in the mortality section, one female (Animal # 4604) treated with 1.0 mg/kg/day had an exceptionally high WBC count (114,600 cells/µL) at Week 35 (Day 245) that increased to 151,800 cells/µL on Day 250. Flow cytometry results showed a large population of CD34+ blast cells and increased monocytes, which suggested acute leukemia.

Table 58: Hematology and coagulation changes in males

Parameter	Dose (mg/kg/day)	Mean (% deviation from control)								
		Week 2	Week 4	Week 8	Week 12	Week 21	Week 27	Week 31	Week 35	Week 39
RDW	0	13.50	13.00	12.80	13.03	13.22	13.33	13.27	13.27	12.40
	1.0	13.94	14.03	14.68* ↑15	15.18* ↑17	14.66* ↑11	15.68 ↑18	14.18 ↑7	14.33* ↑8	13.85* ↑12
Reticulocytes	0	1.147	0.653	0.145	0.380	0.508	0.832	0.467	0.445	0.625
	1.0	1.760* ↑53	1.186* ↑82	0.877* ↑111	0.620* ↑63	0.808* ↑59	1.074 ↑29	0.753 ↑61	0.668 ↑50	0.788 ↑26
WBC	0	12.88	11.33	10.40	11.02	11.60	14.07	11.97	11.90	11.58
	1.0	8.00* ↓38	7.40 ↓35	5.87* ↓44	7.07* ↓36	5.62* ↓52	5.68* ↓60	7.48 ↓38	6.43* ↓46	5.80* ↓50
Lymphocytes	0	8477.5	8499.3	6148.0	8469.3	8319.7	10280.7	8706.3	9300.8	8404.5
	1.0	5616.1 ↓34	4152.9* ↓51	3033.7* ↓51	2822.5* ↓67	2726.6* ↓67	2743.8* ↓73	3155.3* ↓64	2542.0* ↓73	2599.3* ↓69
Fibrinogen	0	NA	NA	NA	220.50	214.33	210.83	233.00	237.50	182.00
	1.0	NA	NA	NA	298.83	326.60* ↑52	341.40* ↑62	324.00* ↑39	294.75*	279.50*

RDW= red cell distribution width NA=Not evaluated

↑= increase ↓=decrease; Significantly different from Control, * p≤0.05

Note: The baseline for fibrinogen in the 1.0 mg/kg/day group was 294.00, which was higher than the control baseline of 217.83.

Table 59: Hematology and coagulation changes in females

Parameter	Dose (mg/kg/day)	Mean (% deviation from control)								
		Week 2	Week 4	Week 8	Week 12	Week 21	Week 27	Week 31	Week 35	Week 39
RDW	0	13.63	13.08	12.82	13.15	12.77	13.10	13.00	12.92	12.23
	1.0	13.74	13.63	14.27* ↑11	15.07* ↑15	13.94* ↑9	14.06	13.95	14.43* ↑12	13.70* ↑12
Reticulocytes	0	0.947	0.778	0.490	0.600	0.573	0.842	0.602	0.510	0.585
	1.0	1.727* ↑82	0.919	0.748 ↑53	0.608	0.554	0.686	0.620	0.723 ↑42	0.627
WBC	0	12.10	10.82	11.75	11.58	12.12	14.62	13.30	11.53	11.60
	1.0	9.27 ↓23	9.13	10.27	9.22 ↓20	7.04* ↓42	8.26* ↓44	9.28 ↓30	7.80 ↓32	10.30
Lymphocytes	0	6160.2	5781.3	6270.8	7026.5	7074.7	8449.5	8184.0	7279.0	6846.0
	1.0	5914.1	5251.4	3751.0 ↓40	3397.5* ↓52	3459.8* ↓51	3800.8* ↓55	3880.5 ↓53	2984.0* ↓59	3290.7 ↓52
Fibrinogen	0	NA	NA	NA	214.33	228.00	212.50	230.50	244.83	164.67
	1.0	NA	NA	NA	309.33* ↑44	265.80	344.80* ↑62	339.75* ↑47	338.75* ↑38	393.00* ↑139

RDW= red cell distribution width
↑= increase ↓=decrease; Significantly different from Control, * p≤0.05

Bone Marrow Cytology

Test-article related effects included lymphoid hypocellularity in several animals administered 1.0 mg/kg/day and acute leukemia in one female (Animal # 4604).

Table 60: Lymphocytes in bone marrow: Surviving main study and recovery animals

Group or Individual		% Lymphocytes	
		Day 274	Day 330 (Recovery)
Group means			
Males	Control	66.6%	64.5%
	1.0 mg/kg/day	34.8%	46.3%
Females	Control	46.0%	34.8%
	1.0 mg/kg/day	24.8%	40.5% ‡
Individual animal values (1.0 mg/kg/day)			
Male	# 4106	12.5%	NA
Females	# 4602	29.0%	NA
	# 4605	20.5%	NA

‡ There was only one recovery female for the 1.0 mg/kg/day group
NA= Not available

Table 61: Lymphocytes in bone marrow: Early deaths in 1.0 mg/kg/day group

Individual animal		Day	% Lymphocytes
Males	# 4103	44	25%
	# 4203	139	10.5%
	# 4205	195	17%
Females	#4603	195	29.5%
	# 4604	253	3%
	# 4701	139	23%

Clinical Chemistry

A female treated with 1.0 mg/kg/day (Animal # 4605) had increases in alkaline phosphatase (ALP) and Gamma-glutamyltransferase (GGT) beginning on Day 147. The values for the other females in the 1.0 mg/kg/day group had values similar to controls. The mean female control values and the values for Animal #4605 are shown in the table below. These increases in hepatobiliary enzymes correlated with bile duct proliferation observed in the histopathology.

Table 62: ALP and GGT levels in female controls and animal # 4605

Parameter	Group or Individual	Mean or individual value (U/L)						
		Day -3	Day 84	Day 147	Day 184	Day 217	Day 245	Day 272
ALP	Control Group (0 mg/kg/day)	368	371	319	336	351	289	280
	# 4605 (1 mg/kg/day)	590	564	1045	1443	2493	2512	3454
GGT	Control Group (0 mg/kg/day)	60	67	59	60	64	59	57
	# 4605 (1 mg/kg/day)	109	167	271	304	319	291	215

Albumin was decreased in some males and females treated with 1.0 mg/kg/day beginning on Day 84. The mean value was significantly decreased for males compared to controls on Day 272. The means the control and 1.0 mg/kg groups and the individual values for some of the 1.0 mg/kg/day animals are in the table below.

Table 63: Albumin levels

Group or Individual		Albumin (g/dL)							
		Day -3	Day 84	Day 128	Day 147	Day 184	Day 217	Day 245	Day 272
Group means									
Males	Control	4.2	4.1	NA	4.0	4.0	4.1	4.2	4.1
	1.0 mg/kg/day	4.2	3.9	NA	3.8	3.8	3.9	4.0	3.7*
Females	Control	4.1	3.8	NA	3.7	3.7	3.8	3.7	3.6
	1.0 mg/kg/day	4.1	3.5	NA	3.4	3.3	3.3	3.4	3.0
Individual animal values (1.0 mg/kg/day)									

Group or Individual		Albumin (g/dL)							
		Day -3	Day 84	Day 128	Day 147	Day 184	Day 217	Day 245	Day 272
Males	# 4102	4.2	4.0	NA	3.8	3.7	3.7	4.1	3.5
	# 4104	4.5	3.9	NA	4.0	3.9	3.7	3.8	3.6
	# 4203	4.4	3.6	3.1	NA	NA	NA	NA	NA
	# 4205	4.4	3.5	NA	2.8	3.0	NA	NA	NA
Females	# 4603	4.1	3.7	NA	3.5	3.3	NA	NA	NA
	# 4604	4.1	3.5	NA	3.3	3.2	3.5	3.5	NA
	# 4605	3.8	3.2	NA	3.0	2.8	2.8	2.7	2.3
	# 4606	4.3	4.0	NA	3.3	3.3	3.0	3.2	3.1
	# 4701	3.8	2.6	2.6	NA	NA	NA	NA	NA

NA= not available * p≤0.05

Urinalysis

Unremarkable

Gross Pathology

Important gross pathology findings related to the morbidity and death of the animals found dead or euthanized early are described in the mortality section above. Other test article-related findings are listed in the table below.

Table 64: Gross pathology findings for 9-month monkey study

Treatment-Related Macroscopic Findings		No. of animals affected							
		Males				**Females**			
Dose (mg/kg/day)		0	0.05	0.10	1.0	0	0.05	0.10	1.0
Number of animals examined		0/4/**2**	0/4/**2**	0/4/**2**	3*/2/**2**	0/4/**2**	0/4/**2**	0/4/**2**	4*/2/**1**
Liver, left medial lobe	Nodule, tan	-	-	-	-	-	-	0/1/**0**	-
Mesentery	Nodule, white hard	-	-	-	-	-	0/1/**0**	-	-
Thymus	Small	-	-	-	2*/0/**0**	-	-	-	0/1/**0**

Number of animals examined and affected: Early deaths*/ Terminal necropsy / **Recovery necropsy**
- = no test-article related changes

Organ Weights

Thymus weights were decreased in both males and females treated with 1.0 mg/kg/day CC-4047 at the main study necropsy on Day 274. The finding was reversible and was not observed at the recovery necropsy on Day 330.

Table 65: Organ weights at main study necropsy on Day 274

Group and Dose		Mean		Percentage deviation from Control					
		Control 0 mg/kg/day		0.05 mg/kg/day		0.10 mg/kg/day		1.0 mg//kg/day	
Sex		Males	Females	Males	Females	Males	Females	Males	Females
Number of animals examined		4	4	4	4	4	4	2	2
Thymus	Absolute (g)	3.9408	2.4278	-	-	-	-	↓85	↓71
	Relative Body weight (g/kg)	1.2483	0.8598	-	-	-	-	↓89	↓70

↓=decrease - = no test-article related changes

Histopathology

Adequate Battery: Yes

Peer Review: Yes

Histological Findings

The test article-related findings are listed in the table below and include acute inflammation of the large intestine (cecum, colon, and rectum) and lymphoid depletion of the lymph nodes, spleen, and thymus. Animal # 4103 (male) treated with 1.0 mg/kg/day had an acute *Staphylococcus aureus* infection involving the tissue surrounding the thoracic and lumbar vertebrae, marrow cavity and meninges of the spinal cord and brain, with hematogenous spread to the lungs and myocardium. The histopathology findings related to this diagnosis are listed in the table. Animal # 4604 (female) treated with 1.0 mg/kg/day had test-article related findings consistent with AML. The histopathology findings for this animal included the bone marrow of all bones being filled with blast cells (listed in table) and leukemic infiltrates in various organs including the brain, cervix, esophagus, eye, kidney, liver, mandibular and mesenteric lymph nodes, oviduct, pancreas, salivary gland, spleen, stomach, thymus, uterus, and vagina.

Table 66: Histopathology findings for 9-month monkey study

Treatment-Related Microscopic Findings			No. of animals affected							
			Males				Females			
Dose (mg/kg/day)			0	0.05	0.10	1.0	0	0.05	0.10	1.0
Number of animals examined			0/4/2	0/4/2	0/4/2	3*/2/2	0/4/2	0/4/2	0/4/2	4*/2/1
Organ	Finding									
Bone, vertebra	Acute inflammation	Moderate	-	-	-	1*/0/0	-	-	-	-
Bone marrow, sternum	Marrow in all bones filled with blast cells		-	-	-	-	-	-	-	1*/0/0
Brain	Acute inflammation in meninges	Mild	-	-	-	1*/0/0	-	-	-	-
	Meningitis and hemorrhage on ventral surface of brain		-	-	-	1*/0/0	-	-	-	-
Cecum	Chronic inflammation	Total	-	-	-	2*/1/0	-	-	-	2*/1/0
		Minimal	-	-	-	1*/1/0	-	-	-	-
		Mild	-	-	-	1*/0/0	-	-	-	1*/1/0
		Moderate	-	-	-	-	-	-	-	1*/0/0
Colon	Chronic inflammation	Total	-	-	-	2*/1/0	-	-	-	3*/1/0
		Minimal	-	-	-	0/1/0	-	-	-	2*/0/0
		Mild	-	-	-	2*/0/0	-	-	-	0/1/0
		Moderate	-	-	-	-	-	-	-	1*/0/0
Duodenum	Villous atrophy	Total	-	-	-	1*/0/0	-	-	-	2*/0/0
		Mild	-	-	-	-	-	-	-	2*/0/0
		Marked	-	-	-	1*/0/0	-	-	-	-
Heart	Acute inflammation in myocardium	Marked	-	-	-	1*/0/0	-	-	-	-
Ileum	Villous atrophy	Total	-	-	-	1*/0/0	-	-	-	1*/0/0
		Mild	-	-	-	-	-	-	-	1*/0/0
		Moderate	-	-	-	1*/0/0	-	-	-	-
Jejunum	Villous atrophy	Moderate	-	-	-	1*/0/0	-	-	-	-
Liver	Prolifera ion of bile	Total	-	-	-	0/1/1	-	-	-	0/2/0

Treatment-Related Microscopic Findings			No. of animals affected							
			Males				Females			
Dose (mg/kg/day)			0	0.05	0.10	1.0	0	0.05	0.10	1.0
Number of animals examined			0/4/2	0/4/2	0/4/2	3*/2/2	0/4/2	0/4/2	0/4/2	4*/2/1
Organ	Finding									
	ductule	Minimal	-	-	-	0/1/0	-	-	-	0/1/0
		Mild	-	-	-	0/0/1	-	-	-	0/1/0
	Granuloma		-	-	-	-	-	-	0/1/0	-
Lung	Acute inflammation in pulmonary artery	Marked	-	-	-	1*/0/0	-	-	-	-
	Acute inflammation in alveolus	Moderate	-	-	-	1*/0/0	-	-	-	-
	Colonies of cocci in thrombus		-	-	-	1*/0/0	-	-	-	-
Mandibular lymph node	Lymphoid depletion of germinal center	Total	-	-	-	3*/1/0	-	-	-	3*/1/0
		Mild	-	-	-	0/1/0	-	-	-	2*/1/0
		Moderate	-	-	-	1*/0/0	-	-	-	-
		Marked	-	-	-	2*/0/0	-	-	-	1*/0/0
Mesenteric lymph node	Lymphoid depletion of germinal center	Total	-	-	-	3*/2/0	-	-	-	3*/1/0
		Mild	-	-	-	0/2/0	-	-	-	2*/0/0
		Moderate	-	-	-	2*/0/0	-	-	-	1*/0/0
		Marked	-	-	-	1*/0/0	-	-	-	0/1/0
Mesentery	Granuloma, mineralized		-	-	-	-	-	0/1/0	-	-
Peyer's patch	Lymphoid depletion	Total	-	-	-	3*/1/0	-	-	-	2*/1/0
		Mild	-	-	-	0/1/0	-	-	-	-
		Moderate	-	-	-	3*/0/0	-	-	-	2*/1/0
Rectum	Chronic inflammation	Total	-	-	-	2*/0/0	-	-	-	2*/1/0
		Minimal	-	-	-	2*/0/0	-	-	-	-
		Mild	-	-	-	-	-	-	-	1*/1/0
		Moderate	-	-	-	-	-	-	-	1*/0/0
Spinal cord, cervical	Acute inflammation in meninges	Marked	-	-	-	1*/0/0	-	-	-	-
Spinal cord, lumbar	Acute inflammation in meninges	Marked	-	-	-	1*/0/0	-	-	-	-
	Necrosis	Marked	-	-	-	1*/0/0	-	-	-	-
Spinal cord, thoracic	Acute inflammation in meninges	Mild	-	-	-	1*/0/0	-	-	-	-
Spleen	Lymphoid depletion of germinal center	Total	-	0/1/0	-	3*/1/0	-	0/1/0	0/1/0	3*/1/0
		Minimal	-	-	-	0/1/0	-	0/1/0	-	0/1/0
		Mild	-	0/1/0	-	1*/0/0	-	-	0/1/0	1*/0/0
		Moderate	-	-	-	1*/0/0	-	-	-	-
		Marked	-	-	-	1*/0/0	-	-	-	1*/0/0
		Severity not listed	-	-	-	-	-	-	-	1*/0/0
Thymus	Lymphoid depletion of cortex/medulla	Total	0/1/1	0/4/0	0/2/0	3*/2/1	0/3/2	0/3/2	0/4/2	3*/2/1
		Minimal	-	0/2/0	-	0/0/1	-	0/0/2	0/0/1	0/0/1
		Mild	0/1/1	0/1/0	0/1/0	-	0/2/2	0/2/0	0/2/1	1*/0/0
		Moderate	-	0/1/0	0/1/0	-	0/1/0	0/1/0	0/2/0	-
		Marked	-	-	-	3*/2/0	-	-	-	2*/2/0

Number of animals examined and affected: Early deaths*/ Terminal necropsy / **Recovery necropsy**
- = no test-article related changes NA= Not Applicable, tissue does not exist in this sex

Toxicokinetics

The toxicokinetics of CC-4047 (0.05, 0.1, and 1.0 mg/kg/day) were evaluated Day 1, 28 (Week 4), Day 194 (Week 28), and Day 272 (Week 39) with samples collected prior to dosing and at 0.5, 2, 4, 8, and 24 hours after dosing. Due to 2 early deaths in the 1.0 mg/kg/day group, the study design was revised to add 2 monkeys (1/sex) to the 1.0 mg/kg/day group. Additional samples were collected from these 2 animals on Day 138 (Week 20), however, due to early euthanasia no Week 39 samples were available from

these animals. Day 1, Day 138, Day 194, and Day 272 plasma samples were analyzed for CC-4047 (racemate) and Day 28 plasma samples were analyzed for CC-4047 enantiomers CC-5083 and CC-6016.

- CC-4047 (racemate) was absorbed after oral administration to monkeys with time of peak plasma concentrations (T_{max}) of 0.5-8 hours
- Systemic exposures (mean AUC_t) increased with an increase in dose, with the exception of the exposures for 0.1 mg/kg/day dose in females, which were similar to the exposures for the 0.05 mg/kg/day dose in females. The increases in exposure were approximately dose proportional or slightly greater than dose proportional on Day 1. On Day 28, Day 194, and Day 272 increases in exposure were approximately dose proportional from 0.05 to 0.1 mg/kg/day in males and greater than dose proportional from 0.1 to 1.0 mg/kg/day in both males and females.
- There was no notable accumulation at the two lower dose levels (0.05 and 0.1 mg/kg/day) with mean accumulation ratios ranging from 0.78 to 1.34 from Day 1 to Day 28, Day 194, and Day 272. At the highest dose level (1.0 mg/kg/day), the mean accumulation ratio ranged from 1.32 to 2.19 from Day 1 to Day 28, Day 194, and Day 272, suggesting a slight accumulation of CC-4047 in both males and females.
- In general, exposure to CC-4047 (AUC_t) was similar in males and females.
- On Day 28, exposure (AUC_t) to CC-6016 (R-enantiomer) was greater by up to 30% than that of CC-5083 (S-enantiomer) in both sexes.

Table 67: Summary of mean toxicokinetic parameters for CC-4047 in male and female monkeys after daily oral administration of CC-4047

(excerpted from Applicant's submission)

Dosing Day	Analyte	Group/ Dose Level (mg/kg/day)	Sex	N	C_{max} (ng/mL)	T_{max}* (hr)	AUC_t (hr • ng/mL)	Dose-Normalized AUC_t[a]	Ratio of AUC_t to Lowest Dose AUC[b]	Rc[c]
Day 1	CC-4047	Group 2	M	6	17.77 (5.725)	2.0 [2.0-2.0]	98.46 (30.84)	1969 (616.8)	1.00	NA
		0.05	F	6	25.32 (8.275)	2.0 [2.0-2.0]	215.1 (126.0)	4302 (2520)	1.00	NA
		Group 3	M	6	31.07 (6.902)	2.0 [0.5-2.0]	242.4 (84.23)	2424 (842.3)	2.46	NA
		0.1	F	6	30.44 (5.085)	2.0 [2.0-2.0]	173.2 (46.60)	1732 (466.0)	0.81	NA
		Group 4	M	7	277.9 (46.32)	4.0 [2.0-4.0]	2892 (709.3)	2892 (709.3)	29.37	NA
		1.0	F	7	224.2 (49.34)	4.0 [4.0-4.0]	2653 (574.9)	2653 (574.9)	12.33	NA
Day 28	CC-5083+ CC-6016	Group 2	M	6	24.31 (5.508)	1.3 [0.5-2.0]	103.9 (29.47)	2078 (589.4)	1.00	1.08 (0.19)
		0.05	F	6	24.51 (5.049)	2.0 [0.5-2.0]	152.0 (85.62)	3040 (1712)	1.00	0.78 (0.26)
		Group 3	M	6	43.87 (11.24)	0.5 [0.5-2.0]	188.9 (47.79	1889 (477.9)	1.82	0.80 (0.13
		0.1	F	6	44.56 (10.56	0.5 [0.5-0.5]	173.8 (36.27)	1738 (362.7)	1.14	1.03 (0.19)
		Group 4	M	7	347.6 (270.9)	4.0 [4.0-8.0]	4194 (2669)	4194 (2669)	40.37	1.45 (0.88)
		1.0	F	7	349.5 (286.2)	4.0 [2.0-4.0]	3669 (2652)	3669 (2652)	24.14	1.32 (0.71)
Day 138	CC-4047	Group 4	M	1	814.5	4.0	11020	11020	NA	4.47
		1.0	F	1	666.2	4.0	9584	9584	NA	2.89
Day 194	CC-4047	Group 2	M	6	24.84 (8.119)	2.0 [0.5-4.0]	132.4 (56.80)	2648 (1136)	1.00	1.33 (0.26)
		0.05	F	6	21.68 (7.329)	3.0 [0.5-4.0]	195.6 (112.7)	3912 (2254)	1.00	0.91 (0.11)
		Group 3	M	6	37.62 (5.969)	2.0 [0.5-4.0]	213.3 (99.06)	2133 (990.6)	1.61	0.87 (0.15)
		0.1	F	6	38.85 (6.548)	2.0 [2.0-2.0]	186.6 (49.03)	1866 (490.3)	0.95	1.09 (0.17)
		Group 4	M	5	431.1 (232.3)	4.0 [4.0-4.0]	5867 (3736)	5867 (3736)	44.31	2.01 (0.87)
		1.0	F	5	370.8 (211.2)	4.0 [2.0-8.0]	4215 (1765)	4215 (1765)	21.55	1.69 (0.66)
Day 272	CC-4047	Group 2	M	6	15.23 (3.400)	3.0 [2.0-4.0]	132.7 (80.59)	2654 (1612)	1.00	1.34 (0.69)
		0.05	F	6	16.94 (4.095)	3.0 [2.0-4.0]	169.9 (88.69)	3398 (1774)	1.00	0.81 (0.11)
		Group 3	M	6	29.64 (8.523)	3.0 [2.0-4.0]	227.3 (130.2)	2273 (1302)	1.71	0.89 (0.28)
		0.1	F	6	26.79 (8.077)	4.0 [2.0-4.0]	211.4 (130.7)	2114 (1307)	1.24	1.16 (0.50)
		Group 4	M	4	554.5 (89.77)	2.0 [2.0-2.0]	5640 (1840)	5640 (1840)	42.50	2.19 (0.51)
		1.0	F	3	751.8 (513.1)	2.0 [2.0-2.0]	6540 (6886)	6540 (6886)	38.49	2.12 (1.78)

C_{max} and AUC_t are presented to 4 significant figures, NA: Not applicable

*Median and [range] for T_{max}

[a] Dose-normalized AUC is calculated as $AUC_t \div$ Dose, expressed as (ng • hr/mL)/(mg/kg)

[b] AUC ratio between the dose and the lowest dose

[c] Rc = AUC_t(Day 28, 138, 194, or 272)/AUC_t(Day 1)

Table 68: Summary of mean toxicokinetic parameters for CC-4047 enantiomers CC-5083 and CC-6016 in male and female monkeys on Day 28 after daily oral administration of CC-4047

(excerpted from Applicant's submission)

Dosing Day	Analyte (enantiomer)	Group/ Dose Level (mg/kg/day)	Gender	N	C_{max} (ng/mL)	T_{max}* (hr)	AUC_t (hr • ng/mL)	S/R AUC_t Ratio[a]
Day 28	CC-5083	Group 2	M	6	12.01 (3.139)	1.3 [0.5-2.0]	43.14 (16.95)	73.34
	(S)	0.05	F	6	11.89 (3.443)	2.0 [0.5-2.0]	65.85 (43.82)	77.62
		Group 3	M	6	22.78 (7.033)	0.5 [0.5-2.0]	85.13 (23.05)	82.01
		0.1	F	6	22.69 (5.405)	0.5 [0.5-0.5]	75.11 (15.94)	76.13
		Group 4	M	7	196.5 (174.5)	4.0 [2.0-8.0]	2165 (1539)	106.65
		1.0	F	7	189.2 (176.6)	4.0 [2.0-4.0]	1732 (1260)	89.46
	CC-6016	Group 2	M	6	12.31 (2.512)	1.3 [0.5-2.0]	58.82 (14.83)	–
	(R)	0.05	F	6	12.80 (1.863)	2.0 [0.5-2.0]	84.84 (43.35)	–
		Group 3	M	6	21.44 (4.303)	2.0 [0.5-2.0]	103.8 (26.01)	–
		0.1	F	6	22.18 (4.847)	0.5 (0.5-2.0)	98.66 (21.19)	–
		Group 4	M	7	158.8 (109.2)	4.0 [4.0-8.0]	2030 (1141)	–
		1.0	F	7	161.2 (109.3)	4.0 [2.0-4.0]	1936 (1404)	–

C_{max} and AUC_t are presented to 4 significant figures
*Median and [range] for T_{max}
[a] S/R AUC_t Ratio = AUC_t(CC-5083)/AUC_t(CC-6016)*100%

Table 69: Histopathology Inventory

Study	1398/115	CC-4047-TOX-013	1398/116	1398/117	1398/126	CC-4047-TOX-006
Species	Rats	Rats	Monkeys	Monkeys	Monkeys	Monkeys
Study duration	28 days	6 months	14 days	28 days‡	28 days	9 months
Adrenals	X*	X*	X*	X*	X*	X*
Aorta		X	X			X
Bone Marrow smear		X			X	X
Bone (femur)	X	X	X	X	X	X
Brain	X*	X*	X*	X*	X*	X*
Cecum	X	X	X	X	X	X
Cervix		X				X
Colon	X	X	X	X	X	X
Duodenum	X	X	X	X	X	X
Epididymis	X*	X*	X*	X*	X*	X*

Study	1398/115	CC-4047-TOX-013	1398/116	1398/117	1398/126	CC-4047-TOX-006
Species	Rats	Rats	Monkeys	Monkeys	Monkeys	Monkeys
Study duration	28 days	6 months	14 days	28 days‡	28 days	9 months
Esophagus	X	X	X	X	X	X
Eye	X	X	X	X	X	X
Fallopian tube						
Gall bladder			X	X	X	X
Gross lesions	X	X	X	X	X	X
Harderian gland		X				
Heart	X*	X*	X*	X*	X*	X*
Ileum	X	X	X	X	X	X
Injection site						
Jejunum	X	X	X	X	X	X
Kidneys	X*	X*	X*	X*	X*	X*
Lachrymal gland			X	X		
Larynx	X					
Liver	X*	X*	X*	X*	X*	X*
Lungs	X	X*	X	X	X	X*
Lymph nodes, cervical						
Lymph nodes mandibular	X	X	X	X	X	X
Lymph nodes, mesenteric	X	X	X	X	X	X
Mammary Gland	X	X		X	X	X
Nasal cavity						
Optic nerves	X	X		X	X	X
Ovaries	X*	X*	X*	X*	X*	X*
Pancreas	X	X	X	X	X	X
Parathyroid	X*	X*	X*	X*	X*	X*
Peripheral nerve						
Pharynx						
Pituitary	X*	X*	X*	X*	X*	X*
Prostate	X*	X*	X	X*	X*	X
Rectum	X	X	X	X		X
Salivary gland	X	X*	X	X	X	X
Sciatic nerve	X	X	X	X	X	X
Seminal vesicles	X	X*	X	X		X
Skeletal muscle		X				X
Skin	X	X	X	X	X	X
Spinal cord	X	X	X	X	X	X
Spleen	X*	X*	X*	X	X*	X*
Sternum	X	X	X	X	X	X
Stomach	X	X	X	X	X	X
Testes	X*	X*	X	X*	X*	X*
Thymus	X	X*	X	X	X	X*
Thyroid	X*	X*	X	X*	X*	X*

Study	1398/115	CC-4047-TOX-013	1398/116	1398/117	1398/126	CC-4047-TOX-006
Species	Rats	Rats	Monkeys	Monkeys	Monkeys	Monkeys
Study duration	28 days	6 months	14 days	28 days‡	28 days	9 months
Tongue		X	X	X		X
Trachea	X	X	X	X	X	X
Urinary bladder	X	X	X		X	X
Uterus	X	X*	X	X*	X	X
Vagina	X	X	X	X		X
Zymbal gland						
Standard List						

X, histopathology performed
*, organ weight obtained
‡, study was originally planned for 28 days but was terminated after 18 days

7 Genetic Toxicology

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

Study title: Evaluation of pomalidomide (CC-4047) in the bacterial reverse mutation with a confirmatory assay

Study no.:	CC-4047-TOX-015
Study report location:	eCTD 4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 12, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Pomalidomide (CC-4047), lot # CML W-376/09-CC2, Purity: 100.8%

Study was reviewed under IND (b) (4) by (b) (4) The review was slightly modified to fit this NDA review:

Key Study Findings

- Pomalidomide did not increase the number of revertant colony counts of any strain in the absence or presence of S-9 activation, therefore, pomalidomide was not mutagenic in this assay.

Methods

Strains:	*Salmonella typhimurium* histidine auxotrophs TA98, TA100, TA1535, and TA1537 and the *Escherichia coli* tryptophan auxotroph WP2*uvr*A

Concentrations in definitive study:	50.0, 160, 500, 1600, and 5000 µg per plate in the presence or absence of S-9
Basis of concentration selection:	Range finding assay at up to 5000 µg/plate
Negative control:	Dimethylsulfoxide (DMSO)
Positive control:	See table below
Formulation/Vehicle:	DMSO
Incubation & sampling time:	52 ± 4 hours

Table 70: Summary of positive control agents for Ames assay

Assay	Chemicals	Concentration (µg/plate)	Responding strains
Nonactivation	2-nitrofluorene (2NF)	1.0	TA98
	Sodium azide (SA)	2.0	TA100, TA1535
	ICR-191 (ICR)	2.0	TA1537
	4-nitroquinoline-N-oxide (4NQO)	1.0	WP2uvrA
S9 activation	2-aminoanthracene (2-AA)	2.5	TA100, TA1535, TA1537
	2-aminoanthracene (2-AA)	25	WP2uvrA
	Benzo[a]pyrene (BP)	2.5	TA98

Study Validity

1. Concentration selection was acceptable because recommended maximum concentration, 5000 µg per plate, was used.
2. The negative control counts fell within the historic control ranges.
3. The positive controls induced a greater than 3-fold increase in mean revertant colony numbers over that of the vehicle control.
4. Triplicate cultures.

Results

Under the conditions of this study, pomalidomide (CC-4047) did not cause a positive increase in the mean number of revertants per plate with any of the tester strains either in the presence or absence of S-9.

Table 71: Confirmatory mutagenicity assay results with S-9

(excerpted from Applicant's submission)

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA98	**Pomalidomide (CC-4047)**	5000	*14.0*	*1.0*	*0.6*	14 P M N, 13 P M N, 15 P M N
		1600	*17.0*	*4.6*	*0.8*	22 P M N, 13 P M N, 16 P M N
		500	*18.3*	*5.0*	*0.8*	19 N, 13 N, 23 N
		160	*18.0*	*1.0*	*0.8*	17 N, 19 N, 18 N
		50.0	*19.0*	*3.6*	*0.9*	16 N, 23 N, 18 N
	Dimethyl Sulfoxide		*22.0*	*6.1*		29 M N, 19 M N, 18 M N
TA100	**Pomalidomide (CC-4047)**	5000	*101.0*	*6.2*	*0.9*	103 P M N, 94 P M N, 106 P M N
		1600	*106.7*	*6.4*	*0.9*	102 P N, 114 P N, 104 P N
		500	*110.3*	*6.4*	*1.0*	115 N, 103 N, 113 N
		160	*114.3*	*28.4*	*1.0*	145 N, 109 N, 89 N
		50.0	*97.0*	*7.0*	*0.9*	104 N, 90 N, 97 N
	Dimethyl Sulfoxide		*113.3*	*20.6*		115 N, 92 N, 133 N
TA1535	**Pomalidomide (CC-4047)**	5000	*10.0*	*2.6*	*0.9*	13 P M N, 9 P M N, 8 P M N
		1600	*10.3*	*4.0*	*0.9*	6 P M N, 14 P M N, 11 P M N
		500	*9.7*	*1.5*	*0.9*	10 N, 11 N, 8 N
		160	*10.3*	*0.6*	*0.9*	11 N, 10 N, 10 N
		50.0	*13.7*	*3.1*	*1.2*	13 N, 17 N, 11 N
	Dimethyl Sulfoxide		*11.3*	*1.5*		13 N, 10 M N, 11 N
TA1537	**Pomalidomide (CC-4047)**	5000	*3.7*	*1.5*	*0.6*	5 P M N, 4 P M N, 2 P M N
		1600	*6.0*	*1.7*	*0.9*	7 P M N, 4 P M N, 7 P M N
		500	*7.7*	*2.1*	*1.2*	6 N, 7 N, 10 N
		160	*9.7*	*2.5*	*1.5*	10 N, 7 N, 12 N
		50.0	*6.7*	*0.6*	*1.1*	6 N, 7 N, 7 N
	Dimethyl Sulfoxide		*6.3*	*1.5*		6 N, 5 N, 8 M N

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
WP2*uvr*A	**Pomalidomide (CC-4047)**	5000	*8.3*	*4.2*	*0.3*	13 P M N, 5 P M N, 7 P M N
		1600	*10.3*	*2.5*	*0.4*	10 P M N, 8 P M N, 13 P M N
		500	*27.3*	*6.5*	*1.0*	34 N, 21 N, 27 N
		160	*29.0*	*5.2*	*1.1*	26 N, 35 N, 26 N
		50.0	*23.7*	*4.5*	*0.9*	19 N, 24 N, 28 N
	Dimethyl Sulfoxide		*27.3*	*6.5*		21 N, 27 N, 34 N
TA98	**BP**	2.5	*432.3*	*29.0*	*19.7*	452 N, 399 N, 446 N
TA100	**2AA**	2.5	*1765.0*	*563.6*	*15.6*	1303 N, 1599 N, 2393 N
TA1535	**2AA**	2.5	*176.7*	*11.7*	*15.6*	187 N, 164 N, 179 N
TA1537	**2AA**	2.5	*103.3*	*22.7*	*16.3*	124 N, 107 N, 79 N
WP2*uvr*A	**2AA**	25.0	*490.3*	*61.1*	*17.9*	420 N, 530 N, 521 N

Key to Positive Controls		Key to Plate Postfix Codes	
BP	Benzo{a}pyrene	P	Precipitation of test article observed
2AA	2-aminoanthracene	M	Plate counted manually
		N	Normal background bacterial lawn

Table 72: Confirmatory mutagenicity assay results without S-9

(excerpted from Applicant's submission)

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA98	**Pomalidomide (CC-4047)**	5000	*5.7*	*3.5*	*0.5*	9 P M N, 6 P M N, 2 P M N
		1600	*11.7*	*1.5*	*1.0*	12 P M N, 10 P M N, 13 P M N
		500	*7.0*	*1.0*	*0.6*	6 N, 8 N, 7 N
		160	*10.7*	*5.0*	*0.9*	16 M N, 10 N, 6 M N
		50.0	*10.0*	*4.0*	*0.8*	14 N, 6 N, 10 N
	Dimethyl Sulfoxide		*12.0*	*5.3*		10 N, 18 N, 8 N
TA100	**Pomalidomide (CC-4047)**	5000	*92.7*	*3.1*	*0.9*	96 P M N, 92 P M N, 90 P M N
		1600	*95.7*	*5.1*	*0.9*	90 P N, 97 P N, 100 P N
		500	*93.3*	*14.0*	*0.9*	107 N, 79 M N, 94 N
		160	*84.0*	*1.7*	*0.8*	86 N, 83 N, 83 N
		50.0	*66.0*	*1.0*	*0.6*	66 N, 67 N, 65 N
	Dimethyl Sulfoxide		*107.0*	*10.4*		119 N, 102 N, 100 N
TA1535	**Pomalidomide (CC-4047)**	5000	*8.7*	*4.0*	*0.7*	5 P M N, 13 P M N, 8 P M N
		1600	*8.3*	*2.5*	*0.7*	6 P M N, 11 P M N, 8 P M N
		500	*10.7*	*4.0*	*0.8*	10 N, 7 M N, 15 M N
		160	*8.7*	*2.1*	*0.7*	11 N, 8 N, 7 N
		50.0	*11.3*	*3.1*	*0.9*	8 N, 14 N, 12 N
	Dimethyl Sulfoxide		*12.7*	*1.2*		12 N, 14 N, 12 N
TA1537	**Pomalidomide (CC-4047)**	5000	*1.0*	*1.0*	*0.2*	0 N P M, 1 N P M, 2 N P M
		1600	*4.7*	*0.6*	*0.9*	4 P N, 5 P N, 5 P N
		500	*5.3*	*1.5*	*1.1*	5 M N, 4 N, 7 N
		160	*4.0*	*1.7*	*0.8*	2 M N, 5 N, 5 N
		50.0	*4.7*	*1.2*	*0.9*	4 M N, 6 N, 4 N
	Dimethyl Sulfoxide		*5.0*	*3.0*		8 N, 5 N, 2 N

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
WP2*uvr*A	**Pomalidomide (CC-4047)**	5000	*7.7*	*4.5*	*0.5*	3 P M N, 8 P M N, 12 P M N
		1600	*14.3*	*7.1*	*0.9*	13 P M N, 8 P M N, 22 P M N
		500	*12.0*	*3.5*	*0.8*	10 N, 10 N, 16 N
		160	*16.0*	*3.6*	*1.0*	17 N, 12 N, 19 N
		50.0	*17.3*	*3.5*	*1.1*	21 N, 17 N, 14 N
	Dimethyl Sulfoxide		*16.0*	*1.7*		14 N, 17 N, 17 N
TA98	**2NF**	1.0	*269.3*	*31.1*	*22.4*	305 N, 255 N, 248 N
TA100	**SA**	2.0	*1128.0*	*14.8*	*10.5*	1118 N, 1121 N, 1145 N
TA1535	**SA**	2.0	*903.7*	*106.7*	*71.3*	989 N, 938 N, 784 N
TA1537	**ICR**	2.0	*386.0*	*43.7*	*77.2*	381 N, 432 N, 345 N
WP2*uvr*A	**4NQO**	1.0	*294.7*	*15.6*	*18.4*	297 N, 278 N, 309 N

Key to Positive Controls		Key to Plate Postfix Codes	
2NF	2-nitrofluorene	P	Precipitation of test article observed
SA	sodium azide	M	Plate counted manually
ICR	ICR-191	N	Normal background bacterial lawn
4NQO	4-nitroquinoline-N-oxide		

7.2 *In Vitro* Assays in Mammalian Cells

Study title: Evaluation of pomalidomide (CC-4047) in the chromosomal aberrations assay in cultured human peripheral blood lymphocytes

Study no.:	CC-4047-TOX-016
Study report location:	eCTD 4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 12, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Pomalidomide (CC-4047), lot # CML W-376/09-CC2, Purity: 100.8%

Study was reviewed under IND (b) (4) by (b) (4) The review was slightly modified to fit this NDA review:

Key Study Findings

- Pomalidomide did not produce an increase in cells with chromosomal aberrations, polyploidy, or endoreduplication in the absence or presence of S-9 activation, therefore, pomalidomide was not clastogenic in this assay.

Methods

Cell line:	Human peripheral blood lymphocytes
Concentrations in definitive study:	103, 129, 161, 201, 252, 315, 393, 492, 614, 768, 960, 1200, and 1500 µg/mL; cultures treated with 252, 315, and 614 µg/mL without metabolic activation (3-hour treatment), 252, 315, and 492 µg/mL without metabolic activation (~22-hour treatment), and 252, 393, and 614 µg/mL with metabolic activation analyzed for chromosomal aberrations
Basis of concentration selection:	Range finding study
Negative control:	DMSO (10.0 µL/mL)
Positive control:	Mitomycin C (MMC, 1.0 µg/mL or 0.3 µg/mL for 3h or ~ 22h treatment, respectively) for the nonactivation series and cyclophosphamide (CP) for the metabolic activation series
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Treatment period: 3 or ~ 20-hours without metabolic activation and 3 -hours with metabolic activation, cultures were

harvested ~22 hours from the initiation of treatment.

Study Validity

Human blood lymphocytes were obtained from a healthy adult donor (non-smoker without a history of radiotherapy, chemotherapy, or drug usage, and lacking current viral infections).

Cells were selected for good morphology and only cells with the number of centromeres equal to the modal number 46 ± 2 (range 44-48) were analyzed.

One hundred cells from each duplicate culture were analyzed for the different types of chromosomal aberrations.

At least 25 cells were analyzed from those cultures that had greater than 25% of cells with one or more aberrations.

The mitotic index was evaluated for cytotoxicity by analyzing the number of mitotic cells in 1000 cells.

All slides were coded prior to analysis for control of bias.

The vehicle controls had 0% cells with –g (without gaps) aberrations, which were within the historical control range (< 5%).

The positive control had 36, 43, and 45% cells with -g aberrations and had significant increase ($p \leq 0.01$) in cells with chromosomal aberrations as compared with the vehicle control cultures.

The high concentrations selected for analysis in the assay had a > 50% reduction in mitotic index as compared to the vehicle control cultures and/or a precipitate was formed at the end of the treatment period.

Results

The stability was established for 8 days under the room temperature and refrigerated storage conditions for formulations at concentrations of 0.01 mg/mL to 191 mg/mL. All values were within 5.5% of initial concentration.

Results of concentration verification analyses revealed that all formulations were within 2.6% of targeted concentrations.

In the assay without metabolic activation with a 3-hour treatment, chromosome aberrations were analyzed from the cultures treated with 252, 315, and 614 µg/mL. The high concentration, 614 µg/mL, had a precipitate at the end of the treatment period and a 34% reduction in mitotic index as compared to the vehicle control cultures. No increase in cells with chromosomal aberrations (0% -g aberrations), polyploidy, or

endoreduplication was observed in the cultures analyzed.

Table 73: Chromosomal aberrations in human lymphocytes without metabolic activation: 3-hour treatment

(excerpted from Applicant's submission)

					# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judge-Ment (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations							Judge-ment (+/-)[d]
												simple				Totals[c]		
											gaps	breaks	chte	chre	mab	-g	+g	
Controls																		
Vehicle:	DMSO	10.0	µL/mL	A	100		100	0	0		3					0	3	
				B	100		100	0	0		2					0	2	
				Total	**200**		**200**				5					0	5	
				Average	**%**	**0**		**0.0**	**0.0**		**2.5**					**0.0**	**2.5**	
Positive:	MMC	1.00	µg/mL	A	50		100	0	0		7	19	8			24	29	
				B	50		100	0	0		4	16	9			21	25	
				Total	**100**		**200**				11	35	17			45	54	
				Average	**%**	--		**0.0**	**0.0**	-	**11.0**	**35.0**	**17.0**			**45.0**	**54.0**	+
Test Article		252	µg/mL	A	100		100	0	0		3					0	3	
				B	100		100	0	0		3					0	3	
				Total	**200**		**200**				6					0	6	
				Average	**%**	**20**		**0.0**	**0.0**	-	**3.0**					**0.0**	**3.0**	-
		315	µg/mL	A	100		100	0	0							0	0	
				B	100		100	0	0		3					0	3	
				Total	**200**		**200**				3					0	3	
				Average	**%**	**22**		**0.0**	**0.0**	-	**1.5**					**0.0**	**1.5**	-
		614	µg/mL	A	100		100	0	0		4					0	4	
				B	100		100	0	0		3					0	3	
				Total	**200**		**200**				7					0	7	
				Average	**%**	**34**		**0.0**	**0.0**	-	**3.5**					**0.0**	**3.5**	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. DMSO = dimethylsulfoxide MMC = Mitomycin C

In the assay without metabolic activation with a 22-hour treatment, chromosomal aberrations were analyzed from the cultures treated with 252, 315, and 492 µg/mL. The high concentration, 492 µg/mL, had a precipitate at the end of the treatment period and a 38% reduction in mitotic index as compared to the vehicle control cultures. No significant increase in cells with chromosomal aberrations (0.5, 0.4, or 1 % -g aberrations, respectively), polyploidy, or endoreduplication was observed in the cultures analyzed. While increases in the number of cells having gaps were observed at the 252 and 315 µg/mL concentrations, these were not concentration dependent.

Table 74: Chromosomal aberrations in human lymphocytes without metabolic activation: 22-hour treatment

(excerpted from Applicant's submission)

					# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judge-Ment (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations							Judge-ment (+/-)[d]
												simple				Totals[c]		
											gaps	breaks	chte	chre	mab	-g	+g	
Controls																		
Vehicle:	DMSO	10.0	µL/mL	A	100		100	0	0		2					0	2	
				B	100		100	0	0		1					0	1	
				Total	**200**		**200**				3					0	3	
				Average	**%**	**0**		**0.0**	**0.0**		**1.5**					**0.0**	**1.5**	
Positive:	MMC	0.300	µg/mL	A	50		100	0	0		11	18	6			21	30	
				B	50		100	0	0		9	19	5			22	27	
				Total	**100**		**200**				20	37	11			43	57	
				Average	**%**	--		**0.0**	**0.0**	-	**20.0**	**37.0**	**11.0**			**43.0**	**57.0**	+
Test Article		252	µg/mL	A	100		100	0	0		5	1				1	6	
				B	100		100	0	0		5					0	5	
				Total	**200**		**200**				10	1				1	11	
				Average	**%**	**4**		**0.0**	**0.0**	-	**5.0**	**0.5**				**0.5**	**5.5**	-
		315	µg/mL	A	100		100	0	0		4					0	4	
				B	150		100	0	0		6	1				1	7	
				Total	**250**		**200**				10	1				1	11	
				Average	**%**	**23**		**0.0**	**0.0**	-	**4.0**	**0.4**				**0.4**	**4.4**	-
		492	µg/mL	A	100		100	0	0		4	1				1	5	
				B	100		100	0	0		3	1				1	3	
				Total	**200**		**200**				7	2				2	8	
				Average	**%**	**38**		**0.0**	**0.0**	-	**3.5**	**1.0**				**1.0**	**4.0**	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. DMSO = dimethylsulfoxide MMC = Mitomycin C

In the assay with metabolic activation, chromosomal aberrations were analyzed from the cultures treated with 252, 393, and 614 µg/mL. The high concentration, 614 µg/mL, had a 51 % reduction in mitotic index as compared with the vehicle control cultures and a precipitate at the end of the treatment period. There were no significant increases in cells with chromosomal aberrations (0, 0.5, or 0% -g aberrations, respectively), polyploidy, or endoreduplication observed in the cultures analyzed.

Table 75: Chromosomal aberrations in human lymphocytes with metabolic activation: 3-hour treatment

(excerpted from Applicant's submission)

				# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judge-Ment (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations: simple gaps	simple breaks	chte	chre	mab	Totals[c] -g	Totals[c] +g	Judge-ment (+/-)[d]
Controls																	
Vehicle:	DMSO	10.0	µL/mL	A 100		100	0	0		1					0	1	
				B 100		100	0	0		2					0	2	
				Total **200**		**200**				3					0	3	
				Average %	**0**		**0.0**	**0.0**		**1.5**					**0.0**	**1.5**	
Positive:	CP	25.0	µg/mL	A 50		100	0	0		11	19	4			20	29	
				B 50		100	0	0		8	14	4	1		16	23	
				Total **100**		**200**				19	33	8	1		36	52	
				Average %	--		**0.0**	**0.0**	-	**19.0**	**33.0**	**8.0**	**1.0**		**36.0**	**52.0**	+
Test Article		252	µg/mL	A 100		100	0	0		2					0	2	
				B 100		100	0	0		1					0	1	
				Total **200**		**200**				3					0	3	
				Average %	**2**		**0.0**	**0.0**	-	**1.5**					**0.0**	**1.5**	-
		393	µg/mL	A 100		100	0	0		2					0	2	
				B 100		100	0	0		1	1				1	2	
				Total **200**		**200**				3	1				1	4	
				Average %	**26**		**0.0**	**0.0**	-	**1.5**	**0.5**				**0.5**	**2.0**	-
		614	µg/mL	A 100		100	0	0		2					0	2	
				B 100		100	0	0		1					0	1	
				Total **200**		**200**				3					0	3	
				Average %	**51**		**0.0**	**0.0**	-	**1.5**					**0.0**	**1.5**	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. DMSO = dimethylsulfoxide CP = Cyclophosphamide

Study title: CC-4047: Mutation at the thymidine kinase (*tk*) locus of mouse lymphoma L5178Y cells (MLA) using the Microtitre fluctuation technique

Study no.:	1398/82
Study report location:	eCTD 4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 19, 1997
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CC-4047, lot # 40563-27-A, Purity: Not provided

Key Study Findings

- CC-4047 did not increase the number of mutant frequencies at the *tk* locus of L5178Y lymphoma cells in the absence or presence of S-9, therefore, CC-4047 was not mutagenic in this mouse lymphoma assay.

Methods

Cell line:	L5178Y TK $^{+/-}$ mouse lymphoma cells
Concentrations in definitive study:	18.75, 37.5, 75, 150, 300, and 600 µg/mL
Basis of concentration selection:	Cytotoxicity range finding study

Negative control:	DMSO
Positive control:	In absence of S-9: 4-nitroquinoline 1-oxide (NQO; 0.05 and 0.10 µg/mL) In presence of S-9: benzo(a)pyrene (BP; 2.0 and 3.0 µg/mL)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	There was a 3 hour drug treatment incubation period and a 2 day expression period for TK mutation. At the end of the expression period, cell densities in the selected cultures were adjusted to 1 x 10^4/mL. TFT (3 µg/mL) was added and cells were incubated until scorable (11-13 days) for TFT resistance.

Study Validity

1) The mutant frequency of the positive controls was at least twice that of the negative controls.

2) The spontaneous mutant frequencies of the negative control cultures were within the normal range (above 60 mutants per 10^6 surviving cells but not more than three times the historical mean value).

3) The plating efficiencies of the negative controls were between the range of 60% to 140% on Day 0 and 70% to 130% on Day 2.

Results

In the absence of S-9, no statistically significant increases in mutant frequency were observed compared to the negative control following treatment at any CC-4047 concentration level tested in Experiment 1 or 2. In experiment 1, there was a weak statistically significant linear trend in the absence of S-9, however, this finding was not reproduced in Experiment 2 and was considered of no biological significance.

In the presence of S-9, a small but statistically significant increase in mutant frequency was observed at the intermediate concentration of 150 µg/mL in Experiment 1. No statistically significant increases in mutant frequency were observed at the higher concentrations in Experiment 1 or at any concentration level in Experiment 2. This finding was not concentration-related and was not reproducible, therefore, it was considered to be of no biological significance.

Marked increases in mutant frequency were observed with the positive controls chemicals NQO and BP. Since no reproducible, concentration-related increases in mutant frequency were observed at any concentration of CC-4047 in the absence or presence of S-9, CC-4047 did not induce mutation at the *tk* locus of L5178Y mouse lymphoma cells. Under the conditions of this study, CC-4047 was not mutagenic.

Table 76: Summary of results of mouse lymphoma assay

(excerpted from Applicant's submission)

Experiment 1

Treatment (μg/mL)	-S-9 %RS#	RTG	MF§		Treatment (μg/mL)	+S-9 %RS#	RTG	MF§	
0		1.00!	133.07!		0		1.00	120.63	
18.75		1.52	117.67	NS	18.75		0.96	127.71	NS
37.5		1.03	169.87	NS	37.5		1.08	114.80	NS
75		1.39	154.63	NS	75		1.00	128.23	NS
150		1.28	183.13	NS	150		0.73	200.72	*
300		1.45	142.17	NS	300		1.08	156.56	NS
600		1.23	179.46	NS	600		1.58	101.75	NS
Linear trend			*		Linear trend			NS	
NQO					BP				
0.05		0.65	749.20		2		0.49	715.14	
0.1		0.77	536.82		3		0.31	731.88	

Experiment 2

Treatment (μg/mL)	-S-9 %RS	RTG	MF§		Treatment (μg/mL)	+S-9 %RS	RTG	MF§	
0	100.00	1.00	239.87		0	100.00	1.00	147.35	
18.75	97.45	0.86	194.45	NS	18.75	92.31	1.08	151.78	NS
37.5	99.37	1.02	228.03	NS	37.5	93.06	0.89	167.15	NS
75	102.29	1.02	218.22	NS	75	108.72	0.96	123.40	NS
150	114.80	1.14	169.21	NS	150	98.63	0.84	176.86	NS
300	79.08	0.85	228.40	NS	300	87.20	0.76	186.34	NS
600	79.60	0.71	177.75	NS	600	89.06	0.75	164.59	NS
Linear trend			NS		Linear trend			NS	
NQO					BP				
0.05	100.26	0.72	615.19		2	75.78	0.51	905.91	
0.1	73.98	0.80	640.56		3	54.81	0.33	1544.87	

§	5-TFT resistant mutants/10^6 viable cells 2 days after treatment
%RS	Percent relative survival adjusted by post treatment cell counts
!	Based on one replicate only
NS	Not significant
*	Comparison of each treatment with control: Dunnett's test (one-sided), significant at 5% level
*, **, ***	Test for linear trend: χ^2 (one-sided), significant at 5%, 1% and 0.1% level respectively
#	Not calculated due to contamination of survival plates (see Appendix 9)

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

Study title: Evaluation of pomalidomide (CC-4047) in the *in vivo* rat bone marrow micronucleus assay

Study no:	CC-4047-TOX-017
Study report location:	eCTD 4.2.3.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 15, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Pomalidomide (CC-4047), lot # CML W-376/09-CC2, Purity: 100.8%

Study was reviewed under IND (b) (4) by (b) (4) The review was slightly modified to fit this NDA review:

Key Study Findings

- Pomalidomide did not induce statistically significant increases in micronucleated PCEs at any dose (500, 1000, and 2000 mg/kg/day) examined, therefore, pomalidomide was not clastogenic in this assay.

Methods

Doses in definitive study:	0, 500, 1000, and 2000 mg/kg/day
Frequency of dosing:	Twice approximately 24 hours apart
Route of administration:	Oral gavage
Dose volume:	10 mL/kg/day
Formulation/Vehicle:	1% w/v carboxymethylcellulose (CMC) in 100 mM acetate buffer, pH 5.5
Species/Strain:	Hsd:SD rats, bone marrow
Number/Sex/Group:	5 males
Satellite groups:	N/A
Basis of dose selection:	Findings in a previous micronucleus assay (CLE study number 1398/86). Adverse clinical signs were not observed in this study in both males and female rats dosed up to 2000 mg/kg/day. Based on these results, males only were tested in the present test.
Negative control:	1% w/v carboxymethylcellulose (CMC) in 100 mM acetate buffer, pH 5.5
Positive control:	Cyclophosphamide (60 mg/kg)

Study Validity

At least 2000 PCEs per animal were analyzed for the frequency of micronuclei.

Cytotoxicity was assessed by scoring the number of PCEs and normochromatic erythrocytes (NCEs) in at least the first 500 total erythrocytes for each animal.

The limit dose, 2000 mg/kg, was used as the high dose.

The PCEs with micronuclei in vehicle controls were within the historical control range (< 0.4%).

Cyclophosphamide induced a statistically significant increase in micronucleated PCEs compared to the vehicle control, which was consistent with historical positive control data.

Results

All dose formulations were homogeneous with the relative standard deviation ranging from 0.5 to 3.5%.

The concentrations of dose formulations were at 93.3 to 109.1% of their respective targeted concentrations.

There was no mortality or significant adverse clinical sign observed. CC-4047 was not cytotoxic to the bone marrow at any doses used.

CC-4047 did not induce statistically significant increases in micronucleated PCEs (0-0.1%) at any dose (500, 1000, and 2000 mg/kg/day) examined.

Table 77: Summary of micronucleus assay data

(excerpted from Applicant's submission)

Treatment	Dose	Harvest Time	% Micronucleated PCEs Mean ± SD Male	Ratio PCE:NCE Mean ± SD Male
Vehicle Control	10 mL/kg/day	24	0.04 ± 0.04	0.62 ± 0.13
Positive Control	60 mg/kg[a]	24	1.27 ± 0.52*	0.57 ± 0.06
Test Article	500 mg/kg/day	24	0.04 ± 0.04	0.71 ± 0.14
	1000 mg/kg/day	24	0.03 ± 0.03	0.64 ± 0.08
	2000 mg/kg/day	24	0.04 ± 0.04	0.67 ± 0.11

* Significantly greater than the corresponding vehicle control, $p \leq 0.01$.
Vehicle Control = 1% w/v carboxymethylcellulose (CMC) in 100 mM acetate buffer, pH 5.5
Positive control = Cyclophosphamide
PCE = Polychromatic erythrocyte
NCE = Normochromatic erythrocyte
[a] Positive control animals dosed once; approximately 24 hours prior to harvest.

8 Carcinogenicity

Carcinogenicity studies have not been conducted.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: A fertility and early embryonic development study in rats administered pomalidomide (CC-4047) orally

Study no.:	CC-4047-TOX-020
Study report location:	eCTD 4.2.3.5.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 21, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CC-4047, lot # CMLW-377/09-CC2 and CMLW-376/09-CC2, Purity: 100.8%

Key Study Findings

- Mean body weight change during gestation (Days 1-13) was lower in females treated with CC-4047 compared to controls
- There were no effects of CC-4047 on premating estrous cyclicity in treated females or reproductive function or fertility indices in treated males or treated and untreated females

- In CC-4047 treated females paired with treated males, the number of viable embryos was significantly decreased and the post-implantation loss and total number of resorptions (early + late) were significantly increased compared to controls at all doses
- There were no effects of CC-4047 on embryo viability in untreated females paired with treated males

Methods

Doses:	0, 25, 250, or 1000 mg/kg/day
Frequency of dosing:	Males: Once daily for 28 days prior to pairing through mating until necropsy (a total of 100 days) Females: Once daily for 14 days prior to pairing through mating until Gestation Day (GD) 7 (a total of 23 to 38 days)
Dose volume:	10 mL/kg/dose
Route of administration:	Oral gavage
Formulation/Vehicle:	1% (w/v) sodium carboxymethylcellulose medium viscosity (400 to 800 cps) in deionized water
Species/Strain:	Crl:CD (SD) rat
Number/Sex/Group:	25/sex/group; treated males and treated females (first pairing) Untreated females (25/group) were paired with treated males (second pairing)
Satellite groups:	None
Study design:	Males treated with vehicle or CC-4047 once daily beginning 28 days prior to pairing were paired with females treated with vehicle or CC-4047 once daily beginning 14 days prior to pairing. Cesarean section was performed on GD 13 in females. After CC-4047-related reproductive effects were observed in females, males were paired a second time with untreated females. Serial blood samples for toxicokinetic analysis were collected on Day 28 of dosing in treated males and Day 14 of dosing in treated females.
Parameters and endpoints evaluated:	Males and females: Clinical signs (cageside and detailed observations), body weights, food consumption, copulatory interval, fertility index, mating index, fecundity index, toxicokinetic analysis, necropsy, and organ weights Females only: Estrous cycle determination, total number of corpora lutea, viable embryos,

number of resorptions, total number of implantations, % pre-implantation loss and % post-implantation loss

Results

Mortality

There were no mortalities in this study.

Clinical Signs

Discolored urine (yellow) was observed during the detailed clinical observations in most animals treated with CC-4047, but was not observed in controls.

Table 78: Clinical signs: Discolored urine

Sex	No. of animals affected (No. of incidences observed)			
	0 mg/kg/day	25 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
Males	0 (0)	25 (2008)	25 (1994)	25 (2121)
Females	0 (0)	24 (289)	25 (274)	25 (361)

Body Weight

In males treated with CC-4047, the mean body weights were comparable to controls throughout the dosing period (premating, pairing, and postmating). In females, the mean body weight change during gestation (Days 1-13) was lower in females treated with CC-4047 compared to controls, with a statistically significant difference in the 1000 mg/kg/day group (12%).

Table 79: Gestation body weight change in females

Study interval (Days)	Body weight change (g)			
	0 mg/kg/day	25 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
0-13	76.1	72.0	70.7	67.0*

* Significantly different from control ($p<0.05$)

Food Consumption

Unremarkable

Toxicokinetics

Samples were collected on Day 28 for males and Day 14 for females at 2 hours after dosing in controls, and at pretest and 1, 2, 4, 8, and 24 hours after dosing in CC-4047 treated animals.

Table 80: Mean toxicokinetic parameters for CC-4047 in female (Day 14) and male (Day 28) rats after daily oral administration of CC-4047 (25, 250, and 1000 mg/kg/day)

(excerpted from Applicant's submission)

Dosing Day	Dosage Group	C_{max} (ng/mL)	t_{max} (h)	AUC_{24h} (ng·h/mL)	Dose-Normalized AUC_t[a]	Ratio of AUC_t to Lowest Dose AUC[b]
Day 14 (Female)	2 (25 mg/kg/day)	2780	4.0	39960	1598	1.00
	3 (250 mg/kg/day)	5010	2.0	65280	261.1	1.63
	4 (1000 mg/kg/day)	6998	2.0	92810	92.81	2.32
Day 28 (Male)	2 (25 mg/kg/day)	1857	4.0	21070	842.8	1.00
	3 (250 mg/kg/day)	3337	4.0	43550	174.2	2.07
	4 (1000 mg/kg/day)	4541	2.0	53890	53.89	2.56

C_{max} and AUC values are presented to 4 significant figures
[a] Dose-normalized AUC is calculated as mean AUC_t ÷ Dose, expressed as (ng•h/mL)/(mg/kg)
[b] AUC ratio between the dose and the lowest dose

Necropsy

Small and soft testis was observed in 2 of 25 males at the 1000 mg/kg/day dose. The finding was unilateral in one male (animal # 395) that mated and impregnated a female in both pairings. In the other male (animal #398), the finding was bilateral and the male did not mate in the first pairing but did mate and impregnate a female in the second pairing.

Organ weights

The absolute epididymal weights in the 25 and 250 mg/kg/day groups and the epididymal weight relative to body weight in the 25 mg/kg/day group were significantly lower than controls, however, epididymal weights were not significantly lower in the 1000 mg/kg/day group.

Gravid uterine weights, absolute and relative to GD 13 body weight, were lower than controls at all doses of CC-4047 and statistically significant at 250 and 1000 mg/kg/day. The lower gravid uterine weights in the treated groups were attributed to the reduction of viable embryos and increase in post-implantation loss observed in the uterine examination.

Table 81: Organ weights for fertility study

Group and Dose		Mean		Mean (Percentage deviation from Control)					
		Control 0 mg/kg/day		25 mg/kg/day		250 mg/kg/day		1000 mg/kg/day	
Sex		Males	Females	Males	Females	Males	Females	Males	Females
Number of animals examined		25	23	25	22	25	24	25	23
Epididymides	Absolute (g)	1.615	NA	1.455** (↓10)	NA	1.485** (↓8)	NA	1.523	NA
	Relative body weight (%)	0.2623	NA	0.2392* (↓9)	NA	0.2491	NA	0.2449	NA
Uterus with cervix	Absolute (g)	NA	9.004	NA	7.759 (↓14)	NA	7.275* (↓19)	NA	6.570** (↓27)
	Relative body weight (%)	NA	2.5740	NA	2.2485 (↓13)	NA	2.1119* (↓18)	NA	1.8855** (↓27)

↓=decrease NA= Not Applicable, tissue does not exist in this sex
* Significantly different from control ($p<0.05$) ** Significantly different from control ($p<0.01$)

Fertility Parameters

Estrous Cycle

There were no effects of CC-4047 on premating estrous cyclicity. The mean cycle length and mean number of cycles over the 15-day premating interval in CC-4047 groups were comparable to controls.

Table 82: Premating estrous cycling

Endpoint	Mean			
	0 mg/kg/day	25 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
Mean cycle length (days)	4.8	4.8	5.2	4.4
No. of cycles (count)	2.0	2.1	1.8	2.2

Reproductive and fertility indices

No effects of CC-4047 on reproductive function or fertility indices were observed in either the first pairing with CC-4047 treated females or the second pairing with untreated females.

Table 83: Reproductive and fertility Parameters: First pairing (with treated females)

Endpoint	0 mg/kg/day	25 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
No. females on study	25	25	25	25
No. females paired	25	25	25	25
No. females mated	25	25	25	25
No. pregnant	25	23	25	24
Female mating index (%)	100.0	100.0	100.0	100.0

Endpoint	0 mg/kg/day	25 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
Female fertility index (%)	100.0	92.0	100.0	96.0
Female fecundity index (%)	100.0	92.0	100.0	96.0
No. males on study	25	25	25	25
No. males paired	25	25	25	25
No. males mated	25	25	24	24
No. males impregnating a female	25	23	24	23
Male mating index (%)	100.0	100.0	96.0	96.0
Male fertility index (%)	100.0	92.0	96.0	92.0
Male fecundity index (%)	100.0	92.0	100.0	95.8
Females with confirmed mating day	24	24	23	23
Mean copulatory interval (days)	3.1	3.2	2.9	3.1

Table 84: Reproductive and fertility Parameters: Second pairing (with untreated females)

Endpoint	0 mg/kg/day	25 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
No. females on study	25	25	25	25
No. females paired	25	25	25	25
No. females mated	25	25	25	25
No. pregnant	24	25	23	25
Female mating index (%)	100.0	100.0	100.0	100.0
Female fertility index (%)	96.0	100.0	92.0	100.0
Female fecundity index (%)	96.0	100.0	92.0	100.0
No. males on study	25	25	25	25
No. males paired	25	25	25	25
No. males mated	23	25	25	25
No. males impregnating a female	23	25	23	25
Male mating index (%)	92.0	100.0	100.0	100.0
Male fertility index (%)	92.0	100.0	92.0	100.0
Male fecundity index (%)	100.0	100.0	92.0	100.0
Females with confirmed mating day	25	25	25	25
Mean copulatory interval (days)	2.8	2.2	2.3	2.6

Uterine and ovarian examination

For the first pairing with CC-4047 treated females, effects of treatment on embryo viability were observed at all dose levels of CC-4047. The number of viable embryos was significantly decreased and the post-implantation loss and total number of resorptions (early + late) were significantly increased compared to controls at all doses. Pre-implantation loss was also increased at all doses, but was not statistically significant.

Table 85: Maternal and developmental observations at uterine examination: First pairing (with treated females)

Endpoint	0 mg/kg/day	25 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
No. females on study	25	25	25	25
No. not pregnant	0	2	0	1
No. pregnant	25	23	25	24
No females with all resorptions	0	0	0	1
No. females with viable embryos (GD 13)	23	22	24	22
No. pregnant females with no confirmed mating date	2	1	1	1
Mean				
Corpora lutea (No. per animal)	17.1	17.4	18.7	17.4
Implantation sites (No. per animal)	15.9	15.3	14.8	15.0
Pre-implantation loss (% per animal)	6.82	12.26	19.67	11.16
Viable embryos (No. per animal)	15.3	11.8*	10.9**	9.1**
Post-implantion loss (% per animal)	3.95	24.30**	30.06**	40.55**
Resorptions: Early + late (No. per animal)	0.6	3.5**	4.0**	5.9**

* Significantly different from control ($p<0.05$)
** Significantly different from control ($p<0.01$)

There were no effects of CC-4047 on embryo viability for the second pairing with untreated females. Results from this pairing indicate that the increase in post-implantation loss seen in the first pairing was not attributable to treatment of the males.

Table 86: Maternal and developmental observations at uterine examination: Second pairing (with untreated females)

Endpoint	0 mg/kg/day	25 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
No. females on study	25	25	25	25
No. not pregnant	1	0	2	0
No. pregnant	24	25	23	25
No females with all resorptions	0	0	0	0
No. females with viable embryos (GD 13)	24	25	23	25
No. pregnant females with no confirmed mating date	0	0	0	0
Mean				
Corpora lutea (No. per animal)	17.3	17.1	16.7	17.4
Implantation sites (No. per animal)	16.4	15.6	15.6	15.3
Pre-implantation loss	5.51	8.67	6.02	12.25

Endpoint	0 mg/kg/day	25 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
(% per animal)				
Viable embryos (No. per animal)	15.3	14.4	14.3	14.4
Post-implantion loss (% per animal)	6.81	7.99	8.13	5.45
Resorptions: Early + late (No. per animal)	1.0	1.3	1.3	0.9

9.2 Embryo-Fetal Development

Study title: An embryo-fetal development study in rats administered pomalidomide (CC-4047) orally

Study no.: CC-4047-TOX-021
Study report location: eCTD 4.2.3.5.2
Conducting laboratory and location: (b) (4)
Date of study initiation: December 28, 2010
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: CC-4047, lot # CMLW-377/09CC2, Purity: 100.8%

Key Study Findings

- Maternal body weight gain was lower in the CC-4047 treated groups than controls starting around GD 12, but this is secondary to the significantly lower gravid uterine weight observed for all CC-4047 treated groups.
- The mean number of resorptions and post-implantation loss were significantly increased and the number of viable fetuses was significantly decreased in all CC-4047 dose groups compared to controls.
- Fetal weights were significantly decreased in all CC-4047 dose groups compared to controls.
- Increases in visceral and skeletal malformations and variations were observed in all the CC-4047 treated groups.
- Embryo-fetal loss and teratogenicity were observed at all doses of CC-4047, therefore, a developmental NOEL could not be determined in this study. These findings occurred in the absence of maternal toxicity.

Methods

Doses: 0, 25, 250, or 1000 mg/kg/day
Frequency of dosing: Once daily gestation days (GD) 6-17
Dose volume: 10 mL/kg/dose
Route of administration: Oral gavage
Formulation/Vehicle: 1% (w/v) sodium carboxymethylcellulose medium viscosity (400 to 800 cps) in deionized

	water
Species/Strain:	Crl:CD (SD) rat
Number/Sex/Group:	22 females/group
Satellite groups:	Toxicokinetics: 3 females/group
Study design:	22 females/group were dosed GD 6-17 and euthanized on GD 20
Parameters and endpoints evaluated:	Females: Clinical signs, body weight, gravid uterine weights, food consumption, necropsy, toxicokinetics, and number of corpora lutea, implantation sites, early and late resorptions, and viable and dead fetuses Fetuses: Fetal weight and external examinations (malformations and variations)

Results

Mortality

There were no mortalities in this study.

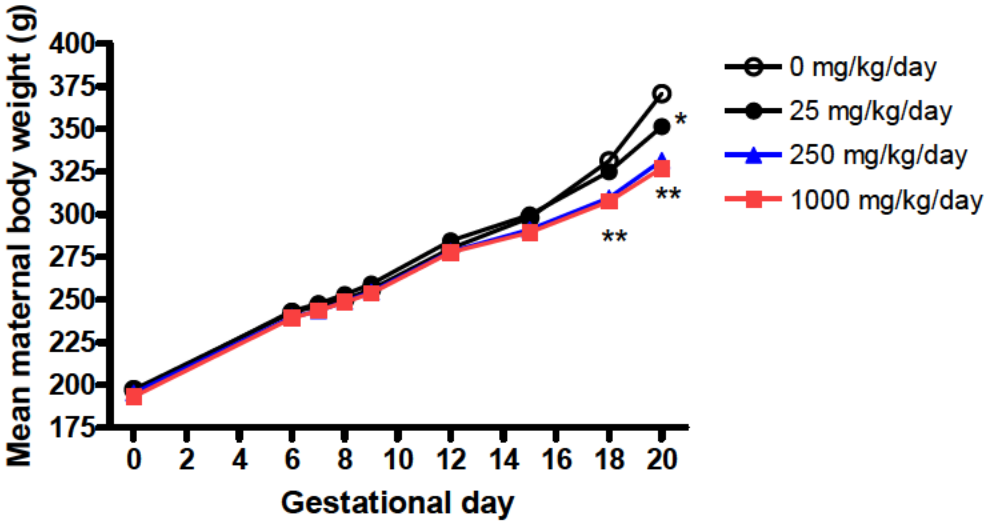
Clinical Signs

A yellow discoloration of the urine was observed at least once during GD 6-20 in all 22 females treated at 1000 mg/kg/day CC-4047. The finding was not observed in controls or females treated at 25 or 250 mg/kg/day.

Body Weight

Maternal body weight gain was lower in the CC-4047 treated groups than controls starting around GD 12. Body weights were statistically lower in the 250 and 1000 mg/kg/day groups on GD 18 (7%) and in all CC-4047 treated groups on GD 20 (5-12%).

Figure 28: Maternal body weight in rats administered CC-4047



* Significantly different from control (p<0.05)
** Significantly different from control (p<0.01)

Body weight change was statistically significant in the 250 and 1000 mg/kg/day groups for the GD 12-15 interval (29-36%) and in all CC-4047 treated groups for GD 15-18 (24-45%), GD 18-20 (33-50%), and GD 6-20 (16-31%) intervals.

Table 87: Maternal mean body weight change (g)

Study interval (GD)	Dose (mg/kg/day)			
	0	25	250	1000
6-9	13.6	15.9	14.7	14.7
9-12	23.9	25.3	23.7	23.9
12-15	18.1	15.2	12.9*	11.6**
15-18	33.4	25.5**	18.7**	18.3**
18-20	39.3	26.4**	21.9**	19.5**
6-20	128.3	108.2**	91.9**	88.0**

* Significantly different from control (p<0.05)
** Significantly different from control (p<0.01)

The gravid uterine weights were significantly lower for all CC-4047 treated groups. The adjusted final body weights and the adjusted body weight change from GD 0 were not lower than controls. Therefore, the lower maternal body weight gain is secondary to the lower gravid uterine weight observed.

Table 88: Mean gravid uterine weight and adjusted body weight and body weight change (g)

Parameter	Dose (mg/kg/day)			
	0	25	250	1000
Gravid uterine weight (g)	70.5	44.0**	28.6**	23.2**
Final body weight (g)	370.8	353.7*	331.7**	331.4**
Adjusted final body weight (g)	300.2	309.7	303.1	308.2
Adjusted body weight change (g) from GD 0	102.9	113.8	107.5	114.1

* Significantly different from control ($p<0.05$)
** Significantly different from control ($p<0.01$)

Food Consumption

Unremarkable

Toxicokinetics

Samples were collected from the toxicokinetic animals on GD 17 at 2 hours after dosing in controls and at predose and 1, 2, 4, 8, and 24 hours after dosing in CC-4047 treated groups. On GD 17, the systemic exposure (mean AUC 24h) for CC-4047 in pregnant female rats increased in a less than dose-proportional manner with an increase of 2.70-fold for a 40-fold increase in dose from 25 to 1000 mg/kg/day.

Table 89: Mean toxicokinetics parameters for CC-4047 on GD 17

(excerpted from Applicant's submission)

	25 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
GD 17			
C_{max} (ng/mL)	2729 (364.3)	4706 (219.9)	6436 (652.3)
T_{max} (h)	4.0 [4.0-4.0]	4.0 [4.0-8.0]	4.0 [2.0-4.0]
AUC_{24h} (ng·h/mL)	34340 (1518)	70000 (8418)	92610 (14410)
Dose-Normalized AUC_{24h} [a]	1374 (60.72)	280.0 (33.67)	92.61 (14.41)
Ratio of AUC_{24h} to the Lowest Dose Mean AUC_{24h} [b]	1.00	2.04	2.70

Values are mean (SD); T_{max} are median and [range]
[a] Dose-normalized AUC is calculated as mean AUC_t/Dose, expressed as (ng·h/mL)/(mg/kg)
[b] AUC ratio between the dose and the lowest dose in this study.

Necropsy

Unremarkable

Cesarean Section Data

The mean number of resorptions and post-implantation loss were significantly increased and the number of viable fetuses was significantly decreased in all CC-4047 dose groups compared to controls.

Table 90: Uterine examination data in rats

Dose (mg/kg/day)	0	25	250	1000
Number of pregnant females	22	22	22	22
Number of females with all resorptions	0	1	1	2
Number of females with viable fetuses	22	21	21	20
Corpora lutea Mean number per female	13.6	13.8	13.6	13.3
Implantation sites Mean number per female	12.2	12.1	12.2	11.8
Pre-implantation loss Mean % per female	9.27	9.97	9.67	9.57
Resorptions (mean number per female)				
Early	0.7	4.8**	7.5**	8.4**
Late	0.0	0.3	0.5*	0.3
Total (early + late)	0.8	5.1**	8.0**	8.7**
Nonviable fetuses Mean number per female	0.0	0.0	0.0	0.0
Post-implantation loss Mean % per female	6.06	42.47**	65.75**	74.10**
Viable fetuses Mean number per female/mean litter size	11.4	7.0**	4.2**	3.1**

* Significantly different from control ($p<0.05$)
** Significantly different from control ($p<0.01$)

Offspring

Fetal weights were significantly decreased in all CC-4047 dose groups compared to controls.

Table 91: Fetal sex ratio and weights

Dose (mg/kg/day)	0	25	250	1000
Fetal sex ratio Mean % male fetuses per female	48.4	57.8	53.0	61.8
Mean fetal weight				
Males	4.18	3.85**	3.78**	3.81**
Females	3.98	3.59**	3.47**	3.60**
Males + females	4.08	3.72**	3.61**	3.73**

** Significantly different from control ($p<0.01$)

An external examination was conducted on all viable fetuses. One half of the viable fetuses from each litter were fixed in Bouin's solution and examined for soft tissue defects (visceral examination). The other half of each litter was eviscerated and fixed in alcohol for skeletal examination. No clear CC-4047-related effects were observed in the external examinations. Malformations of a defect in the abdominal wall (omphalocele) and malrotated hind limb were observed in the same fetus at 1000 mg/kg/day.

Numerous visceral and skeletal malformations and variations were observed in the CC-4047 treated groups and are presented in the tables below.

Table 92: Visceral malformations and variations in rats

Dose (mg/kg/day)		0	25	250	1000
Number of litters evaluated		22	21	18	18
Number of fetuses evaluated		126	78	41	35
Total malformations	Number of litters (%)	0 (0.0)	10 (47.6)**	7 (38.9)**	13 (72.2)**
	Number of fetuses (%)	0 (0.0)	11 (14.1)	10 (24.4)	16 (45.7)
Total variations	Number of litters (%)	2 (9.1)	16 (76.2)**	15 (83.3)**	16 (88.9)**
	Number of fetuses (%)	2 (1.6)	36 (46.2)	27 (65.9)	25 (71.4)
Malformations					
Absent urinary bladder	Number of litters (%)	0 (0.0)	6 (28.6)**	5 (27.8)*	5 (27.8)*
	Number of fetuses (%)	0 (0.0)	6 (7.7)	7 (17.1)	5 (14.3)
Dilated aortic arch	Number of litters (%)	0 (0.0)	0 (0.0)	0 (0.0)	3 (16.7)
	Number of fetuses (%)	0 (0.0)	0 (0.0)	0 (0.0)	3 (8.6)
Retroesophageal aortic arch	Number of litters (%)	0 (0.0)	0 (0.0)	0 (0.0)	3 (16.7)
	Number of fetuses (%)	0 (0.0)	0 (0.0)	0 (0.0)	3 (8.6)
Right sided aortic arch	Number of litters (%)	0 (0.0)	0 (0.0)	1 (5.6)	4 (22.2)*
	Number of fetuses (%)	0 (0.0)	0 (0.0)	1 (2.4)	6 (17.1)
Extra azygous vein	Number of litters (%)	0 (0.0)	0 (0.0)	2 (11.1)	3 (16.7)
	Number of fetuses (%)	0 (0.0)	0 (0.0)	2 (4.9)	3 (8.6)
Small pulmonary trunk	Number of litters (%)	0 (0.0)	0 (0.0)	1 (5.6)	3 (16.7)
	Number of fetuses (%)	0 (0.0)	0 (0.0)	1 (2.4)	3 (8.6)
Absent thyroid	Number of litters (%)	0 (0.0)	2 (9.5)	1 (5.6)	1 (5.6)
	Number of fetuses (%)	0 (0.0)	2 (2.6)	1 (2.4)	2 (5.7)
Variations					
Increased renal pelvic cavitation in kidney	Number of litters (%)	1 (4.5)	11 (52.4)**	8 (44.4)**	6 (33.3)*
	Number of fetuses (%)	1 (0.8)	21 (26.9)	14 (34.1)	10 (28.6)
Renal papillae undeveloped in kidney	Number of litters (%)	0 (0.0)	12 (57.1)**	12 (66.7)**	9 (50.0)**
	Number of fetuses (%)	0 (0.0)	23 (29.5)	20 (48.8)	13 (37.1)

Dose (mg/kg/day)		0	25	250	1000
Number of litters evaluated		22	21	18	18
Number of fetuses evaluated		126	78	41	35
Dilated ureter	Number of litters (%)	1 (4.5)	14 (66.7)**	14 (77.8)**	10 (55.6)**
	Number of fetuses (%)	1 (0.8)	25 (32.1)	24 (58.5)	15 (42.9)
Absent innominate artery	Number of litters (%)	0 (0.0)	1 (4.8)	1 (5.6)	6 (33.3)**
	Number of fetuses (%)	0 (0.0)	1 (1.3)	1 (2.4)	7 (20.0)

* Significantly different from control (p<0.05) ** Significantly different from control (p<0.01)
The number of fetuses was not statistically analyzed

Table 93: Skeletal malformations and variations in rats

Dose (mg/kg/day)		0	25	250	1000
Number of litters evaluated		22	21	21	18
Number of fetuses evaluated		125	75	51	33
Total malformations	Number of litters (%)	1 (4.5)	10 (47.6)**	10 (47.6)**	9 (50.0)**
	Number of fetuses (%)	1 (0.8)	13 (17.3)	12 (23.5)	13 (39.4)
Total variations	Number of litters (%)	17 (77.3)	20 (95.2)	21 (100.0)	16 (88.9)
	Number of fetuses (%)	39 (31.2)	64 (85.3)	46 (90.2)	27 (81.8)
Malformations					
Lumbar vertabae Fused centra	Number of litters (%)	0 (0.0)	6 (28.6)**	4 (19.0)*	3 (16.7)
	Number of fetuses (%)	0 (0.0)	8 (10.7)	4 (7.8)	4 (12.1)
Lumbar vertabae Fused neural arches	Number of litters (%)	0 (0.0)	1 (4.8)	3 (14.3)	3 (16.7)
	Number of fetuses (%)	0 (0.0)	1 (1.3)	3 (5.9)	4 (12.1)
Lumbar vertabae Misaligned neural arches	Number of litters (%)	0 (0.0)	3 (14.3)	5 (23.8)	3 (16.7)
	Number of fetuses (%)	0 (0.0)	3 (4.0)	6 (11.8)	3 (9.1)
Thoracic vertabae Fused centra	Number of litters (%)	0 (0.0)	1 (4.8)	1 (4.8)	2 (11.1)
	Number of fetuses (%)	0 (0.0)	1 (1.3)	1 (2.0)	2 (6.1)
Thoracic vertabae Fused neural arches	Number of litters (%)	1 (4.5)	1 (4.8)	2 (9.5)	3 (16.7)
	Number of fetuses (%)	1 (0.8)	1 (1.3)	2 (3.9)	3 (9.1)
Thoracic vertabae Misaligned neural arches	Number of litters (%)	0 (0.0)	1 (4.8)	2 (9.5)	2 (11.1)
	Number of fetuses (%)	0 (0.0)	1 (1.3)	2 (3.9)	2 (6.1)
Variations					
Lumbar vertabae misaligned centra	Number of litters (%)	1 (4.5)	3 (14.3)	6 (28.6)	1 (5.6)

Dose (mg/kg/day)		0	25	250	1000
Number of litters evaluated		22	21	21	18
Number of fetuses evaluated		125	75	51	33
	Number of fetuses (%)	1 (0.8)	3 (4.0)	8 (15.7)	1 (3.0)
13th rib absent	Number of litters (%)	0 (0.0)	2 (9.5)	6 (28.6)**	5 (27.8)*
	Number of fetuses (%)	0 (0.0)	3 (4.0)	8 (15.7)	5 (15.2)
Irregular ossification of ribs	Number of litters (%)	0 (0.0)	4 (19.0)	5 (23.8)	2 (11.1)
	Number of fetuses (%)	0 (0.0)	4 (5.3)	6 (11.8)	2 (6.1)
Small ribs	Number of litters (%)	0 (0.0)	7 (33.3)**	9 (42.9)**	4 (22.2)*
	Number of fetuses (%)	0 (0.0)	8 (10.7)	12 (23.5)	5 (15.2)
Misaligned sternebrae	Number of litters (%)	3 (13.6)	9 (42.9)	8 (38.1)	5 (27.8)
	Number of fetuses (%)	3 (2.4)	13 (17.3)	8 (15.7)	7 (21.2)
Not ossified sternebrae	Number of litters (%)	12 (54.5)	20 (95.2)**	20 (95.2)**	15 (83.3)
	Number of fetuses (%)	22 (17.6)	61 (81.3)	41 (80.4)	25 (75.8)

* Significantly different from control ($p<0.05$) ** Significantly different from control ($p<0.01$)
The number of fetuses was not statistically analyzed

Study title: Developmental and reproductive toxicity screening study for effects on embryo-fetal development in rabbits

Study no.: 0329DC35.001
Study report location: eCTD 4.2.3.5.2
Conducting laboratory and location: (b) (4) (b) (4)
Date of study initiation: June 19, 1997
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: CC-4047, lot # 40563-12-A
Thalidomide, lot # 574-574-96-005

Study was briefly reviewed under IND (b) (4) by (b) (4). The review was slightly modified to fit this NDA review:

In this study, thalidomide was used as a positive control. Four pregnant females were treated with 100 mg/kg CC-4047 or 250 mg/kg of thalidomide on gestation Days 8-10.

Rabbits were euthanized and the uterine examinations were conducted on gestation Day 29. Following treatment with CC-4047, there were no premature deaths or deliveries, no maternal toxicity or clinical signs, no difference in body weights, in mean incidence of gravidity, mean total corpora lutea, or mean preimplantation or post implantation losses compared with controls. Thalidomide treatment produced abnormal curvature or rotation of the forelimbs or hindlimbs, abnormal digits, or missing digits.

Study title: Oral screen study for effects on embryo-fetal development in New Zealand White rabbits (SEG II)

Study no.:	0329DC35.002
Study report location:	eCTD 4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 29, 2001
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	CC-4047, lot # 60895-05 Thalidomide, lot # 574-574-00-003

Study was briefly reviewed under IND (b) (4) by (b) (4) The review was slightly modified to fit this NDA review:

This study was conducted in New Zealand White rabbits. Pregnant females (4 per group) were treated with 100 mg/kg or 250 mg/kg of CC-4047 from gestation Days 6-18. Three other groups (4/group) were treated with thalidomide at 10, 100, or 250 mg/kg/day. Rabbits were euthanized and the uterine examinations were conducted on gestation Day 29.

Key Study Findings

- There was no maternal toxicity for any of the groups tested. Both CC-4047 and thalidomide at 250 mg/kg induced 25% and 33% increase in incidence of abortion respectively. One litter from the CC-4047 250 mg/kg aborted.
- Effects observed at both 100 and 250 mg/kg of CC-4047: reduced fetal body weight, limb rotation, shortened limbs, umbilical hernia, swollen joints, missing or abnormal digits.
- The incidence of malformations was 31% and 91% of viable tissues at 100 mg/kg and 250 mg/kg CC-4047, respectively.

Conclusions: CC-4047 produces a dose-dependent increase in abortions, malformations, and alterations after oral administration of 100 and 250 mg/kg to pregnant rabbits from gestation Days 6 to 18.

Study title: Oral (stomach tube) developmental toxicity study of CC-4047 in rabbits

Study no.:	CC-4047-TOX-008
Study report location:	eCTD 4.2.3.5.2
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	February 10, 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CC-4047, batch # 61749-06, Purity: 100% Thalidomide, batch # 574-574-04-016, Purity: 98.8%

Key Study Findings

- CC-4047 produced similar effects to the positive control (180 mg/kg/day thalidomide) including decreases in maternal body weight change, increased post-implantation loss, and increases in gross external, visceral, and skeletal malformations and alterations.
- CC-4047 was maternally toxic at the mid and high doses (100 and 250 mg/kg/day) with decreased body weight gain, changes in hematology and clinical chemistry, and decreased spleen and thymus weights.
- The gravid uterine weights were lower at 100 and 250 mg/kg/day CC-4047 than in the vehicle control group.
- Post-implantation loss was increased in the 100 and 250 mg/kg/day groups compared to the vehicle control group with increases in the number of early, late, and total resorptions, % resorbed conceptuses per litter, and number of females with resorptions.
- Increases in fetal cardiac anomalies, principally the malformation of interventricular septal defect, were observed at all doses of CC-4047.
- The 100 and 250 mg/kg/day doses of CC-4047 produced significant increases in the litter and fetal incidences of gross external, visceral, and skeletal malformations, including limb malformations similar to those observed in the thalidomide positive control group.
- Teratogenicity was observed at all doses of CC-4047.

Methods

Doses:	0, 10, 100 or 250 mg/kg/day CC-4047; 180 mg/kg/day thalidomide
Frequency of dosing:	Once daily gestation days (GD) 7-19
Dose volume:	5 mL/kg/dose
Route of administration:	Oral via stomach tube

Formulation/Vehicle:	1% (w/v) sodium carboxymethylcellulose medium viscosity (400 to 800 cps) in deionized water
Species/Strain:	New Zealand White rabbit
Number/Sex/Group:	20 females/group for vehicle control and CC-4047 treated groups; 5 females/group for thalidomide positive control group
Satellite groups:	None
Study design:	20 females/group were dosed with vehicle or CC-4047 (10, 100, or 250 mg/kg/day) GD 7-19 and were euthanized on GD 29. The day of mating was considered to be GD 0. A positive control group of 5 females was dosed with 180 mg/kg/day of thalidomide on the same dosing schedule.
Parameters and endpoints evaluated:	Females: Clinical signs, body weight, gravid uterine weights, food consumption, hematology, clinical chemistry, urinalysis, organ weights, necropsy, and number of corpora lutea, implantation sites, early and late resorptions, and live and dead fetuses Fetuses: Fetal weight and external examinations (malformations and variations)

Results

Mortality

One female (animal #5864) in the 100 mg/kg/day group was euthanized on GD 15 (prior to the administration of the ninth daily dosage) due to body weight loss, severely reduced food consumption, and adverse clinical signs (soft or liquid feces, green discolored feces, scant feces, ungroomed coat, decreased motor activity, and dehydration. All tissues were reported as normal at necropsy and the litter consisted of 8 embryos.

Clinical Signs

Ungroomed coat was observed in all CC-4047 treated groups and green urine was observed in multiple animals in the 250 mg/kg/day CC-4047 group.

Table 94: Clinical signs in pregnant rabbits

Clinical signs	No. of animals affected (No. of incidences observed)				
	Vehicle control 0 mg/kg/day	180 mg/kg/day Thalidomide	10 mg/kg/day CC-4047	100 mg/kg/day CC-4047	250 mg/kg/day CC-4047
Green urine	0 (0)	0 (0)	0 (0)	0 (0)	7 (18)**
Ungroomed coat	0 (0)	0 (0)	3 (13)*	6 (21)**	2 (6)

* Significantly different from vehicle control ($p<0.05$)
** Significantly different from vehicle control ($p<0.01$)

Body Weight

Maternal body weights were lower in the 100 and 250 CC-4047 treated groups than vehicle controls starting around GD 20. Body weight change (weight gain) was significantly lower in the 100 and 250 mg/kg/day CC-4047 groups and the 180 mg/kg/day thalidomide group for the GD 7-28 interval (29-38%) and the GD 7-29 interval with the adjusted body weight for GD 29 (GD 29 body weight minus the gravid uterine weight; 39-52%). The gravid uterine weights were also lower in the 100 and 250 mg/kg/day CC-4047 groups (9 and 11%, respectively) and the 180 mg/kg/day thalidomide group (18%) than in the vehicle control group.

Figure 29: Maternal weight in rabbits administered CC-4047

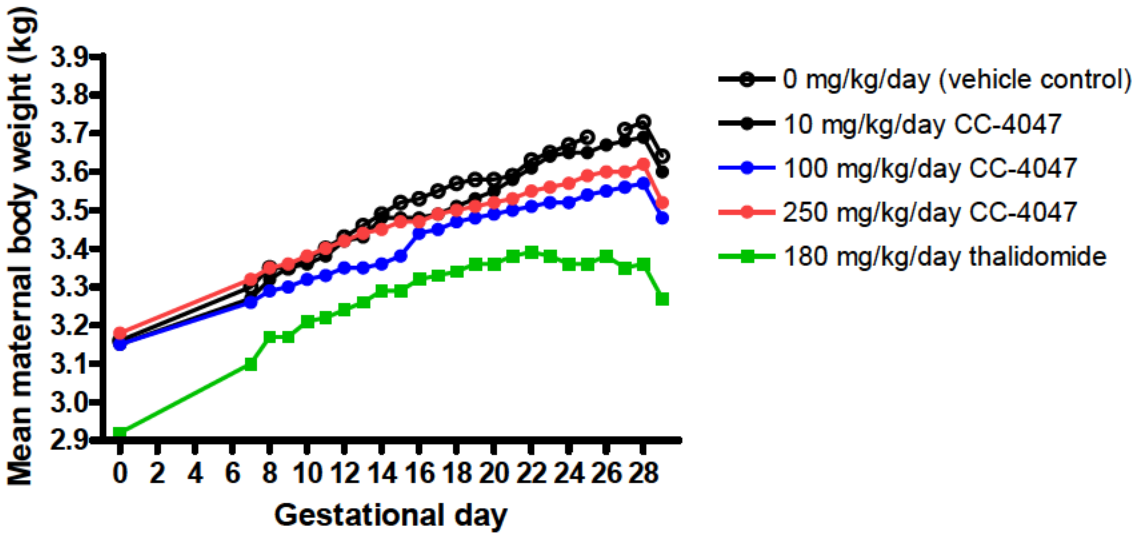


Table 95: Maternal mean body weight change (kg)

Study interval (GD)	Group				
	Vehicle control 0 mg/kg/day	180 mg/kg/day Thalidomide	10 mg/kg/day CC-4047	100 mg/kg/day CC-4047	250 mg/kg/day CC-4047
7-28	0.42	0.26*	0.42	0.30**	0.30**
7-29C	0.33	0.16*	0.32	0.20**	0.20**

* Significantly different from vehicle control ($p<0.05$)
** Significantly different from vehicle control ($p<0.01$)
29C=corrected/adjusted maternal body weight (GD 29 body weight minus the gravid uterine weight)

Table 96: Mean gravid uterine weight and adjusted maternal body weight

Parameter	Group				
	Vehicle control 0 mg/kg/day	180 mg/kg/day Thalidomide	10 mg/kg/day CC-4047	100 mg/kg/day CC-4047	250 mg/kg/day CC-4047
Gravid uterine weight (g)	510.68	417.48	513.15	465.03	452.18
Maternal body weight on GD 29 (kg)	3.64	3.27	3.60	3.48	3.52
Adjusted maternal body weight on GD29 (kg)	3.13	2.86	3.08	3.01	3.07

Food Consumption

The mean absolute (g/day) and relative (g/kg/day) maternal food consumption was reduced by 12-19% compared to vehicle controls in the 100 mg/kg/day CC-4047 group and the 180 mg/kg/day thalidomide group. Food consumption was not reduced in the 250 mg/kg/day CC-4047 group.

Hematology and Clinical Chemistry

RBC, hemoglobin, and % hematocrit were significantly decreased in the 250 mg/kg/day CC-4047 group compared to the vehicle control group. Calcium and inorganic phosphorus were significantly increased in the 250 mg/kg/day CC-4047 group and triglycerides were significantly decreased in the 100 and 250 mg/kg/day CC-4047 groups compared to the vehicle control group. Cholesterol and calcium were significantly increased in the 180 mg/kg/day thalidomide positive control group compared to the vehicle control group.

Table 97: Hematology and clinical chemistry changes in rabbit embryo-fetal development study

Parameter	Mean	Percentage deviation from vehicle control			
	Vehicle control 0 mg/kg/day	180 mg/kg/day Thalidomide	10 mg/kg/day CC-4047	100 mg/kg/day CC-4047	250 mg/kg/day CC-4047
RBC	4.865	-	-	-	↓8*
Hemoglobin	10.61	-	-	-	↓8*
% Hematocrit	32.08	-	-	-	↓9*
Cholesterol	17.5	↑59**	-	-	-
Calcium	9.49	↑17**	-	-	↑9*
Inorganic phosphorus	4.15	-	-	-	↑18**
Triglycerides	45.2	-	-	↓27**	↓32**

↑= increase ↓=decrease - = no test-article related changes
* Significantly different from vehicle control ($p<0.05$) ** Significantly different from vehicle control ($p<0.01$)

Urinalysis

Unremarkable

Organ weights

Spleen and thymus weights were lower in the 100 and 250 mg/kg/day CC-4047 groups compared to the vehicle control group.

Table 98: Organ weights for rabbit embryo-fetal development study

Group and Dose		Mean		Mean (Percentage deviation from vehicle control)		
		Vehicle control 0 mg/kg/day	180 mg/kg/day Thalidomide	10 mg/kg/day CC-4047	100 mg/kg/day CC-4047	250 mg/kg/day CC-4047
Number of animals examined		20	4	19	18	19
Spleen	Absolute (g)	1.5	1.4	1.4	1.2 (↓20)*	1.1 (↓27)**
	Relative body weight (%)	0.040	0.040	0.037	0.035 (↓12.5)	0.032 (↓20)**
Thymus	Absolute (g)	1.90	1.85	1.81	1.69 (↓11)	1.68 (↓12)
	Relative body weight (%)	0.050	0.058	0.049	0.048 (↓4)	0.047 (↓6)

↓=decrease * Significantly different from vehicle control ($p<0.05$) ** Significantly different from vehicle control ($p<0.01$)

Toxicokinetics

Toxicokinetics were not conducted in this study, but were conducted in the range finding study in rabbits (Study CC-4047-TOX-007). The mean pharmacokinetic parameters for 10, 100, and 250 mg/kg/day in that study are presented in the table below.

Table 99: Mean pharmacokinetic parameters for CC-4047 in maternal plasma

(excerpted from Applicant's submission)

Gestation Day	Dosage Group	C_{max} (ng/mL)	T_{max}[1] (hr)	T_{last}[1] (hr)	AUC_t (hr • ng/mL)	AUC_∞ (hr • ng/mL)	$t_{½}$ (hr)
Day 7	II (5 mg/kg)	73.26 (22.99)	1.0 [0.5-4.0]	24.0 [10.0-24.0]	318.0 (16.43)	352.2 (NA)	2.5 (NA)
	III (10 mg/kg)	138.7 (70.74)	1.0 [0.5-1.0]	24.0 [10.0-24.0]	951.7 (453.0)	776.0 (426.4)	2.3 (0.3)
	IV (100 mg/kg)	313.9 (112.2)	4.0 [2.0-4.0]	24.0 [NA]	3545 (978.0)	4048 (1017)	7.8 (2.5)
	V (250 mg/kg)	343.9 (141.4)	4.0 [1.0-4.0]	24.0 [NA]	4556 (2149)	8155 (3113)	12.8 (4.8)
Day 19	II (5 mg/kg)	61.68 (24.84)	0.5 [NA]	24.0 [10.0-24.0]	270.7 (62.60)	308.4 (76.62)	5.1 (2.2)
	III (10 mg/kg)	71.88 (7.118)	0.5 [0.5-1.0]	10.0 [10.0-24.0]	417.9 (9.301)	458.7 (64.76)	3.6 (0.1)
	IV (100 mg/kg)	217.4 (77.46)	0.5 [NA]	24.0 [NA]	2787 (1229)	NA (NA)	NA (NA)
	V (250 mg/kg)	385.1 (163.5)	0.5 [NA]	24.0 [NA]	3328 (1535)	5841 (897.5)	14.4 (4.0)

1. Median and [range] for T_{max}, T_{last}
NA = Not applicable

Necropsy

One female in the 10 mg/kg/day group had a uterine abnormality (see cesarean section data below).

Cesarean Section Data

One female (animal #5836) in the 10 mg/kg/day group had a uterine abnormality (right uterine horn had five twists present between implantation sites three and four) that resulted in resorption or death of all conceptuses in the litter. There were 2 early resorptions and 11 dead fetuses. Since this observation was observed in only this one rabbit at the low dose and was not observed at higher doses, it was considered to be unrelated to CC-4047 by the Applicant. The data for this rabbit and litter were excluded from group summarization and statistical analyses. The data were also excluded for animal #5864 in the 100 mg/kg/day group that was euthanized on GD 15.

The uterine examination data are presented in the table below. The means for corpora lutea, implantation sites, and resorptions were provided along with the % resorbed conceptuses/litter and number of females with resorptions. The % pre-implantation loss and % post-implantation loss were not provided or presented in the study report, however, post-implantation loss was discussed in the text of the results.

Post-implantation loss was increased in the 100 and 250 mg/kg/day CC-4047 groups and the 180 mg/kg/day thalidomide group (positive control) compared to the vehicle control group with increases in the number of early, late, and total resorptions, %

resorbed conceptuses per litter, and number of females with resorptions. Only the increase in early resorptions at 250 mg/kg/day CC-4047 was statistically significant. The mean number of live fetuses/mean litter size tended to be lower in the 250 mg/kg/day CC-4047 and 180 mg/kg/day thalidomide groups, but were not statistically significant.

Table 100: Uterine examination data in rabbits

Parameter	Vehicle control	Thalidomide 180 mg/kg/day	CC-4047 (mg/kg/day)		
			10	100	250
Number of pregnant females	20	4	20	19	19
Unscheduled sacrifice	0	0	0	1	0
Excluded due to uterine abnormality	0	0	1	0	0
Number of pregnant females with data	20	4	19	18	19
Corpora lutea (mean)	8.6	8.0	9.0	8.6	8.4
Implantation sites (mean)	8.4	8.0	8.8	8.6	8.2
Resorptions					
Early resorptions: Number	1	3	5	11	14
Mean	0.0	0.8	0.2	0.6	0.7*
Late resorptions: Number	3	3	1	7	7
Mean	0.2	0.8	0.0	0.4	0.4
Total (early + late): Number	4	6	6	18	21
Mean	0.2	1.5	0.3	1	1.1
Mean % resorbed conceptuses/litter	2	18.2	1.9	9.9	11.7
Females with resorptions					
Number	3	2	3	5	7
%	15	50	15.7	27.8	36.8
Dead fetuses (number)	0	0	0	0	0
Live fetuses					
Number	165	26	163	136	134
Mean / mean litter size	8.2	6.5	8.6	7.6	7.0

* Significantly different from vehicle control ($p<0.05$)

Offspring

Fetal body weights tended to be lower in the 100 and 250 mg/kg/day CC-4047 groups and the 180 mg/kg/day thalidomide group than the vehicle control group, but the differences were not statistically significant.

Table 101: Rabbit fetal sex ratio and weights

Parameter	Vehicle control	Thalidomide 180 mg/kg/day	CC-4047 (mg/kg/day)		
			10	100	250
Mean % live male fetuses/litter	45.7	44.0	47.3	51.4	46.8
Fetal body weights					
Mean live fetal weight (g)/litter	43.24	39.18	42.96	40.63	41.49
Males	43.15	39.25	43.49	40.71	41.95
Females	43.18	38.90	42.21	40.55	40.79

The dead fetuses from the female with the abnormal uterus in the 10 mg/kg/day (animal # 5836) were examined. One fetus had a minor variation of a displaced nasal midline

suture at skeletal examination. Another had a short tail with only 9 caudal vertebrae present, which is a malformation that appears attributable to CC-4047 based on the data in the CC-4047 dose groups. Data on malformations and variations observed in the live fetuses are presented in the tables below.

Table 102: Summary of alterations (malformations and variations) in rabbit fetuses

Group	Vehicle control	Thalidomide 180 mg/kg/day	CC-4047 (mg/kg/day)		
			10	100	250
Number of litters evaluated	20	4	19	18	19
Number of fetuses evaluated	165	26	163	136	134
Number of litters with fetuses with any alteration observed (%)	13 (65.0)	4 (100.0)	16 (84.2)	17 (94.4)**	19 (100.0)**
Number of fetuses with any alteration observed (%)	26 (15.8)	25 (96.2)**	29 (17.8)	76 (55.9)*	119 (88.9)**
Mean % fetuses with any alteration/litter	16.6	95.0**	19.0	56.7**	89.8**

* Significantly different from control ($p<0.05$)
** Significantly different from control ($p<0.01$)

Table 103: External malformations in rabbits

Group		Vehicle control	Thalidomide 180 mg/kg/day	CC-4047 (mg/kg/day)		
				10	100	250
Number of litters evaluated		20	4	19	18	19
Number of fetuses evaluated		165	26	163	136	134
Eye: Bulge depressed	Number of litters (%)	0 (0.0)	1 (25.0)**	0 (0.0)	2 (11.1)	0 (0.0)
	Number of fetuses (%)	0 (0.0)	1 (3.8)**	0 (0.0)	2 (1.5)	0 (0.0)
Fore and/or hindlimbs: Flexed	Number of litters (%)	1 (5.0)	3 (75.0)**	0 (0.0)	0 (0.0)	5 (26.3)**
	Number of fetuses (%)	1 (0.6)	4 (15.4)**	0 (0.0)	0 (0.0)	7 (5.2)**
Fore and/or hindlimbs: Rotated	Number of litters (%)	0 (0.0)	3 (75.0)**	0 (0.0)	1 (5.6)	5 (26.3)**
	Number of fetuses (%)	0 (0.0)	10 (38.5)**	0 (0.0)	1 (0.7)	13 (9.7)**
Fore and/or hindlimbs: Digit unattached	Number of litters (%)	0 (0.0)	2 (50.0)**	0 (0.0)	0 (0.0)	4 (21.0)**
	Number of fetuses (%)	0 (0.0)	2 (7.7)**	0 (0.0)	0 (0.0)	6 (4.5)**
Fore and/or hindlimbs: Digit absent	Number of litters (%)	0 (0.0)	1 (25.0)**	0 (0.0)	0 (0.0)	3 (15.8)**
	Number of fetuses (%)	0 (0.0)	1 (3.8)**	0 (0.0)	0 (0.0)	3 (2.2)**
Tail: Short	Number of litters (%)	0 (0.0)	3 (75.0)**	1 (5.3)	2 (11.1)	5 (26.3)**
	Number of fetuses (%)	0 (0.0)	7 (26.9)**	1 (0.6)	4 (2.9)	14 (10.4)**

** Significantly different from control ($p<0.01$)

Table 104: Visceral malformations and variations in rabbits

Group		Vehicle control	Thalidomide 180 mg/kg/day	CC-4047 (mg/kg/day) 10	100	250
Number of litters evaluated		20	4	19	18	19
Number of fetuses evaluated		165	26	163	136	134
Malformations						
Eyes: Small	Number of litters (%)	0 (0.0)	1 (25.0)**	0 (0.0)	1 (5.6)	0 (0.0)
	Number of fetuses (%)	0 (0.0)	2 (7.7)**	0 (0.0)	1 (0.7)	0 (0.0)
Heart: Interventricular septal defect	Number of litters (%)	0 (0.0)	2 (50.0)**	4 (21.0)*	3 (16.7)	8 (42.1)**
	Number of fetuses (%)	0 (0.0)	3 (11.5)**	4 (2.4)	5 (3.7)	16 (11.9)**
Heart: Large	Number of litters (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.3)
	Number of fetuses (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.5)
Vessels: Innominate artery absent	Number of litters (%)	0 (0.0)	2 (50.0)**	0 (0.0)	3 (16.7)*	5 (26.3)**
	Number of fetuses (%)	0 (0.0)	3 (11.5)**	0 (0.0)	3 (2.2)*	5 (3.7)**
Vessels: Right subclavian passes dorsal to the trachea and esophagus	Number of litters (%)	0 (0.0)	2 (50.0)**	0 (0.0)	2 (11.1)	5 (26.3)**
	Number of fetuses (%)	0 (0.0)	3 (11.5)**	0 (0.0)	2 (1.5)	5 (3.7)**
Vessels: Right subclavian arises to the left of the left subclavian	Number of litters (%)	0 (0.0)	2 (50.0)**	0 (0.0)	2 (11.1)	5 (26.3)**
	Number of fetuses (%)	0 (0.0)	3 (11.5)**	0 (0.0)	2 (1.5)	5 (3.7)**
Vessels: Aorta distended	Number of litters (%)	0 (0.0)	0 (0.0)	0 (0.0)	3 (16.7)**	4 (21.0)**
	Number of fetuses (%)	0 (0.0)	0 (0.0)	0 (0.0)	4 (2.2)	9 (6.7)**
Vessels: Pulmonary artery constricted	Number of litters (%)	0 (0.0)	1 (25.0)**	0 (0.0)	2 (11.1)	1 (5.3)
	Number of fetuses (%)	0 (0.0)	1 (3.8)**	0 (0.0)	3 (2.2)	2 (1.5)
Vessels: Persistent truncus arteriosis	Number of litters (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (10.2)
	Number of fetuses (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (2.2)**
Vessels: Pulmonary artery distended	Number of litters (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.3)
	Number of fetuses (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)
Diaphragm: Diaphragmatic hernia	Number of litters (%)	0 (0.0)	2 (50.0)**	0 (0.0)	1 (5.6)	3 (15.8)
	Number of fetuses (%)	0 (0.0)	4 (15.4)**	0 (0.0)	1 (0.7)	4 (3.0)**
Liver: Lobe absent	Number of litters (%)	0 (0.0)	1 (25.0)**	0 (0.0)	0 (0.0)	0 (0.0)

Group		Vehicle control	Thalidomide 180 mg/kg/day	CC-4047 (mg/kg/day)		
				10	100	250
Number of litters evaluated		20	4	19	18	19
Number of fetuses evaluated		165	26	163	136	134
	Number of fetuses (%)	0 (0.0)	1 (3.8)**	0 (0.0)	0 (0.0)	0 (0.0)
Kidneys: Absent	Number of litters (%)	0 (0.0)	2 (50.0)**	0 (0.0)	3 (16.7)	6 (31.6)**
	Number of fetuses (%)	0 (0.0)	2 (7.7)**	0 (0.0)	3 (2.2)	10 (7.5)**
Kidneys: Low set	Number of litters (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (15.8)**
	Number of fetuses (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (3.0)**
Ureter: Absent	Number of litters (%)	0 (0.0)	0 (0.0)	0 (0.0)	2 (11.1)	6 (31.6)**
	Number of fetuses (%)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.5)	9 (6.7)**
Variations						
Brain: Ventricle dilation (slight)	Number of litters (%)	0 (0.0)	3 (75.0)**	0 (0.0)	5 (27.8)**	5 (26.3)**
	Number of fetuses (%)	0 (0.0)	4 (15.4)**	0 (0.0)	12 (8.8)**	7 (5.2)*
Brain: Ventricle dilation (moderate)	Number of litters (%)	0 (0.0)	2 (50.0)**	0 (0.0)	2 (11.1)	7 (36.8)**
	Number of fetuses (%)	0 (0.0)	3 (11.5)**	0 (0.0)	4 (2.9)	11 (8.2)**
Lungs: Intermediate lobe absent	Number of litters (%)	0 (0.0)	2 (50.0)**	0 (0.0)	2 (11.1)	9 (47.4)**
	Number of fetuses (%)	0 (0.0)	5 (19.2)**	0 (0.0)	2 (1.5)	20 (14.9)**
Kidneys: Large	Number of litters (%)	0 (0.0)	1 (25.0)**	0 (0.0)	0 (0.0)	1 (5.3)
	Number of fetuses (%)	0 (0.0)	1 (3.8)**	0 (0.0)	0 (0.0)	1 (0.7)
Kidneys: Displaced	Number of litters (%)	0 (0.0)	2 (50.0)**	0 (0.0)	0 (0.0)	2 (10.5)
	Number of fetuses (%)	0 (0.0)	2 (7.7)**	0 (0.0)	0 (0.0)	2 (1.5)
Kidneys: Pelvis dilation slight	Number of litters (%)	0 (0.0)	1 (25.0)**	0 (0.0)	0 (0.0)	0 (0.0)
	Number of fetuses (%)	0 (0.0)	1 (3.8)**	0 (0.0)	0 (0.0)	0 (0.0)

* Significantly different from control ($p<0.05$)
** Significantly different from control ($p<0.01$)

The various skeletal malformations observed in the CC-4047 treated groups are listed in the table below. Of note are the combinations of skeletal malformations observed in two of the fetuses; these skeletal malformations are listed in the table.

- One fetus (5853-8) in the 100 mg/kg/day group that had a shortened tail had multiple malformations of the lower spine including fused arches of the 6th lumbar and 1st sacral vertebrae, fused arches of the 13th thoracic and the 1st lumbar

vertebrae, fused centra of the 5th and 6th lumbar and 1st sacral vertebrae, fused centra of the 1st through 3rd sacral and 1st caudal vertebrae, fused 1st and 2nd caudal vertebrae, and not ossified arches of the 2nd and 3rd sacral vertebrae. This fetus also had tibias that were not ossified, a bent fibula and not ossified pubis.

- One fetus (5868-11) in a 250 mg/kg/day group litter with another fetus with a short tail (5868-3) had unilateral ossification of the 1st, fused 1st and 2nd and small arch of the 1st lumbar vertebrae. These malformations were considered to be associated with this dose of CC-4047 because of the pattern of effect.

Table 105: Skeletal malformations and variations in rabbits

Group		Vehicle control	Thalidomide 180 mg/kg/day	CC-4047 (mg/kg/day) 10	100	250
Number of litters evaluated		20	4	19	18	19
Number of fetuses evaluated		165	26	163	136	134
Malformations						
Forelimb: Metacarpal misaligned	Number of litters (%)	0 (0.0)	1 (25.0)**	0 (0.0)	0 (0.0)	1 (5.3)
	Number of fetuses (%)	0 (0.0)	1 (3.8)**	0 (0.0)	0 (0.0)	4 (3.0)**
Forelimb: Metacarpal not ossified	Number of litters (%)	0 (0.0)	2 (50.0)**	0 (0.0)	0 (0.0)	3 (15.8)**
	Number of fetuses (%)	0 (0.0)	3 (11.5)**	0 (0.0)	0 (0.0)	7 (5.2)**
Forelimb: Extra phalanx	Number of litters (%)	0 (0.0)	2 (50.0)**	0 (0.0)	1 (5.6)	2 (10.5)
	Number of fetuses (%)	0 (0.0)	2 (7.7)**	0 (0.0)	2 (1.5)	2 (1.5)
Forelimb: Phalanx not ossified	Number of litters (%)	0 (0.0)	1 (25.0)**	0 (0.0)	0 (0.0)	3 (15.8)**
	Number of fetuses (%)	0 (0.0)	2 (7.7)**	0 (0.0)	0 (0.0)	5 (3.7)**
Forelimb: Phalanx misaligned	Number of litters (%)	0 (0.0)	2 (50.0)**	0 (0.0)	0 (0.0)	1 (5.3)
	Number of fetuses (%)	0 (0.0)	2 (7.7)**	0 (0.0)	0 (0.0)	4 (3.0)**
Forelimb: Digit absent	Number of litters (%)	0 (0.0)	1 (25.0)**	0 (0.0)	0 (0.0)	3 (15.8)**
	Number of fetuses (%)	0 (0.0)	1 (3.8)**	0 (0.0)	0 (0.0)	3 (2.2)**
Hindlimb: Tibia short	Number of litters (%)	0 (0.0)	3 (75.0)**	0 (0.0)	0 (0.0)	4 (21.0)**
	Number of fetuses (%)	0 (0.0)	8 (30.8)**	0 (0.0)	0 (0.0)	6 (4.5)**
Hindlimb: Tibia not ossified	Number of litters (%)	0 (0.0)	2 (50.0)**	0 (0.0)	1 (5.6)	4 (21.0)**
	Number of fetuses (%)	0 (0.0)	3 (11.5)**	0 (0.0)	1 (0.7)	7 (5.2)**
Hindlimb: Fibula short	Number of litters (%)	0 (0.0)	2 (50.0)**	0 (0.0)	0 (0.0)	0 (0.0)
	Number of fetuses (%)	0 (0.0)	6 (23.1)**	0 (0.0)	0 (0.0)	0 (0.0)

Group		Vehicle control	Thalidomide 180 mg/kg/day	CC-4047 (mg/kg/day)		
				10	100	250
Number of litters evaluated		20	4	19	18	19
Number of fetuses evaluated		165	26	163	136	134
Hindlimb: Fibula bent	Number of litters (%)	0 (0.0)	2 (50.0)**	0 (0.0)	1 (5.6)	4 (21.0)**
	Number of fetuses (%)	0 (0.0)	2 (7.7)**	0 (0.0)	1 (0.7)	6 (4.5)**
Hindlimb: Metatarsal not ossified	Number of litters (%)	0 (0.0)	1 (25.0)**	0 (0.0)	0 (0.0)	1 (5.3)
	Number of fetuses (%)	0 (0.0)	1 (3.8)**	0 (0.0)	0 (0.0)	1 (0.7)
Hindlimb: Phalanx not ossified	Number of litters (%)	0 (0.0)	1 (25.0)**	0 (0.0)	0 (0.0)	1 (5.3)
	Number of fetuses (%)	0 (0.0)	1 (3.8)**	0 (0.0)	0 (0.0)	1 (0.7)
Caudal vertebrae: Misaligned	Number of litters (%)	2 (10.0)	4 (100.0)**	7 (36.8)	14 (77.8)**	18 (94.7)**
	Number of fetuses (%)	2 (1.2)	11 (42.3)**	11 (6.7)	21 (15.4)	45 (33.6)**
Caudal vertebrae: Small	Number of litters (%)	1 (5.0)	0 (0.0)	4 (21.0)	1 (5.6)	5 (26.3)
	Number of fetuses (%)	1 (0.6)	0 (0.0)	4 (2.4)	1 (0.7)	6 (4.5)
Caudal vertebrae: Fused	Number of litters (%)	0 (0.0)	4 (100.0)**	1 (5.3)	7 (38.9)*	12 (63.2)**
	Number of fetuses (%)	0 (0.0)	8 (30.8)**	2 (1.2)	11 (8.1)*	20 (14.9)**
Skull: Small eye socket	Number of litters (%)	0 (0.0)	1 (25.0)**	0 (0.0)	1 (5.6)	0 (0.0)
	Number of fetuses (%)	0 (0.0)	1 (3.8)**	0 (0.0)	1 (0.7)	0 (0.0)
Variations						
Skull: Irregular ossification	Number of litters (%)	8 (40.0)	4 (100.0)**	10 (52.6)	14 (77.8)**	17 (89.5)**
	Number of fetuses (%)	15 (9.1)	19 (73.1)**	12 (7.4)	41 (30.1)*	52 (38.8)**
Skull: Irregular ossification of nasals	Number of litters (%)	8 (40.0)	3 (75.0)	10 (52.6)	11 (61.1)	15 (78.9)
	Number of fetuses (%)	15 (9.1)	4 (15.4)	11 (6.7)	31 (22.8)**	22 (16.4)
Skull: Nasals fused	Number of litters (%)	0 (0.0)	0 (0.0)	0 (0.0)	2 (11.1)	2 (10.5)
	Number of fetuses (%)	0 (0.0)	0 (0.0)	0 (0.0)	4 (2.9)**	2 (1.5)
Skull: Nasals contain an internasal	Number of litters (%)	1 (5.0)	2 (50.0)**	2 (10.5)	4 (11.1)	5 (26.3)
	Number of fetuses (%)	1 (0.6)	3 (11.5)**	3 (0.6)	7 (3.7)	6 (4.5)
Skull: Irregular ossification of frontals	Number of litters (%)	0 (0.0)	4 (100.0)**	1 (5.3)	4 (22.2)	10 (57.9)**
	Number of fetuses (%)	0 (0.0)	17 (65.4)**	1 (0.6)	8 (6.6)	18 (14.9)**
Skull: Frontals	Number of	0 (0.0)	4 (100.0)**	1 (5.3)	4 (22.2)	9 (6.7)**

Group		Vehicle control	Thalidomide 180 mg/kg/day	CC-4047 (mg/kg/day)		
				10	100	250
Number of litters evaluated		20	4	19	18	19
Number of fetuses evaluated		165	26	163	136	134
contain an interfrontal	litters (%)					
	Number of fetuses (%)	0 (0.0)	16 (57.7)**	1 (0.6)	8 (5.1)*	15 (11.9)**
Skull: Frontals fused	Number of litters (%)	0 (0.0)	1 (25.0)**	0 (0.0)	3 (16.7)**	4 (21.0)**
	Number of fetuses (%)	0 (0.0)	1 (3.8)**	0 (0.0)	3 (2.2)**	4 (3.0)**
Skull: Interparietals incompletely ossified	Number of litters (%)	1 (5.0)	2 (50.0)**	0 (0.0)	4 (22.2)	9 (47.4)**
	Number of fetuses (%)	1 (0.6)	2 (7.7)**	0 (0.0)	4 (2.9)	17 (12.7)**
Skull: Parietal contains a hole	Number of litters (%)	0 (0.0)	1 (25.0)**	0 (0.0)	1 (5.6)	3 (15.8)
	Number of fetuses (%)	0 (0.0)	1 (3.8)**	0 (0.0)	2 (1.5)	6 (4.5)**
Hyoid: Small alae	Number of litters (%)	0 (0.0)	2 (50.0)**	0 (0.0)	1 (5.6)	1 (5.3)
	Number of fetuses (%)	0 (0.0)	2 (7.7)**	0 (0.0)	1 (0.7)	1 (0.7)
Hyoid: Angulated alae	Number of litters (%)	2 (10.0)	3 (75.0)**	3 (15.8)	2 (11.1)	4 (21.0)
	Number of fetuses (%)	3 (1.8)	7 (26.9)**	3 (1.8)	2 (1.5)	7 (5.2)
Sternal centra: Fused	Number of litters (%)	2 (10.0)	1 (25.0)	0 (0.0)	7 (38.9)	15 (78.9)**
	Number of fetuses (%)	2 (1.2)	1 (3.8)	0 (0.0)	14 (10.3)	39 (29.1)**
Pelvis: Pubis not ossified	Number of litters (%)	0 (0.0)	1 (25.0)**	0 (0.0)	1 (5.6)	3 (15.8)
	Number of fetuses (%)	0 (0.0)	2 (7.7)**	0 (0.0)	1 (0.7)	5 (3.7)**
Pelvis: Pubis incompletely ossified	Number of litters (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (10.5)
	Number of fetuses (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.5)

* Significantly different from control ($p<0.05$)
** Significantly different from control ($p<0.01$)

9.3 Prenatal and Postnatal Development

No prenatal and postnatal development studies were submitted.

10 Special Toxicology Studies

Study title: A 28-day immunotoxicity study of CC-4047 administered by nasogastric gavage to cynomolgus monkeys followed by a 30-day recovery period

Study no.:	CC-4047-TOX-019
Study report location:	eCTD 4.2.3.7.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 15, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CC-4047, lot # CMLW-376/09-CC2, Purity: 100.8%

Key Study Findings

- CC-4047 did not produce changes in granulocyte and monocyte function or natural killer cell function.
- CC-4047-related findings included lymphoid depletion of the thymus, spleen, and mandibular and mesenteric lymph nodes, alterations to the primary and secondary humoral immune response demonstrated by reductions in anti-KLH IgM and IgG antibody production, and decreases in circulating peripheral lymphocytes correlating to bone marrow lymphocyte hypocellularity.

Methods

Doses:	0 or 2 mg/kg/day
Frequency of dosing:	Once daily for 28 days; 30-day recovery period
Route of administration:	Nasogastric gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	1% w/v sodium carboxymethylcellulose sodium salt in deionized water and 100 mM acetate buffer
Species/Strain:	Cynomolgus monkeys
Number/Sex/Group:	Main study: 4/sex/group Recovery: 2/sex/group
Age:	Males: 2.9-6.0 years at initiation of dosing Females: 3.4-5.3 years at initiation of dosing
Weight:	Males: 3.3-5.4 kg on Day -1 Females: 2.7-3.7 kg on Day -1
Satellite groups:	None

Results

This study was conducted to investigate the potential immunotoxicology of CC-4047 and the effect on the primary T-cell dependent antibody response to an immunogen. A 28-day toxicology study with a 30-day recovery period was conducted with the typical toxicology study assessments and immunotoxicity assessments including natural-killer cell activity assay, peripheral blood immunophenotyping, peripheral blood granulocytes and monocytes function, and T-cell dependent antibody response analyses. One female (Animal # 2606) treated with 2 mg/kg/day was euthanized moribund on Day 22 due to reduced food consumption, watery feces, and weight loss (~14%) beginning on Day 14. There were no test article-related changes in food consumption, body weight, clinical chemistry parameters, granulocyte and monocyte function, or natural killer cell function. Test article-related findings included reduced mean thymus weight and marked lymphoid depletion of the thymus, spleen, and mandibular and mesenteric lymph nodes. There were alterations to the primary and secondary humoral immune response demonstrated by reductions in anti-KLH IgM and IgG antibody production. Additionally, there were mild to moderate decreases in circulating peripheral lymphocytes correlating to mild to moderate bone marrow lymphocyte hypocellularity. The mean percent of baseline values of each cell population is presented in the table below. Most of the test-article-related findings were fully recovered at the end of the 30-day recovery period with the exception of the decreased CD20+ B-lymphocytes (partial recovery observed), reduced thymus weights, minimal expansion of the paracortex in the mandibular lymph node, and mild germinal center lymphoid depletion in the mesenteric lymph node.

Table 106: Mean percent of baseline values of each cell population for combined males and females on Day 27

(excerpted from Applicant's submission)

	Total T-lymphocytes (CD3+)	T-cytotoxic lymphocytes (CD3+/CD8+)	T-helper lymphocytes (CD3+/CD4+)	Monocytes (CD3-/CD14+)	B-lymphocytes (CD20+)	NK-cells (CD3-/CD16+)
0 mg/kg/day (Control Group)	85 ± 16	82 ± 17	88 ± 14	96 ± 21	105 ±27	83 ± 26
2 mg/kg/day (CC-4047)[a]	48 ± 13	53 ± 21	48 ± 10	54± 22	9 ± 5	63 ± 22

[a] Includes data from animal euthanized early (Day 22)

11 Integrated Summary and Safety Evaluation

Pomalidomide (CC-4047) is one of several structural analogues of thalidomide (called IMiDs) known for their immunomodulatrory effects. Currently there is one approved thalidomide analogue, lenalidomide (Revlimid®), which is indicated for the treatment of multiple myeloma, in combination with dexamethasone, in patients who have received at least one prior therapy and patients with transfusion-dependent anemia due to low- or intermediate-1-risk myelodysplastic syndromes (MDS) associated with a deletion 5q abnormality with or without additional cytogenetic abnormalities. Pomalidomide has

also been developed for the treatment of multiple myeloma and the proposed indication is in combination with dexamethasone for the treatment of patients with relapsed and refractory multiple myeloma who have received at least two prior regimens of established benefit, including both lenalidomide and bortezomib, and have demonstrated disease progression on the last therapy. Nonclinical pharmacology, pharmacokinetic, and toxicology studies have been submitted to support the approval of pomalidomide for this indication.

Pharmacology

In vitro and *in vivo* studies were conducted to investigate the pharmacologic and anti-tumor activity of pomalidomide. Cereblon was identified as the direct molecular target modulating the anti-proliferative and some immunomodulatory effects of pomalidomide. Cereblon affects polyubiquitination of proteins, targeting them for destruction in the proteosome and its expression was required in T cells for pomalidomide-induction of IL-2 and TNF-α. Pomalidomide and lenalidomide exhibited similar binding affinity for cereblon and the expression of this protein in myeloma cell lines was shown to be required for pomalidomide to exert its effects. Decreased levels of cereblon correlated with acquisition of resistance to lenalidomide in different cell lines.

Pomalidomide was also shown to affect the cell cycle producing G_1 arrest in different cell lines and to induce apoptosis by down regulating CDK4 and CDK6 responses with no effect on CDK2. CDK regulation was associated with decreases in retinoblastoma protein phosphorylation and decreased levels of other proteins that are important regulators of cell growth and differentiation in myeloma cell lines. The immunomodulatory effects of pomalidomide included the inhibition of pro-inflammatory cytokines and chemokines in lipopolysaccharide stimulated human peripheral blood mononuclear cells including IL-12, IL-1β, IL-6, TNF-α, GM-CSF production, MCP-1, and MIP-1α, and increased production of IL-10. Pomalidomide did not have an effect on IL-8 or RANTES (regulated upon activation, normal T-cell expressed, and secreted) production. Using a similar lipopolysaccharide stimulated human peripheral blood mononuclear cells, pomalidomide inhibited COX-2 expression (64%) and inhibited PGE_2 production with an IC_{50} of 50 μM (study not reviewed). Pomalidomide displayed inhibition 20 times and 2 times higher than lenalidomide for TNF-α and IL-12, respectively. Pomalidomide was shown in an *in vitro* clonogenic assay to have potential for inhibiting erythroid progenitors, but it did not inhibit megakaryocytic or myeloid progenitors. When CC-4047 was present during CD34+ cell expansion and maturation phases, it blocked the generation of dendritic cell progenitors and increased the differentiation of CD34+ cells into the granulocytic lineage. Results suggested that pomalidomide could modulate lineage-specific expression and subsequent myeloid differentiation.

Pomalidomide was 10 times more effective than lenalidomide in reducing tumor volumes in SCID mice bearing human multiple myeloma tumors. Pomalidomide displayed higher (5.8 times) sprout formation inhibition, an indicator of anti-angiogenic activity, than lenalidomide in a human umbilical cord vessel ring assay. However,

lenalidomide demonstrated significant anti-angiogenic activity at doses 10 times lower than pomalidomide in an *in vivo* angiogenesis matrigel plug assay.

The combination of pomalidomide and dexamethasone showed anti-neoplastic activity in KMS-12-BM and H929 multiple myeloma cells previously made resistant to lenalidomide and pomalidomide. Synergistic effects of pomalidomide and dexamethasone on cell proliferation and viability of lenalidomide-resistant cell lines were accompanied by synergistic molecular responses consistent with the G_1 cell cycle. These included reduced phosphorylation of retinoblastoma protein 1 (pRB1), decreased expression of interferon regulatory factor 4 (IRF-4) and anti-apoptotic factors Survivin and B-cell lymphoma 2 (Bcl-2), and increased expression of p27 and the pro-apoptotic factor BIM.

Safety Pharmacology

Pomalidomide showed no evidence of acute effects on neurological, pulmonary or cardiovascular function. Single doses up to 2000 mg/kg (12,000 mg/m^2) in rats produced no effects on neurological and pulmonary function. Cardiovascular and pulmonary functions were concurrently evaluated in anesthetized beagle dogs and conscious monkeys wearing telemetry jackets. Changes observed at the high dose in dogs (25 mg/kg; 500 mg/m^2) were of a similar trend and intensity as those in the control group, but more variable. One female dog exhibited an adverse reaction at 500 mg/m^2 that resulted in an increased rate of respiration accompanied with decreases in blood pressure and femoral artery blood flow. Monkeys wearing telemetry jackets were dosed in a Latin square design with a 7-day washout interval between doses. Pulse pressure was slightly lower (~ 10%) at the 10 mg/kg (120 mg/m^2) dose compared with the control treatment during the first seven hours after treatment. Cardiovascular effects of pomalidomide were also assessed *in vitro*. To assess the potential for delayed repolarization and prolongation of the QT interval, the effects of pomalidomide (7.9 and 87.5 µM) were studied in a hERG assay in human embryonic kidney (HEK293) cells expressing the hERG potassium channel. Pomalidomide produced less than 1% inhibition of the hERG current, suggesting a low potential to block *in vivo* cardiac I_{Kr}.

Phamacokinetics

Absorption, distribution, metabolism and excretion were evaluated *in vivo* in rats and monkeys and in *in vitro* models using a racemate of pomalidomide (S and R enantiomers) and/or individual enantiomers. Oral bioavailability of the racemate of pomalidomide in rats was calculated at 13% with corresponding half-lives of 5.5 and 6.2 hours after single oral (100 mg/kg; 600 mg/m^2) or intravenous (2.5 mg/kg;15 mg/m^2) doses, respectively. The similar half-lives suggested comparable elimination phases for both routes of administration. Oral bioavailability of the racemate of pomalidomide in monkeys was 15% with corresponding mean half-lives of 25.0 and 6.7 hours after single oral (100 mg/kg; 1200 mg/m^2) or intravenous (10 mg/kg;120 mg/m^2) doses, respectively. A detailed evaluation of the pharmacokinetics of the pomalidomide enantiomers in monkeys showed interconversion between enantiomers. Exposure to

CC-6016 (R-enantiomer) was almost twice that of CC-5083 (S-enantiomer), and clearance values for CC-5083 was approximately twice that of CC-6016 following intravenous dosing of individual enantiomers.

In an *in vitro* protein binding study, plasma protein binding ranged from 15 to 40% and from 16 to 55% for the pomalidomide R- and S-enantiomers, respectively, when pomalidomide was incubated with plasma from human, monkey, rat, mouse and rabbit origin. Protein binding of the pomalidomide R- and S-enantiomers was similar in mouse and rabbit plasma, the binding of R- was higher than the S-enantiomer in rat plasma, and the protein binding of S- was higher than the R-enantiomer in monkey and human plasma. A tissue distribution study in Long-Evans pigmented rats demonstrated that pomalidomide-derived radioactivity was widely distributed to most tissues and blood through 8 hours after a single 100 mg/kg (600 mg/m^2), 200 µCi/kg oral dose of radioactive pomalidomide. The highest concentrations were measured in the alimentary canal (GI tract) and organs of excreta (renal cortex, medulla, and urinary bladder). Moderate concentrations were found in the bile, liver, endocrine glands, secretory glands, brown adipose, and pigmented skin, lymph nodes and thymus.

In the dose-range finding embryo-fetal development study in rabbits (study not reviewed), fetal plasma pomalidomide concentrations were approximately 50% of the maternal C_{max} after multiple dosing to pregnant rabbits, indicating that pomalidomide crosses the placenta. In a lacteal transfer study following a single oral dose of pomalidomide (10 mg/kg; 60 mg/m^2) in rats, the mean milk concentration ratios ranged from 0.63 to 1.5 for up to 24 hours after dosing, indicating that pomalidomide is excreted into milk in rats.

Pomalidomide metabolism was assessed in monkeys following intravenous and oral administration of radioactive doses. No significant differences in metabolic profiles from urine, feces and plasma between the two different routes of administration suggested minimal first pass metabolism in the intestine. Pomalidomide was extensively metabolized through hydroxylation, glucuronidation, hydrolysis, and N-dealkylation-deamination with ≤10% and ≤ 3% of the radioactive dose excreted in urine and feces, respectably, attributed to the parent compound. More than 15 metabolites were identified in urine and feces; however, the contribution of the 6 major metabolites to systemic exposure was not significant. Plasma samples from other studies were used to understand the exposure of pomalidomide metabolites produced in humans and in animal species used in toxicological studies. All five previously identified human metabolites, M11, M12, M13, M16 and M17, were detectable in rat and monkey plasma pooled from toxicological studies. The percent distribution of the metabolites was comparable in plasma samples from monkeys and humans. Radioactive pomalidomide was excreted rapidly and completely primarily in urine (intravenous, 71.6%; oral, 72.9%) and in feces (intravenous, 11.6%; oral, 13.1%). The high urinary excretion after oral dosing implied substantial oral bioavailability.

In vitro studies were conducted to evaluate the ability of pomalidomide to inhibit or induce cytochrome P450 enzymes and the potential for drug-drug interactions.

Pomalidomide did not induce or cause either direct or time-dependent inhibition of cytochrome P450 enzymes in microsomes prepared from freshly isolated and cultured human hepatocytes exposed to pomalidomide. Pomalidomide was a substrate for P-glycoprotein in MDCK-MDR1 transfected and wild type cells, but did not inhibit P-glycoprotein efflux of the known P-glycoprotein substrate digoxin in MDCK-MDR1 transfected and wild type cells.

General Toxicology

Pomalidomide was assessed in repeat dose oral toxicology studies including 7-, 28- and 90-day and 6-month studies in rats and 28-day, 13-week, and 9-month studies in cynomolgus monkeys. In the rat studies, pomalidomide was well tolerated and did not produce any clear toxicity at doses up to 2000 mg/kg/day (12,000 mg/m^2/day) for 28 days, 1500 mg/kg/day (9000 mg/m^2/day) for 90 days, or 1000 (6000 mg/m^2/day) for 180 days (6 months). In the pivotal 6-month rat study, pomalidomide (50, 250, or 1000 mg/kg/day; 300, 1500, or 6000 mg/m^2/day) was administered by oral gavage daily for 180 days with a 28-day recovery period. Some toxicity was observed including changes in hematology (fibrinogen increased and platelets and lymphocytes decreased) and clinical chemistry (decreases in total protein, albumin, and globulin and increases in phosphorus and potassium in females), however, there were no clear dose-response relationships and the values were within the range for historical control values provided by the Applicant. Additionally, there were no pomalidomide-related histopathology findings in the study. Pomalidomide was absorbed and systemic exposures increased in a less than dose-proportional manner.

Immunosupression effects of pomalidomide were observed in the cynomologus monkeys including decreases in platelets, white blood cell count, and lymphocytes in serum, lymphoid hypocellularity in the bone marrow, and lymphoid depletion or atrophy of various lymphoid tissues (mandibular and mesenteric lymph noses, Peyer's patch, spleen and thymus). Decreases in red blood cell count, hemoglobin, and hematocrit, increases in fibrinogen, and decreases in albumin and total protein were also observed. Additionally, pomalidomide produced moribundity and mortality in the 28-day, 13-week, and 9-month studies. In one 28-day study, all doses (30, 100 or 300 mg/kg/day; 360, 1200, and 3600 mg/m^2/day) produced hemopoietic and lymphoid system toxicity that lead to mortalities and the termination of treatment after 18 days. Doses of 0.05, 0.2, 2, or 10 mg/kg/day (0.6, 2.4, 24, or 120 mg/m^2/day) were administered daily by nasogastric intubation in the 13-week study and dosing of the 120 mg/m^2/day group was discontinued at the end of Week 5 due to adverse clinical signs and hematology changes. Clinical signs observed in this and/or other studies included watery feces, sunken eyes, hunched posture, piloerection, decreased body weight, and decreased food consumption.

In the pivotal 9-month monkey study, pomalidomide (0.05, 0.1, or 1 mg/kg/day; 0.6, 1.2, or 12 mg/m^2/day) was administered by nasogastric gavage daily for 39 weeks with an 8-week recovery period. Treatment with the highest dose (12 mg/m^2/day) produced morbidity resulting in early euthanization of 3 males and 3 females. The principle

causes of morbidity were chronic inflammation of the large intestine with or without villous atrophy of the small intestine, *Staphylococcus aureus* infection, and acute myeloid leukemia. The acute *Staphylococcus aureus* infection occurring in one male involved the tissue surrounding the thoracic and lumbar vertebrae, marrow cavity and meninges of the spinal cord and brain, with hematogenous spread to the lungs and myocardium. One female had drug-related findings consistent with acute myeloid leukemia (AML) including an exceptionally high WBC count (114,600 cells/µL) consisting primarily of atypical cells, a large population of CD34+ blast cells, increased monocytes, bone marrow filled with blast cells, and leukemic infiltrates in various organs. Based on the rarity of this type of neoplasm in nonhuman primates, the known association of neoplasms and immunosuppression in humans, and the demonstrated immunotoxicity in the study, the neoplasm in this animal was attributed to the immunosuppressive effects of pomalidomide. Chronic inflammation of the large intestine and irreversible proliferation of intrahepatic bile ducts were observed in surviving monkeys at 12 mg/m^2/day.

Cynomolgus monkeys were clearly more sensitive to pomalidomide than rats. No clear toxicities were observed with repeated daily administration of high doses of pomalidomide (up to 6000 mg/m^2/day for 6 months) in the rat, while daily administration of a dose of 12 mg/m^2/day for 9 months produced mortality in the monkey. These results are consistent with the general toxicology results for lenalidomide, in which little toxicity was observed at 1800 mg/m^2/day for 26 weeks in rats and mortality was observed at doses of 48 and 72 mg/m^2/day prior to 20 weeks in a 52 week study in monkeys. This suggests that rodents are not as sensitive as non-rodents to the effects of lenalidomide and pomalidomide assessed in general toxicology studies.

Genetic Toxicology

Pomalidomide was negative in the battery of genetic toxicology studies. Pomalidomide was tested for mutagenicity in an *in vitro* reverse mutation (Ames) assay and an *in vitro* mouse lymphoma assay. In the reverse mutation assay, pomalidomide (50-5000 µg/plate) did not increase the number of revertant colony counts of any strain in the absence or presence of S-9 activation, therefore, was not mutagenic in this assay. In the mouse lymphoma assay, there was a weak statistically significant linear trend in the absence of S-9 and a small but statistically significant increase in mutant frequency observed at the intermediate concentration of 150 µg/mL in the presence of S-9 in one experiment. These findings were not reproducible in a second experiment. Therefore, pomalidomide did not induce mutation at the *tk* locus of L5178Y mouse lymphoma cells.

Pomalidomide was tested for clastogenicity in an *in vitro* chromosomal aberrations assay in cultured human peripheral blood lymphocytes and an *in vivo* rat bone marrow micronucleus assay. Pomalidomide did not produce an increase in cells with chromosomal aberrations, polyploidy, or endoreduplication in the absence or presence of S-9 activation. Additionally, pomalidomide did not induce statistically significant increases in micronucleated polychromatic erythrocytes (PCEs) at any dose (500, 1000, or 2000 mg/kg/day; 3000, 6000, or 12,000 mg/m^2/day) examined in the rat bone marrow

micronucleus assay. These results indicate that pomalidomide is neither mutagenic or clastogenic under the conditions of the genotoxicity studies conducted.

Carcinogenicity

Carcinogenicity studies have not been conducted.

Reproductive and Developmental Toxicology

A fertility and early embryonic development study was conducted with oral pomalidomide in the Crl:CD (SD) rat with treated males mated to treated females. Males were treated with pomalidomide (0, 25, 250, or 1000 mg/kg/day; 0, 150, 1500, or 6000 mg/m^2/day) once daily for 28 days prior to pairing through mating until necropsy, and females were treated with pomalidomide (0, 25, 250, or 1000 mg/kg/day; 0, 150, 1500, or 6000 mg/m^2/day) once daily for 14 days prior to pairing through mating until gestation day (GD) 7. Cesarean section was performed on GD 13 in females. After pomalidomide-related reproductive effects were observed in females, males were paired a second time with untreated females. Pomalidomide had no effect on premating estrous cyclicity in treated females or reproductive function or fertility indices in treated males or treated and untreated females. The mean body weight change during gestation (Days 1-13) was lower in females treated with pomalidomide compared to controls and corresponded to lower gravid uterine weights and the reduction of viable embryos and increase in post-implantation loss observed in the uterine examination. In pomalidomide treated females paired with treated males, the number of viable embryos was significantly decreased and the post-implantation loss and total number of resorptions (early + late) were significantly increased compared to controls at all doses. Pre-implantation loss was also increased at all doses, but was not statistically significant. There were no effects of pomalidomide on embryo viability in untreated females paired with treated males, indicating that the increase in post-implantation loss seen in the first pairing was not attributable to the treatment of the males. Hence, while the number of pregnancies was not affected, exposure of females to pomalidomide resulted in a decreased number of embryos.

The embryo-fetal development effects of pomalidomide were studied in the rat and rabbit. In the rat study, female Crl:CD (SD) rats were administered pomalidomide (0, 25, 250, or 1000 mg/kg/day; 0, 150, 1500, or 6000 mg/m^2/day) by oral gavage once daily on GD 6-17 and euthanized on GD 20. Maternal body weight gain was lower in the pomalidomide treated groups than controls starting around GD 12, but this finding was secondary to a significantly lower gravid uterine weight for all pomalidomide-treated groups. Additionally, there were no effects of pomalidomide on food consumption. The reduction in body weight gain appears to be due to the reduction of viable fetuses and increase in post-implantation loss observed in the uterine examination. Therefore, maternal toxicity was not observed in this study. The mean number of resorptions and post-implantation loss were significantly increased and the number of viable fetuses was significantly decreased in all pomalidomide dose groups compared to controls. Fetal weights were significantly decreased in all pomalidomide dose groups compared

to controls. Increases in visceral and skeletal malformations and variations were observed in all the pomalidomide treated groups. Visceral malformations included absent urinary bladder and absent thyroid observed at all doses. Increases in aortic arch malformations (right-sided aortic arch, dilated arch, retroesophageal arch, extra azygous vein, and small pulmonary trunk) were observed at 6000 mg/m^2/day. Visceral variations observed in pomalidomide groups included significant increases in renal pelvic cavitation and undeveloped renal papillae in the kidney and dilated ureter at all doses and significant increases in absent innominate artery at 6000 mg/m^2/day. Skeletal malformations included fused centra, fused neural arches, and misaligned neural arches of the lumbar and thoracic vertebrae. Skeletal variations included significant increases in small ribs at all doses, 13th rib at 1500 and 6000 mg/m^2/day, and not ossified sternebrae at 150 and 1500 mg/m^2/day. Embryo-fetal loss and teratogenicity were observed at all doses of pomalidomide.

In the pivotal rabbit embryofetal development study, female New Zealand White rabbits were administered pomalidomide (0, 10, 100, or 250 mg/kg/day; 0, 120, 1200, or 3000 mg/m^2/day) orally via stomach tube once daily on GD 7-19 and euthanized on GD 29. A positive control group of 5 females was dosed with 180 mg/kg/day (2160 mg/m^2/day) of thalidomide on the same dosing schedule. Pomalidomide produced similar effects to the positive control (2160 mg/m^2/day thalidomide) including decreases in maternal body weight gain, increased post-implantation loss, and increases in gross external, visceral, and skeletal malformations and variations. Pomalidomide was maternally toxic at the mid and high doses (1200 and 3000 mg/m^2/day) with decreased body weight gain, changes in hematology and clinical chemistry, and decreased spleen and thymus weights. The gravid uterine weights were lower at 1200 and 3000 mg/m^2/day pomalidomide than in the vehicle control group. Post-implantation loss was increased in the 1200 and 3000 mg/m^2/day groups compared to the vehicle control group with increases in the number of early, late, and total resorptions, % resorbed conceptuses per litter, and number of females with resorptions. Only the increase in early resorptions at 3000 mg/m^2/day pomalidomide was statistically significant. The mean number of live fetuses/mean litter size tended to be lower in the 3000 mg/m^2/day pomalidomide group, but were not statistically significant. Fetal body weights also tended to be lower in the 1200 and 3000 mg/m^2/day pomalidomide groups than the vehicle control group, but the differences were not statistically significant. The 1200 and 3000 mg/m^2/day doses of pomalidomide produced significant increases in the litter and fetal incidences of gross external, visceral, and skeletal malformations, including limb malformations similar to those observed in the thalidomide positive control group. External malformations included flexed and/or rotated fore and/or hindlimbs, unattached or absent digit of the fore and/or hindlimbs, and short tail. Visceral malformations were observed in the heart (interventricular septal defect), vessels (absent innominate artery, distended aorta, abnormal placement of the right subclavian artery, and constricted pulmonary artery), diaphragm (diaphragmatic hernia), kidney (absent kidney and low set kidney), and ureter (absent ureter). Increases in fetal cardiac anomalies, principally the malformation of interventricular septal defect, were observed at all doses of pomalidomide. Visceral variations included slight and moderate ventricle dilation in the brain and absent intermediate lobe in the lungs. There were numerous skeletal

malformations including those associated with short tail (misaligned and fused caudal vertebrae) and limb anomalies (not ossified metacarpal, misaligned phalanx and metacarpal, absent digit, not ossified phalanx, and short, not ossified or bent tibia) observed externally. Skeletal variations include irregular ossification of the skull and fused sternal centra. Teratogenicity was observed at all doses of pomalidomide.

Pomalidomide produced post-implantation loss and was clearly teratogenic in both the rat and rabbit. Based on these findings and to be consistent with the labeling for thalidomide and lenalidomide, pregnancy category X is recommended. Celgene Corporation has submitted a REMS plan for pomalidomide that includes a restricted distribution program similar to those for thalidomide and lenalidomide.

For the animal:human conversions for pomalidomide labeling, the human AUC (AUC_{24h}) of 402 ng•h/mL on week 4 for the recommended clinical dose of 4 mg/day was used.

Tabulated Summary of Safety Pharmacology Studies

Study #/Organ System	Method of Administration	Species/ cells	Doses/ concentrations	Gender/n	Findings
CC-4047-TOX-011 Central Nervous System	Oral	Sprague-Dawley rat	0, 250, 1000, or 2000 mg/kg (1500, 6000, or 12,000 mg/m^2)	Males and Females 10/sex/group	No effects
CC-4047-TOX-014 Respiratory	Oral	Sprague-Dawley rat	0, 250, 1000, or 2000 mg/kg (1500, 6000, or 12,000 mg/m^2)	Males 8/group	No effects
CC-4047-TOX-009 Cardiovascular	*In vitro*	HEK293 cells expressing hERG gene	7.9 or 87.5 µM	NA/n=3-4	0.9% inhibition of hERG channel. IC_{50} was not determined
1398-110 Cardiovascular and respiratory	IV	Beagle dog anesthetized	2.5, 10 and 25 mg/kg (50, 200, and 500 mg/m^2)	Males and Females 2/sex/group	50 and 200 mg/m^2: No effects. 500 mg/m^2: 1 ♀ had adverse reactions. Infusion stopped at 7 min: ↑ respiration rate, ↓ blood pressure, ↓ arterial blood flow. Animal artificially ventilated to infusion completion (45 to 120 min). Large red patches on lungs indicative of possible pulmonary embolism.

Study #/Organ System	Method of Administration	Species/ cells	Doses/ concentrations	Gender/n	Findings
CC-4047-TOX-012 Cardiovascular	Oral	Cynomolgus monkeys	0, 0.2, 2, and 10 mg/kg (0, 2.4, 24, and 120 mg/m^2)	Males / n=4	120 mg/m^2: ↓ Pulse pressure (10%; 3 mm Hg)

Tabulated Summary of General Toxicology Studies

Repeat Dose Toxicity Studies				
Species	**Route Duration**	**N/sex/ dose**	**mg/kg/day (mg/m^2/day)**	**Significant findings**
Rat (Study 1398/114)	Oral Daily x 7 days	5 (MS)	100 (600) 300 (1800) 1000 (6000) 2000 (12,000) 3000 (18,000) 5000 (30,000)	Doses up to 30,000 mg/m^2/day for 7 days were well tolerated There was no dose response for the toxicokinetics for the 12,000, 18,000, and 30,000 mg/m^2/day doses, indicating saturation for absorption
Rat (Study 1398/115)	Oral Daily x 28 days (1 month)	10 (MS)	300 (1800) 800 (4800) 2000 (12,000)	No toxicity was observed at doses up to 12,000 mg/m^2/day for 28 days
Rat (Study CC-4047-TOX-001)	Oral Daily x 90 days (3 months)	10 (MS)	100 (600) 500 (3000) 1500 (9000)	9000 mg/m^2/day: ↓ body weight in males
Rat (Study CC-4047-TOX-013)	Oral Daily x 180 days (6 months) 1-month recovery period	15 (MS) 5 (Recov.)	50 (300) 250 (1500) 1000 (6000)	Doses up to 6000 mg/m^2/day for 180 days did not produce clear toxicity in the rat
Monkey (Study 1398/116)	Oral Daily x 7 to 14 days	2 (MS)	MTD phase: 100 (1200) 300 (3600) 600 (7200) 1200 (14,000) Fixed dose phase: 50 (600) 300 (3600) 1200 (14,000)	3600 mg/m^2/day: yellow discolored urine and feces, hunched posture, piloerection, ↓ body weight, ↓ RBC and WBC 14,000 mg/m^2/day: ↓ body weight, ↓ RBC and WBC
Monkey (Study 1398/117)	Oral Daily x 28 days (1 month)	3 (MS)	30 (360) 100 (1200) 300 (3600)	All doses produced hemopoietic and lymphoid system toxicity that lead to early mortalities; treatment was terminated after 18 days At all doses: mortality, soft/loose feces, yellow discolored urine, hunched posture, piloerection, subdued behavior, tremors, facial and limb swelling, bleeding from gums, sunken eyes, ↓ body weight, ↓ platelets and WBC, ↑ total bilirubin, urea, creatinine, total cholesterol, and triglycerides, ↓ calcium, glucose, and total protein levels, ↑ liver, spleen, and adrenal

Repeat Dose Toxicity Studies				
Species	**Route Duration**	**N/sex/ dose**	**mg/kg/day (mg/m^2/day)**	**Significant findings**
				weights, small thymus, bone marrow toxicity, lymphoid atrophy in mandibular and mesenteric lymph nodes, ileum, and spleen, thymus atrophy
Monkey (Study 1398/126)	Oral Daily x 28 days (1 month)	3 (MS)	0.2 (2.4) 2 (24)	24 mg/m^2/day: mortality (1 male), facial swelling, loose feces, ↓ WBC, papillary mineralization in kidneys
Monkey (Study CC-4047-TOX-002)	Oral Daily x 91 days (13 weeks; ~3 months)	5 (Control, MS) 3 (CC-4047, MS) 2 (Recov., high dose only)	0.05 (0.6) 0.2 (2.4) 2 (24) 10 (120)	Dosing of the 120 mg/m^2/day group was discontinued at the end of Week 5 due to adverse clinical signs and hematology changes. For this group, 3 animals/sex were necropsied on Week 6 and 2 animals/sex were used to assess recovery and necropsied on Week 13/14. 24 mg/m^2/day: ↑ incidence of watery stool, sunken eyes (1 female), ↓ albumin and total protein, band cell neutrophils, ↓ B-lymphocytes, NK cells, and monocytes, ↓ thymus weights, ↓ liver weights, bone marrow toxicity (hypercellularity, myeloid series immaturity, and ↓ megakaryocytes), lymphoid depletion or lymphoid hyperplasia in spleen, ↑ thymus toxicity 120 mg/m^2/day: ↑ incidence of watery stool, sunken eyes (3 females), ↓ body weight, ↓ food consumption, ↓ albumin and total protein, ↓ glucose, ↑ ALP, band cell neutrophils, Dohle bodies in neutrophils, ↑ fibrinogen, ↓ RBC, hemoglobin, and hematocrit, reticulocytosis, ↓ B-lymphocytes, NK cells, and monocytes, ↓ T-lymphocytes and T-helper lymphocytes, ↓ thymus weights, bone marrow toxicity (hypercellularity, myeloid series immaturity, and ↓ megakaryocytes), lymphoid depletion or lymphoid hyperplasia in spleen, ↑ thymus toxicity
Monkey (Study CC-4047-TOX-006)	Oral Daily x 273 days (39 weeks; 9 months) 8-week recovery period	4 (MS) 2 (Recov.)	0.05 (0.6) 0.1 (1.2) 1 (12)	Due to 2 early deaths in the 12 mg/m^2/day group, 2 monkeys (1/sex) were added to the group 0.6 mg/m^2/day: lymphoid depletion of spleen (1 male and 1 female), lymphoid depletion of thymus (↑ incidence in males over controls) 1.2 mg/m^2/day: watery feces, lymphoid depletion of spleen (1 female), lymphoid depletion of thymus (slightly ↑ incidence/severity over controls) 12 mg/m^2/day: mortality (3 males and 3 females), watery feces, inappetence, dehydration, hunched posture, ↓ body weight in 2 males, ↓ food consumption, crescent-shaped opacity on optic discs, ↓ heart rate, ↑ red cell distribution width and reticulocytes, ↓ WBC and lymphocytes, ↑ fibrinogen, lymphoid hypocellularity in bone marrow, ↑ ALP and GGT in 1 female, ↓ albumin, small thymus, ↓ thymus weight, chronic inflammation of large intestine (cecum, colon, rectum), villous atrophy

Repeat Dose Toxicity Studies				
Species	**Route Duration**	**N/sex/ dose**	**mg/kg/day (mg/m^2/day)**	**Significant findings**
				of small intestine (duodenum, ileum, jejunum), proliferation of bile ductile, lymphoid depletion of mandibular and mesenteric lymph nodes, Peyer's patch, spleen, and thymus, *Staphylococcus aureus* infection (involving inflammation of tissue surrounding the thoracic and lumbar vertebrae, marrow cavity and meninges of the spinal cord and brain, with hematogenous spread to lungs and myocardium) in 1 male, acute myeloid leukemia (↑ WBC, ↑ monocytes, CD34+ blast cells, bone marrow filled with blast cells, leukemic infiltrates in various organs) in 1 female

MS=Main study
Recov.=Recovery groups

Tabulated Summary of Genetic Toxicology Studies

Title	Study #	Without Metabolic Activation	With Metabolic Activation
Evaluation of pomalidomide (CC-4047) in the bacterial reverse mutation with a confirmatory assay	CC-4047-TOX-015	Negative for mutagenicity at concentrations of 50-5000 µg/plate for *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2*uvr*A	Negative for mutagenicity at concentrations of 50-5000 µg/plate for *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2*uvr*A
CC-4047: Mutation at the thymidine kinase (*tk*) locus of mouse lymphoma L5178Y cells (MLA) using the Microtitre fluctuation technique	1398/82	Negative for mutagenicity at concentrations of 18.75-600 µg/mL	Negative for mutagenicity at concentrations of 18.75-600 µg/mL
Evaluation of pomalidomide (CC-4047) in the chromosomal aberrations assay in cultured human peripheral blood lymphocytes	CC-4047-TOX-016	Negative for clastogenicity at concentrations of 252-614 µg/mL for the 3-hour treatment and 252-492 µg/mL for the 22-hour treatment	Negative for clastogenicity at concentrations of 252-614 µg/mL for the 3-hour treatment
Evaluation of pomalidomide (CC-4047) in the *in vivo* rat bone marrow micronucleus assay	CC-4047-TOX-017	Negative for clastogenicity in male rats at doses of 500, 1000, or 2000 mg/kg/day (3000, 6000, or 12,000 mg/m^2/day) at 24 hrs	

Tabulated Summary of Reproductive and Developmental Toxicity Studies

	Fertility and Early Embryonic Development	Embryonic Fetal Development	
Study #	**CC-4047-TOX-020**	**CC-4047-TOX-021**	**CC-4047-TOX-008**
Title	A fertility and early embryonic development study in rats administered pomalidomide (CC-4047) orally	An embryo-fetal development study in rats administered pomalidomide (CC-4047) orally	Oral (stomach tube) developmental toxicity study of CC-4047 in rabbits
Methods	Males treated daily beginning 28 days prior to pairing were paired with females treated daily beginning 14 days prior to pairing. Cesarean section was performed on GD 13 in females. After CC-4047-related reproductive effects were observed in females, males were paired a second time with untreated females.	Females dosed daily from GD 6-17 and euthanized on GD 20	Females dosed daily from GD 7-19 and euthanized on GD 29 A positive control group was treated with thalidomide on the same dosing schedule
Key Findings	No effects on premating estrous cyclicity or reproductive performance or fertility Treated females paired with treated males: ↓ number of viable embryos and ↑ post-implantation loss and total number of resorptions (early + late) at all doses Untreated females paired with treated males: No effects on embryo viability	↑ number of resorptions and post-implantation loss and ↓ number of viable fetuses at all doses Fetal weight ↓ at all doses ↑ in visceral and skeletal malformations and variations observed at all doses No NOEL for embryo-fetal developmental toxicity including teratogenicity	Pomalidomide was maternally toxic at the mid and high doses (1200 and 3000 mg/m^2/day) with ↓ body weight gain, changes in hematology and clinical chemistry, and ↓ spleen and thymus weights. ↑ post-implantation loss at 1200 and 3000 mg/m^2/day with increases in the number of early, late, and total resorptions, % resorbed conceptuses per litter, and number of females with resorptions. ↑ in fetal cardiac anomalies, principally the malformation of interventricular septal defect, were observed at all doses The 1200 and 3000 mg/m^2/day doses of pomalidomide produced significant ↑ in the litter and fetal incidences of gross external, visceral, and skeletal malformations,

	Fertility and Early Embryonic Development	Embryonic Fetal Development	
Study #	**CC-4047-TOX-020**	**CC-4047-TOX-021**	**CC-4047-TOX-008**
			including limb malformations similar to those observed in the thalidomide positive control group. No NOEL for embryo-fetal developmental toxicity including teratogenicity
Species	Crl:CD (SD) rat	Crl:CD (SD) rat	New Zealand White rabbit
Doses	0, 25, 250,1000 mg/kg/day (0, 150, 1500, 6000 mg/m^2/day) pomalidomide	0, 25, 250,1000 mg/kg/day (0, 150, 1500, 6000 mg/m^2/day) pomalidomide	0, 10, 100, 250 mg/kg/day (0, 120, 1200, 3000 mg/m^2/day) pomalidomide 180 mg/kg/day (2160 mg/m^2/day) thalidomide
Mortality and Clinical Signs	No mortalities Clinical signs: Discolored urine (yellow) at all doses	No mortalities Clinical signs: Discolored urine (yellow) at 6000 mg/m^2/day	Mortality: One female treated at 1200 mg/m^2/day was euthanized on GD 15 due to body weight loss, ↓ food consumption and clinical signs including abnormal feces, ungroomed coat, decreased motor activity, and dehydration Clinical signs: Ungroomed coat observed at all doses and green urine observed at 3000 mg/m^2/day
Body Weight/Food Consumption	↓ body weight change GD 1-13 in treated females, significant at 6000 mg/m^2/day	↓ maternal body weight gain starting around GD 12; gravid uterine weights were significantly ↓ for all doses, but the adjusted body weight change from GD 0 were not lower than controls	↓ maternal body weight gain GD 7-28 and GD 7-29 (with adjusted body weight on GD 29) at 1200 and 3000 mg/m^2/day and in thalidomide control group; gravid uterine weights also ↓ in same groups ↓ food consumption at 1200 mg/m^2/day and thalidomide control group

GD= Gestational day ↑= increase ↓=decrease

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

/s/

BRENDA J GEHRKE
12/11/2012

PEDRO L DEL VALLE
12/11/2012

HALEH SABER
12/12/2012

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 204026 Applicant: Celgene Corporation Stamp Date: April 10, 2012

Drug Name: Pomalidomide NDA Type: NME

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?	✓		NDA is submitted in the eCTD format
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	✓		Electronic submission
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	✓		
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)?	✓		• Carcinogenicity: Not conducted/submitted and not required • Mutagenicity: Submitted • Teratogenicity: Submitted, both rat and rabbit • Effects on fertility: Submitted • Juvenile studies: Not conducted and not required • Acute dose animal studies: Submitted • Repeat dose animal studies: Submitted, studies include 6 month rat and 9 month monkey • ADME: Submitted • Safety Pharmacology: Submitted
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).	✓		Oral formulations were used in pivotal clinical and nonclinical studies
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?	✓		Same route of administration

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
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?	✓		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	✓		
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?	✓		A labeling review will be conducted after review of the submitted nonclinical program
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)			No impurity issues have been identified at this time
11	Has the applicant addressed any abuse potential issues in the submission?			Not applicable
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable

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

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

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

Brenda J. Gehrke, Ph.D.	5/29/2012
Reviewing Pharmacologist	Date
Pedro Del Valle, Ph.D.	5/29/2012
Reviewing Pharmacologist	Date
Haleh Saber, Ph.D.	5/29/2012
Team Leader/Supervisor	Date

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

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/s/

BRENDA J GEHRKE
05/29/2012

PEDRO L DEL VALLE
05/29/2012

HALEH SABER
05/29/2012