CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-880

PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	21-880
SERIAL NUMBER:	001
DATE RECEIVED BY CENTER:	04/07/2005
DRUG NAME:	Revlimid®
INDICATION:	Myelodysplastic syndrome (MDS)
SPONSOR:	Celgene Corporation
	86 Morris Avenue, Summit, NJ 07901
DOCUMENTS REVIEWED:	Electronic submission
REVIEW DIVISION:	Division of Drug Oncology Products
	(HFD-150)
PHARM/TOX REVIEWER:	M. Anwar Goheer, Ph.D.
	Kimberly Benson, Ph.D.
PHARM/TOX SUPERVISOR:	John K. Leighton, Ph.D., D.A.B.T.
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PROJECT MANAGER:	Carl Huntley, R. Ph., MBA.

Date of review submission to Division File System (DFS): 5 October 2005

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: The non-clinical studies submitted to this NDA provide sufficient information to support the use of lenalidomide (Revlimid®) in patients with transfusion-dependent anemia due to low- or intermediate-1-risk myelodysplastic syndromes (MDS) associated with a deletion 5q cytogenetic abnormality with or without additional cytogenetic abnormalities.

B. Recommendation for nonclinical studies: Adequate reproductive toxicity assessment, specifically embryo-fetal developmental toxicity in two species, needs to be conducted.

C. Recommendations on labeling: A separate review will be conducted.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings: Lenalidomide (3-(4'aminoisoindoline-1-one)-1-piperidine-2, 6-dione; CC-5013; IMiD-3 and Revlimid®) is a thalidomide analogue. It is a racemic mixture of S (-) and R (+) forms. The *in vitro* and *in vivo* characterization of pharmacological properties of lenalidomide had demonstrated that the drug inhibits the secretion of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6 and IL-12) and increases the secretion of anti-inflammatory cytokine (IL-10) from peripheral blood mononuclear cells (PBMC), induces T-cell proliferation (IL-2, IFN-γ), inhibits cell proliferation (MM, Burkitt's lymphoma) and inhibits angiogenesis (Knight-R, Semin Oncol 2005; 32:24-30 & Dredge et al., Microvasc Res. 2005; 69:56-63). Lenalidomide inhibits the expression of cyclooxygenase-2 (COX-2) but did not affect COX-1 *in vitro*. This may translate into adverse effects that need to be fully explored in clinical trials. In addition to these immune effects, there is evidence that thalidomide and its analogues may act directly on tumor cells, via inducing apoptosis or G1 growth arrest.

The oral administration of lenalidomide at dose levels of 3, 6 and 12 g/m^2 produced no effects on behavior or general activity in male rats. Intravenous administration of the drug at doses up to 400 mg/m^2 did not produce any significant effect on cardiovascular and respiratory systems of the anesthetized dog. *In vitro*, lenalidomide inhibited the cloned human potassium channel (hERG) current by 8% only at the highest concentration tested (787 μM).

Lenalidomide did not inhibit or induce any of the major cytochrome P450 isozymes *in vitro* and *in vivo* indicating limited potential for P450-related drug-drug interactions. Distribution of radioactivity in the fetal tissues of pregnant rat was low after oral administration but fetal brain showed more activity than maternal brain. The highest

concentrations were found in the kidney (cortex and medulla), liver, spleen and the mucosa of the GI tract of rats.

During traditional toxicity assessment, lenalidomide was administered to rodents (mice, rats) and non rodents (monkeys) for 1, 7, and 28 days and 13, 26, and 52 weeks. Single dose administration of lenalidomide up to 6 g/m^2 in mice and 12 g/m^2 in rats did not cause any adverse effects. Daily oral administration of lenalidomide at 6 g/m^2 to rats for 28 days was associated with moderate to severe tubular nephropathy/nephritis, which was attributed to precipitation of the lenalidomide in the kidney. Once daily oral administration of lenalidomide to rats at doses of 450, 900 or 1800 mg/m^2/day for 26 weeks was mainly associated with reduced body weight gain (12% ↓) for high dose males and reversible pelvic mineralization in the kidney of all treated animals.

Oral administration of lenalidomide to cynomolgus monkeys at dose levels of 12, 24, 48, or 72 mg/m^2/day for 52 weeks was associated with hemorrhage in multiple organs, gastrointestinal tract inflammation and lymphoid and bone marrow atrophy. Dosing at 48 and 72 mg/m^2/day was discontinued after 20 weeks of treatment due to toxicity and mortalities. A reversal of the macroscopic and microscopic findings seen in decedent and the terminal sacrifice was noted in 7 week treatment-free recovery animals. It is clear that this species is much more sensitive to lenalidomide than rodents.

Lenalidomide did not induce mutation in the Ames test, chromosome aberrations in cultured human peripheral blood lymphocytes, or mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells. Lenalidomide did induce micronuclei in the polychromatic erythrocytes of the bone marrow of male rats.

Reproductive and developmental toxicity: Reproductive studies were conducted with lenalidomide, examining the effects on fertility and early embryo development, embryo-fetal development, and pre-and post-natal development. Only the embryo-fetal development studies are required for drugs with oncologic indications. These studies have not been adequately conducted at this time. The first study, conducted in a rat, showed very slight maternal toxicity and no fetal malformations. The rat, however, is not an adequate species for the full assessment of lenalidomide's developmental effects, given the structural similarity to thalidomide. Historical data indicates that the rat is not sensitive to the full range of thalidomide's teratogenic effects.

An additional developmental study was conducted in the rabbit, with a concurrent thalidomide dose group. This study had a confounding variable with some rabbits not eating prior to the study and all these rabbits had a negative outcome in the study. Additionally, the highest dose tested did not meet the standard criteria for sufficient drug exposure.

B. Pharmacologic activity: Both lenalidomide and thalidomide have been shown to increase the secretion of anti-inflammatory cytokine IL-10 from LPS-stimulated PBMC, stimulates T-cells proliferation and production of IL-2 and IFN-γ. Both inhibit the secretion of pro-inflammatory cytokines TNF-α, IL-1β, and IL-6. In addition to these immune effects, there is evidence that thalidomide and its analogues may act directly on tumor cells, via inducing apoptosis or G1 growth arrest. Exact mechanisms of action however remain unknown.

C. Nonclinical safety issues relevant to clinical use: Inflammation of the gastrointestinal tract and atrophy of the bone marrow, thymus, and lymphoid tissues were observed during repeat dose toxicity studies (up to 12 months) in cynomolgus monkeys. Embryo-fetal developmental toxicity has not been adequately addressed. The structural similarity of lenalidomide to thalidomide, a known human teratogen, suggests developmental risk. Lenalidomide also inhibits expression of COX-2 *in vitro* but not COX-1. This finding should be fully explored in clinical trials.

2.6 *PHARMACOLOGY/TOXICOLOGY REVIEW*

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-880
Review number: 1
Sequence number/date/type of submission: 001 / 12-22-2004 / NDA
Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: Celgene Corporation
86 Morris Avenue, Summit
New Jersey 07901.

Manufacturer for drug substance:

/

Reviewer name: M. Anwar Goheer, Ph.D.

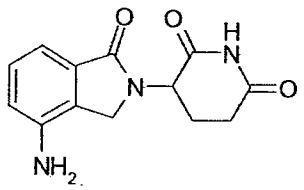
Division name: Division of Oncology Drug Products

HFD #: HFD-150

Review completion date: September 27, 2005

Drug:

Trade name: REVLIMID®
Generic name: Lenalidomide
Code name: CC-5013, CDC-501, IMiD 3
Chemical name: 3-(4'-amino-1,3-dihydro-1-oxo-2*H*-isoindol-2-yl)-2,6-piperidinedione,
3-(4'-amino-1-oxo-1,3-dihydro-2*H*-isoindol-2-yl) piperidine-2,6-dione.
CAS registry number: 191732-72-6
Molecular formula/molecular weight: $C_{13}H_{13}N_3O_3$ / 259.25
Structure:



Relevant INDs/NDAs/DMFs: DMF Numbers: —
IND numbers: 60,100, —

Drug class: Immunomodulator / anti-angiogenesis

Indication: Transfusion-dependent anemia due to low- or intermediate-1-risk myelodysplastic syndromes (MDS) associated with a deletion 5q cytogenetic abnormality with or without additional cytogenetic abnormalities.

Clinical formulation: Composition of lenalidomide capsules

			5 mg Capsule	10 mg Capsule
Ingredient	**Quality Standard**	**Function**	**Theoretical Weight per Capsule (mg)**	
Lenalidomide[a]	In-house	Active	5.0	10.0
Lactose Anhydrous[b]	NF/EP			
Microcrystalline Cellulose	NF/EP			
Croscarmellose Sodium	NF/EP			
Magnesium Stearate	NF/EP			
Total Fill Weight	---	---	200.0	400.0
White Capsule Shells (Size 2) Imprinted with Black Ink[c]	In-house	---	1 Capsule	---
Pale Yellow Body/Blue Green Cap Capsule Shells (Size 0) Imprinted with Black Ink[c]	In-house	---	---	1 Capsule

[a]

[b]

[c] The capsule shells are supplied by Information pertaining to the components and source of gelatin in the capsule shells is provided in P.4.

(Excerpted from the sponsor's submission)

Route of administration: Oral

Proposed use: "Lenalidomide (10 mg daily) is indicated for the treatment of patients with transfusion-dependent anemia due to low- or intermediate-1-risk myelodysplastic syndromes associated with a deletion 5q cytogenetic abnormality with or without additional cytogenetic abnormalities."

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Data reliance : Except as specifically identified below, all data and information discussed below and necessary for approval of NDA 21-880 are owned by Celgene Corporation or are data for which Celgene has obtained a written right of reference. Any information or data necessary for approval of NDA 21-880 that Celgene 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

described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that Celgene does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of NDA 21-880.

Studies reviewed within this submission:

2.6.2 PHARMACOLOGY

2.6.2.2 Primary pharmacodynamics

Mechanism of action:

1. Amino- Substituted thalidomide analogs: Potent inhibitors of TNF-α production.
2. Inhibition of TNF-α production by PBMC and elevation of IL-2 and MIP-3a production by T cells by CC-4047, CC-5013, and C-11006 in vitro.
3. 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. Elevation of IL-2 production by CC-4047, CC-5013, and CC-11006 from human and rat whole blood stimulated with Concanavalin A.
5. Cytokine profiling for five classes of IMiDs in primary human PBMCs and $CD4^+$ T lymphocytes.
6. Anti- Inflammatory effects of CC-4047, CC-5013 and CC-11006 on G-CSF, IL-10, and COX-2 Expression by LPS-stimulated PBMC.
7. Effect of the IMiD CC-5013 on Akt phosphorylation in the Jurkat T cell line.
8. Effect of the PDE4 Inhibitors CC-10004, CC-10082 (cilomilast), CC-11050 and CC-14064 (roflumilast), and the IMID CC-5013 on IL-6 production by human, rat, mouse, and monkey whole blood stimulated with LPS in vitro.
9. Immunomodulatory drugs (IMiDs™) inhibit expression of cyclooxygenase-2 from TNF-α, IL-1ß and LPS stimulated human PBMC in a partially IL-10- dependent manner.
10. Immunomodulatory analogs of thalidomide inhibit growth of Hs Sultan cells and angiogenesis in vivo.
11. Thalidomide and its analogues inhibit lipopolysaccharide-mediated induction of cyclooxygenase-2.
12. 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.

13. Effects of IMiDs on proliferation of breast cancer, NSCLC, CML and NHL cell lines in vitro.
14. Anti- proliferative activity of CC-4047, CC-5013, CC-5079, and CC-10004 against the non-Hodgkin's B lymphoma cell line Farage in vitro.
15. Effect of CC-10004 and CC-5013 on proliferation of the mouse CLL line LNC, alone and in combination with vincristine.
16. Inhibition of endothelial cell migration by thalidomide, CC-4047, and CC-5013.
17. Effect of CC-5013 on HIF-1 alpha expression and VEGF production in PC-3 cells.
18. Lenalidomide inhibits angiogenesis in vitro and reduces lung metastasis of mouse melanoma cells in an animal model.
19. Novel thalidomide analogues display anti-angiogenic activity independently of immunomodulatory effects.

2.6.2.3 Secondary pharmacodynamics

1. Addition of immunomodulatory drugs CC-5013 or CC-4047 to Rituximab enhances anti-tumor activity in a severe combined immunodeficiency (SCID) mouse lymphoma model.
2. Use of ImiD3, a thalidomide analog, as an adjunct to therapy for experimental tuberculous meningitis.
3. Thalidomide and its analogs overcome drug resistance of human multiple myeloma cells to conventional therapy.
4. IMiDs augment fetal hemoglobin synthesis and can be used for the treatment of hemoglobin disorders like sickle cell anemia and ß-thalassemia.
5. Thalidomide and thalidomide analogue drug costimulate virus- specific $CD8^+$ T cells in vitro.

2.6.2.4 Safety Pharmacology See previous reviews for details; a brief summary of these studies is presented in this section.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.2 Methods of Analysis

1. Validation for the determination of CC-5013 in rat plasma (Heparin anticoagulant) using ~ for sample preparation and liquid chromatography with mass spectrometric detection.
2. Validation of an analytical procedure for the determination of the enantiomers of CC-5013 in rat plasma (Heparin) using solid phase extraction and liquid chromatography with — mass spectrometric detection.

3. Validation of an analytical procedure for the determination of CC-5013 in rabbit plasma (Heparin) using — , and liquid chromatography with — mass spectrometric detection.
4. Validation for the determination of CC-5013 in dog plasma (Heparin anticoagulant) using — or sample preparation and liquid chromatography with mass spectrometric detection.
5. Validation for the determination of CC-5013 in primate plasma (Heparin anticoagulant) using — for sample preparation and liquid chromatography with mass spectrometric detection.

2.6.4.3 Absorption

1. [^{14}C] CC-5013: A study of absorption and excretion following oral and intravenous administration to the rat.
2. [^{14}C] CC-5013: A study of absorption, excretion and metabolism following oral and intravenous administration to the cynomolgus monkey.

2.6.4.4 Distribution

1. [^{14}C] CC-5013: Quantitative whole-body autoradiography following a single oral administration (150 mg/kg) to the rat.

2.6.4.7 Pharmacokinetic drug interactions

1. CC- 5013: Effect on cytochrome P450 and related parameters in the male and female Sprague Dawley rat following oral (gavage) administration at 0, 75, 150 and 300 mg/ kg/ day for 26 weeks.
2. CC- 5013: Effect on cytochrome P450 and related parameters in the male and female cynomolgus monkey following oral (gavage) administration at 0, 1 and 2 mg/ kg/ day for 52 weeks.
3. Identification of the cytochrome P450 enzymes responsible for the *in-vitro* metabolism of (^{14}C)-CC-5013 in human liver microsomes.
4. Identification of human P450 isozymes involved in the metabolism of CC-1088 and CC-5013.
5. Effects of CC-1088 and CC-5013 on selected cytochrome P450 activities in human liver microsomes: Prediction of drug interactions.
6. Metabolism of (^{14}C)-CC-5013 in isolated human hepatocytes.
7. Comparison of chemical degradation pathways of lenalidomide and thalidomide.

2.6.6 TOXICOLOGY

2.6.6.3 Repeat-dose toxicity

1. CC- 5013: 7- day oral (gavage) administration range-finding study in the mouse.
2. CC- 5013: 7 day oral (gavage) range-finding toxicity study in the rat.

3. CC- 5013: 28 day oral (gavage administration) toxicity study in the rat.
4. CC- 5013: 13 week oral (gavage administration) toxicity study in the rat.
5. CC- 5013: 26 week oral (gavage) administration toxicity study in the rat with a 4 week treatment- free period.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

1. CC- 5013: Oral (gavage) study of fertility and early embryonic development in the rat (Segment 1).

Embryofetal development

1. CC- 5013: Oral (gavage) range-finding study of embryo-foetal development in the rat.
2. CC- 5013: Oral (gavage) study of embryo-foetal development in the rat.
3. Preliminary study of CC- 5013 embryo-foetal development (Segment II) in the non-pregnant New Zealand white rabbit.
4. CC- 5013: Oral (gavage) range-finding study of embryo-foetal development in the rabbit.
5. CC- 5013: Oral (gavage) study of embryo-foetal development in the rabbit.

Prenatal and postnatal development

1. CC- 5013: Oral (gavage) study of pre-and postnatal development in the rat.

Studies Previously Reviewed: See Appendix A for studies reviewed by Dr. Anwar Goheer on 4/27/2000, 6/2/2000, & 2/14/2001 and Appendix B for studies reviewed by Dr. Anthony Proakis on 6/06/01, 10/29/02, 12/23/02, & 3/31/05,

2.6.3 PHARMACOLOGY

2.6.2.2 Primary pharmacodynamics

Mechanism of action:

1. Amino-substituted thalidomide analogs: Potent inhibitors of TNF-α production.
2. Differential cytokine modulation and T cell activation by two distinct classes of thalidomide analogues that are potent inhibitors of TNF-α.
3. Thalidomide and its analogs overcome drug resistance of human multiple myeloma cells to conventional therapy.

2.6.2.4 Safety pharmacology

Neurological effects:

1. CC-5013: Effects on general activity and behaviour in the rat following oral administration.

Cardiovascular effects:

1. Effects of CC-5013 on cloned hERG channels expressed in mammalian cells.
2. CC- 5013: Cardiovascular and respiratory effects in the anaesthetised dog following intravenous administration.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.3 Absorption

1. CC- 5013: A study to determine the oral bioavailability in the rat, dog and monkey.
2. CC- 5013: In vitro binding to plasma proteins in rat, rabbit, monkey and human.
3. CC-1088, CC-4047, CC-5013 and CC-7025: Comparative absorption by the Caco-2 cell line.

2.6.4.5 Metabolism

1. Metabolism of (^{14}C)-CC-5013 in isolated human hepatocytes.

2.6.6 TOXICOLOGY

2.6.6.2 Single-dose toxicity

1. CC- 5013: Single dose oral toxicity study in the mouse (approximation of the minimum lethal dose level).
2. CC- 5013: Single dose intravenous toxicity study in the mouse (approximation of the minimum lethal dose level).
3. CC- 5013: Single dose oral toxicity study in the rat (approximation of the minimum lethal dose level).
4. CC- 5013: Single dose intravenous toxicity study in the rat (approximation of the minimum lethal dose level).

2.6.6.3 Repeat-dose toxicity

1. CC-5013: 7 day oral (gavage) range-finding toxicity study in the rat.
2. CC-5013: 28 day oral (gavage administration) toxicity study in the rat.
3. CC-5013: 13 week oral (gavage administration) toxicity study in the rat.
4. CC-5013: 28 day oral (gavage administration) toxicity study in the monkey.
5. CC-4047 & CC-5013: 28 day oral (gavage administration) toxicity study in the monkey.
6. CC-5013: 13 week oral (gavage administration) toxicity study in the monkey.
7. CC-5013: 52 week oral (gavage) administration toxicity study in the monkey with a 7 week treatment free period.

6.6.6.4 Genetic toxicology

1. CC-5013: Reverse mutation in four histidine-requiring strains of Salmonella typhimurium and two tryptophan-requiring strains of Escherichia coli.
2. CC-5013: Mutation at the thymidine kinase (tk) locus of mouse lymphoma l5178y cells (MLA) using the microtitre^r fluctuation technique.
3. CC-5013: Induction of chromosome aberrations in cultured human peripheral blood lymphocytes.
4. CC-5013: Induction of micronuclei in the bone marrow of treated rats.

2.6.6.6 Reproductive and developmental toxicology

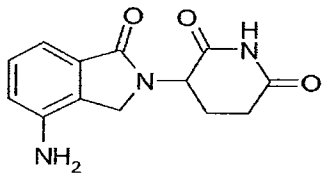
1 Developmental and reproductive toxicity screening study for effects on embryo-fetal development in rabbits.

2.6.2 PHARMACOLOGY

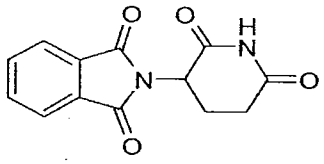
2.6.2.1 Brief summary

Structural analogues of thalidomide have been synthesized and examined for inhibition of TNF-α production. The tested compounds can be classified into two classes. One class of compounds (selective cytokine inhibitory drugs, SelCIDs™) are phosphodiesterase 4 inhibitors, inhibit TNF-α production, increase IL-10 production (in LPS-induced PBMC), and have little effect on T cell activation. The second class of compounds (immunomodulatory drugs, IMiDs™), similar to thalidomide, are not phosphodiesterase 4 inhibitors, markedly stimulate T cell proliferation, and IL-2 and IFN-γ production. In addition to these immune effects, there is evidence that thalidomide and its analogues act directly on tumor cells, via inducing apoptosis or G1 growth arrest.

Lenalidomide (CC-5013) is a thalidomide analogue. It is a racemic mixture of S (-) and R (+) forms.



Lenalidomide



Thalidomide

Angiogenesis, the formation of new blood vessels by pre- existing endothelial cells (EC), depends mainly on proper activation, proliferation, adhesion, migration and maturation of EC (Griffioen & Molema, Pharmacol Rev 2000; 52:237-268). Therefore, inhibition of EC growth, adhesion and migration, and growth factor expression are putative anti-angiogenic targets. A series of preclinical studies had been performed to support the clinical evaluation of CC-5013 in cancer patients.

Lenalidomide inhibited TNF-α, IL-1β, IL-6 and IL-12 in LPS-stimulated PBMC (Muller et al., Bioorg Med Chem lett 1999; 9:1625-1630). It enhanced IFN-γ and IL-2 production in anti-CD3 stimulated $CD4^+$ T cells (Schafer et al., J Pharmacol Exp Ther 2003; 305:1222-1232). Lenalidomide increased fetal hemoglobin in human $CD34^+$ progenitor cells. It inhibited the expression of COX-2, but not COX-1 protein in LPS-, TNF-α, and IL-1β stimulated PBMC. Neutralizing antibody to IL-10 but not IL-1β or TNF-α partially reversed the inhibitory effect of CC-5013 on COX-2 expression. These results suggest that the anti-tumor effects of lenalidomide may be due to in part to elevation of IL-10 production and its subsequent inhibition of COX-2 expression. Lenalidomide inhibited the growth of Namalwa cells (a human B cell lymphoma cell line with a deletion of one chromosome 5) but less effective in inhibiting growth of KG-1 cells (human myeloblastic cell line, also with a deletion of one chromosome 5) and other cell lines without chromosome 5 deletions. Patients with an isolated interstitial deletion of chromosome 5q31 may benefit from this immunomodulatory / antiangiogenic drug.

2.6.2.2 Primary pharmacodynamics

Mechanism of action:

1. **Amino-substituted thalidomide analogs: Potent inhibitors of TNF-α production.** Muller et al., Bioorg Med Chem Lett 1999; 9:1625-1630. See Appendix A for details.

TNF-α inhibition in LPS stimulated human PBMC and whole blood by thalidomide analogues.

Compound Name	TNF-α Inhibit (%). At 100 μM	TNF-α $IC_{50\ (nM)}$	Whole blood TNF-α $IC_{50\ (nM)}$
CC-4047	95	13	25
CC-4047	99	3.9	14
CC-4047	85	93	73
CC-5013	74	100	480

2. **Inhibition of TNF-α production by PBMC and elevation of IL-2 and MIP-3a production by T cells by CC-4047, CC-5013, and C-11006 in vitro.** Study Number: 5043- 152- 5119- 172.

Final IC_{50} and EC_{50} values

	CC-4047	CC-5013	CC-11006
Human PBMC 1-day TNF-α IC_{50} (μM)	0.013	0.100	0.050
Human T cell 2-day IL-2 EC_{50} (μM)	0.010	0.150	0.11
Human T cell 2-day MIP-3α EC_{50} (μM)	0.069	1.2	12
Human T cell 3-day IL-2 EC_{50} (μM)	0.0075	0.15	15

(Excerpted from the sponsor's submission)

3. **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.** Study Number: 5196- 175.

Blood samples from three individual human donors and three rats were used to compare the ability of IMiDs to inhibit TNF-α production by LPS. TNF-α production was inhibited in human blood as shown below.

Compound	Human Whole Blood TNF-α IC_{50} (μM)	Rat Whole Blood TNF-α IC_{50} (μM)
CC-4047	0.14	12
CC-5013	13	>100
CC-11006	0.53	73

(Excerpted from the sponsor's submission)

IC_{50} for lenalidomide (CC-5013) in rat whole blood could not be calculated.

4. **Elevation of IL-2 production by CC-4047, CC-5013, and CC-11006 from human and rat whole blood stimulated with Concanavalin A.** Study Number: 5197- 189- 5226- 016.

Blood samples from three individual human donors and three rats were used to compare the ability of IMiDs to elevate IL-2 production. Human and rat heparinized whole blood was plated on 96 well flat-bottom tissue culture plates. After 1 hour of incubation, Concanavalin A (5 μg/mL final concentration) was added. IL-2 production was measured after 2 hours of incubation. Average EC_{150} (effective concentration at 150%) were calculated using GraphPad Prism 4.00 software. Results are shown below.

IL-2 EC_{150} values of IMiDs in human whole blood

Compound	EC_{150} (μM)
CC-4047	0.012
CC-5013	0.07
CC-11006	0.2

Rat IL-2 was not elevated by any compound.

5. Cytokine profiling for five classes of IMiDs in primary human PBMCs and $CD4^+$ T lymphocytes. Study Number: PD365.

Purified hPBMCs were pre-treated with IMiDs for 30 minutes, stimulated with LPS for 24 hours and TNF-α, IL-1β, IL-10, IL-12, MCP-1, Rantes, IL-6, IL-8, and MIP-1α measured. T cells from PBMC were also purified with anti-$CD4^+$ microbeads. $CD4^+$T cells were co-stimulated with anti-CD3 plus IMiDs for 40 h and IL-2, IFN-γ, TNF-α, IL-8, IL-10, MIP-1α, and Rantes measured.

Results:

IMiDs showed diverse biological activities including inhibition of cytokine expression, augmentation of cytokine expression or no effect in different cell system as shown below. The authors also observed significant differences in TNF-α, inhibitory activity compared to historical data.

Compounds tested in this profiling, their putative classification, and historical experimental data

CC	TNF-α IC50 (μM)	IL-2 EC50 (μM)	MUTZ-1 IC50 (μM)	Angiogenesis IC50 (μM)	Classification
2001	194	>250	>100	0.11	(1) Regular IMiD
4047	0.013	0.0103	0.12	0.33	(1) Regular IMiD
5013	0.1	0.146	2.5	approx 0.7	(1) Regular IMiD
11006	0.05	0.114	2.3	9	(1) Regular IMiD
15005	0.04	0.022	0.3	approx 0.08	(1) Regular IMiD
12018	0.0104	>50	32	ND	(2) Weak IL-2
14024	0.0072	11.5	0.76	ND	(2) Weak IL-2
15093	approx 200	0.18	>100	ND	(3) Strong IL-2
15066	8.7	0.037	2.6	ND	(3) Strong IL-2
8097	>>100	>>10(day3)	ND	3.7	(4) Anti-Angiogenic
15192	approx 200	>>10	ND	approx 5.2	(4) Anti-Angiogenic
4059	156	>>10	>100	approx 8	(5) Anti-Angiogenic/ Cytomorphic

(Excerpted from the sponsor's submission)

IMiDs modulated cytokines in LPS stimulated PBMCs

IMiDs	TNF-α	IL-1β	IL-6	IL-12	MCP-1	MIP-1α	IL-8	IL-10	Rantes
2001	↓	∅	∅	↓	∅	∅	∅	↑	↑
4047	↓	↓	↓	↓	↓	↓	∅	↑	↑
5013	↓	↓	↓	↓	↓	↓	∅	↑	↑
11006	↓	↓	↓	↓	↓	↓	∅	↑	↑
15005	↓	↓	↓	↓	↓	↓	∅	↑	↑
12018	∅	∅	∅	↓	∅	∅	∅	↑	↑
14024	↓	↓	↓	↓	↓	↓	∅	↑	↑
15093	↓	↓	↓	↓	∅	∅	∅	↑	↑
15066	↓	↓	↓	↓	↓	↓	∅	↑	↑
8097	∅	∅	∅	∅	∅	∅	∅	↑	↑
15192	↓	↓	∅	↓	∅	∅	∅	↑	↑
4059	↓	∅	∅	↓	∅	∅	∅	↑	↑

(Excerpted from the sponsor's submission)

IMiDs modulated cytokines in anti-CD3 stimulated CD4+ T cells

IMiDs	IFN-γ	IL-2	IL-10	MIP-1α	Rantes	TNF-α	IL-8
2001	∅	∅	∅	∅	∅	∅	∅
4047	↑	↑	↓	∅	↑	∅	∅
5013	↑	↑	↓	∅	↑	∅	∅
11006	↑	↑	↓	∅	↑	∅	∅
15005	↑	↑	↓	∅	↑	∅	∅
12018	↑	∅	↓	∅	↑	∅	∅
14024	∅	↑	↓	∅	↑	∅	∅
15093	↑	↑	↓	∅	↑	∅	∅
15066	↑	↑	↓	∅	↑	∅	∅
8097	∅	∅	∅	∅	∅	∅	∅
15192	∅	∅	∅	∅	∅	∅	∅
4059	∅	∅	∅	∅	∅	∅	∅

(Excerpted from the sponsor's submission)

6. **Anti- Inflammatory effects of CC-4047, CC-5013 and CC-11006 on G-CSF, IL-10, and COX-2 expression by LPS-stimulated PBMC.** Study Number: 5127-53.

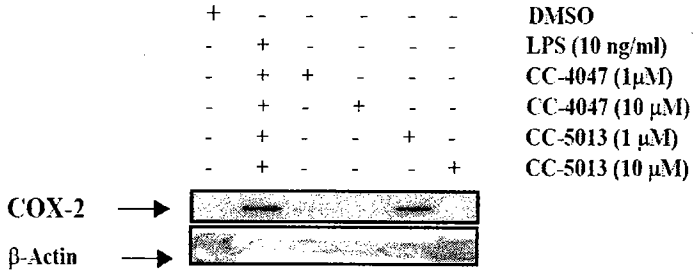
The effects of IMiDs on G-CSF, IL-10, and COX-2 production were measured in human PBMC. PBMC were pretreated with IMiDs and stimulated with LPS (10 ng/mL). G-CSF inhibition and IL-10 stimulation values in LPS-stimulated human PBMC are shown in the table below.

Assay	CC-4047 (μM)	CC-5013 (μM)
G-CSF IC_{50} (μM) LPS Stimulated hPBMC	0.10	>100
IL-10 Production EC_{50} (μM) LPS Stimulated hPBMC (100% = 10 μM compound)	0.079	0.39

(Excerpted from the sponsor's submission)

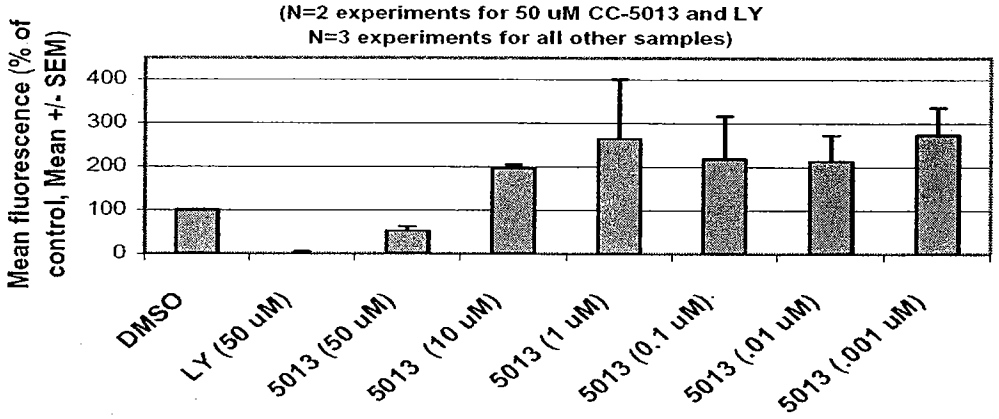
COX-2 measurement: PMBC were pretreated with IMiDs, stimulated with LPS, cells were lysed, and transferred electrophoretically to a polyvinylidene fluoride membrane. Proteins were probed by anti-COX-2 Ab [illegible] and visualized by enhanced chemiluminescence.

CC-5013 and CC-4047 inhibited the expression of COX-2 in LPS-stimulated PBMC. Data are representative from three PBMC donors.



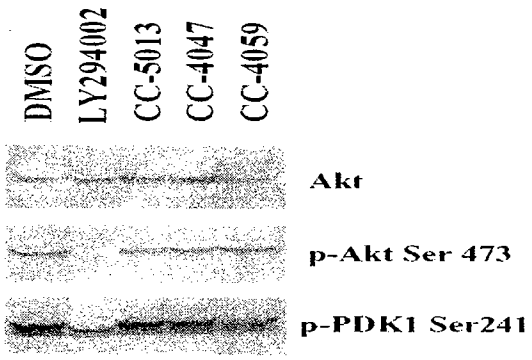
7. Effect of the IMiD CC- 5013 on Akt phosphorylation in the Jurkat T cell line. Study Number: 5191- 107.

CC-5013 at low levels (1nM-10 μM) increased expression of phospho-Akt (Thr308) in unactivated Jurkat T cells by 2-3 fold as shown below.



(Excerpted from the sponsor's submission)

There was no effect of IMiDs on phosphorylation of Akt at the Ser473 or on the phosphorylation of PDK1 at the Ser241 in Jurkat T cells. LY294002 (PI-3-kinase inhibitor) inhibited Akt and PDK1 phosphorylation as shown below.

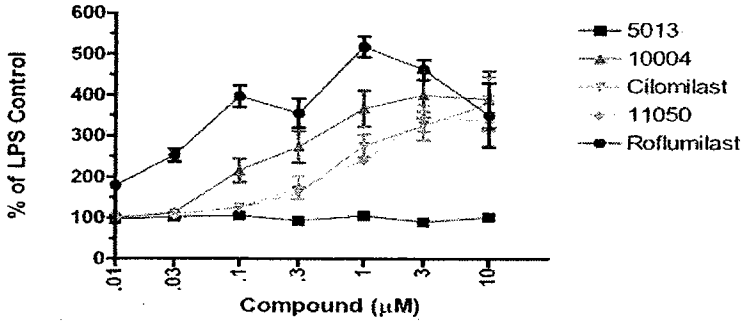


(Excerpted from the sponsor's submission)

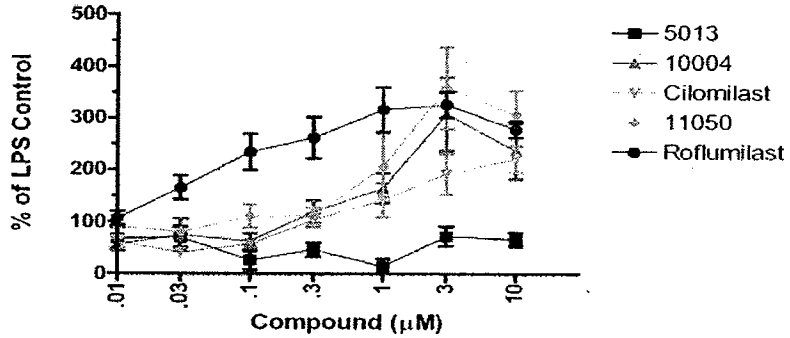
8. **Effect of the PDE4 inhibitors CC-10004, CC-10082 (cilomilast), CC-11050 and CC-14064 (roflumilast), and the IMID CC-5013 on IL- 6 production by human, rat, mouse, and monkey whole blood stimulated with LPS in vitro.** Study Number; 5265- 117.

- The PDE4 inhibitors CC- 10004, CC- 10082 (cilomilast), CC- 11050, and CC-14064 (roflumilast) caused a dose-dependent increase in IL- 6 production from lipopolysaccharide-stimulated whole blood from mouse (3-5 fold) and rat (2-3 fold).
- These PDE4 inhibitors had essentially no effect on human IL- 6 production, and partially inhibited monkey IL- 6 production (maximum of 50%).
- Lenalidomide (CC-5013, non-PDE4 inhibitor) did not elevate LPS-induced IL-6 production from the whole blood of any species tested (mouse, rat, monkey) and inhibited IL-6 production from LPS-stimulated human whole blood.

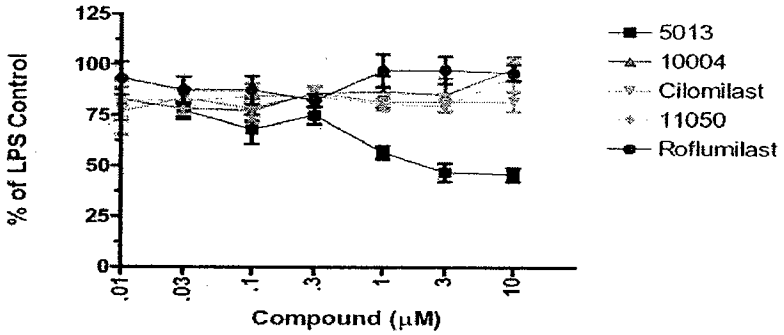
Summary of LPS (10 µg/ml) induced IL-6 production by whole mouse blood
(Mean ± SEM from 3 pools, 7 mice per pool)



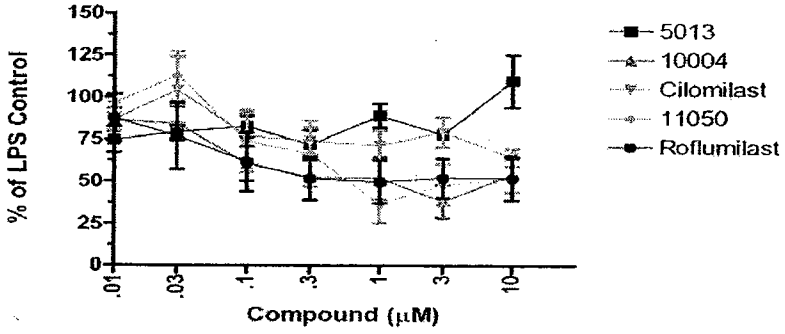
Summary of LPS (10 µg/ml) induced IL-6 production by whole rat blood
(Mean ± SEM from 3 pools, 2 rats per pool)



Summary of LPS (1 ng/ml) induced IL-6 production in whole human blood
(Mean ± SEM from 3 donors)



Summary of LPS (1 ng/ml) induced IL-6 production in whole monkey blood
(Mean±SEM from 3 donors)



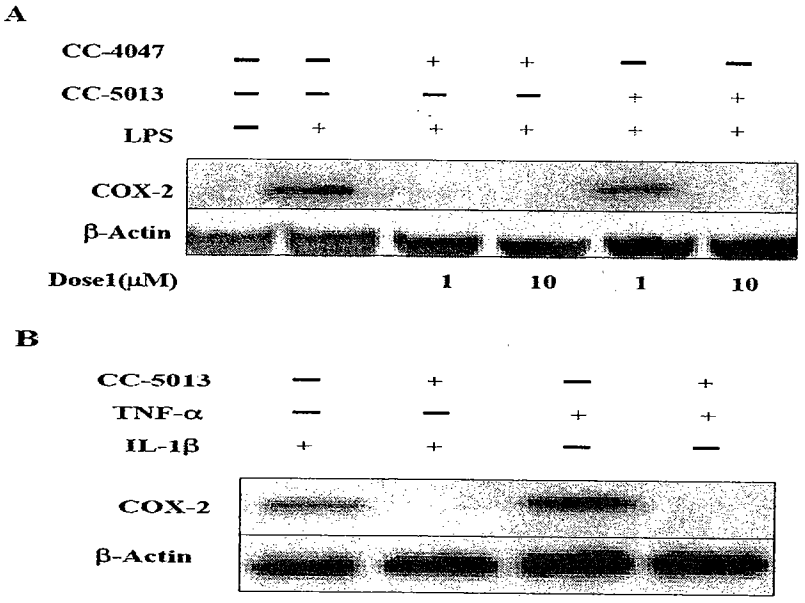
(Excerpted from the sponsor's submission)

9. **Immunomodulatory drugs (IMiDs ™) inhibit expression of cyclooxygenase-2 from TNF-α, IL-1ß and LPS stimulated human PBMC in a partially IL-10-dependent manner.** Study Number: 5116- 86

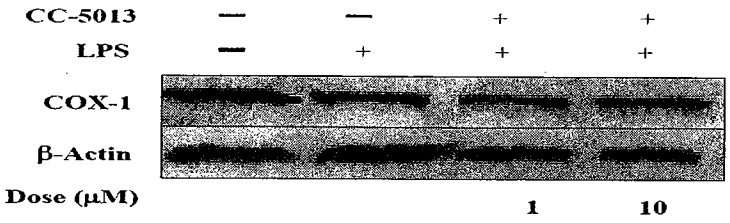
- CC-5013 and CC-4047 inhibited the expression of COX-2 protein from LPS, IL-1ß and TNF-α stimulated PBMC. COX-1 expression was not affected.
- CC-5013 increased the expression of IL-10 mRNA and protein from LPS stimulated PBMC.
- Neutralizing antibody to IL-10 but not IL-1ß or TNF-α partially reversed the inhibitory effects of IMiDs on COX-2 expression.
- IMiDs inhibited the synthesis of PGE_2 from LPS stimulated PBMC in a dose dependent manner.

Effect of CC- 5013 and CC- 4047 on the expression of COX- 1 and COX- 2 proteins from LPS stimulated PBMC.

A and B. PBMC were pretreated with IMiDs (1 or 10 μM) for one hour and stimulated with LPS (10 ng/ml), TNF-α (10 ng/ml) or IL-1ß (15 ng/ml) for 24 hours and harvested the cell lysates for Western blot analysis. Antibodies specific for human COX- 2 and ß- actin were used to detect protein expression.
C. Antibody specific to COX- 1 was used to detect the expression of COX- 1 protein. Data shown below are representative of 3 similar experiments.



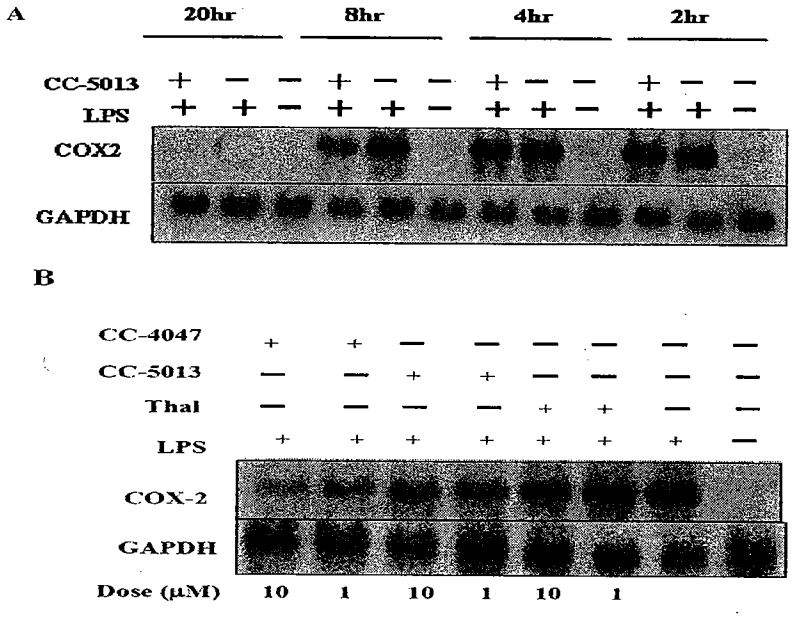
C



(Excerpted from the sponsor's submission)

Effects of IMiDs on expression of COX-2 by PBMC stimulated with LPS.

PBMC from healthy donors were pretreated for one hour with either thalidomide or IMiDs, stimulated with LPS (10 ng/ml) and total RNA was harvested at different time points as indicated in each figure and 20 μg of each sample was subjected to Northern blot analysis.
A. Effects of CC-5013 (10 μM) on expression of COX-2 at various time points.
B. Dose response effects of thalidomide, CC-5013 and CC-4047 on expression of COX-2 mRNA.



(Excerpted from the sponsor's submission)

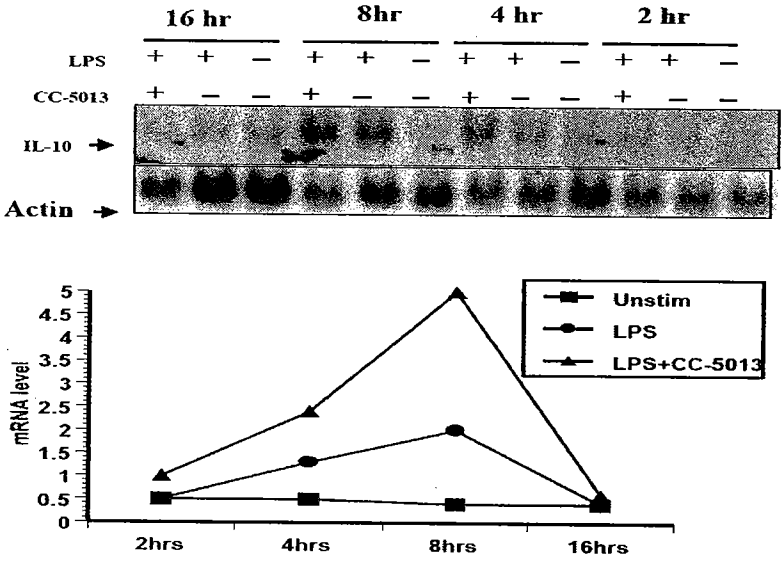
CC- 5013 enhances the expression of IL-10 mRNA and protein in a dose dependent manner.

PBMC from healthy donors were pretreated one hour with CC-5013, stimulated with LPS (10 ng/mL) and total RNA was harvested at various time points as indicated in graph and 20 µg of each sample was subjected to Northern blot analysis

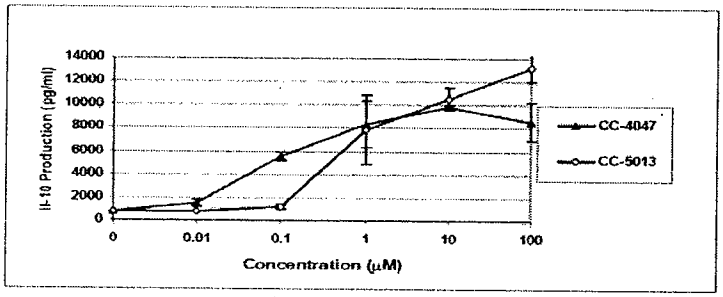
A. Effects of CC- 5013 on expression of IL- 10 mRNA over time

B. PBMC were pretreated with CC- 5013 at 1 µM, stimulated with LPS (10 ng/ ml) for 48 hours and supernatants analyzed for IL-10 production by ELISA. Data shown are representative of 3 similar experiments.

A



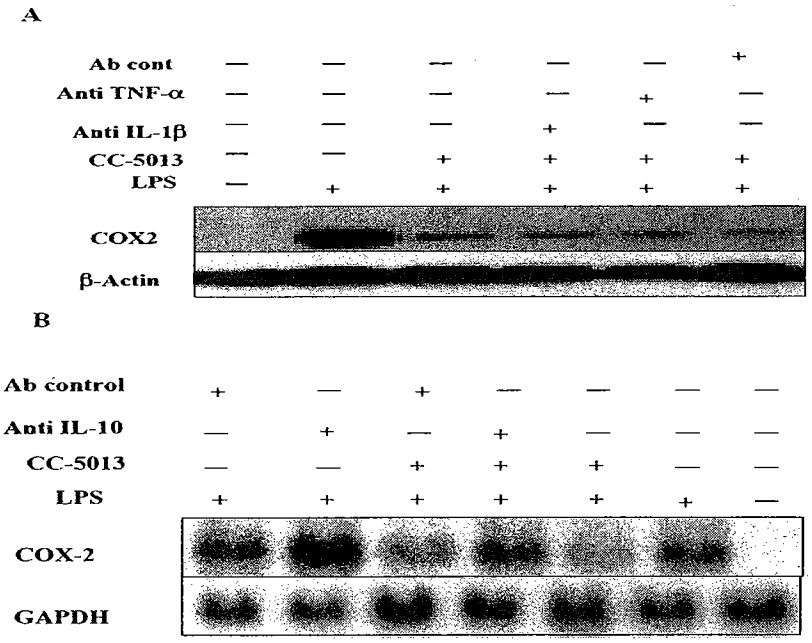
B



(Excerpted from the sponsor's submission)

Anti- IL-10 antibody increase expression of COX-2 protein and mRNA

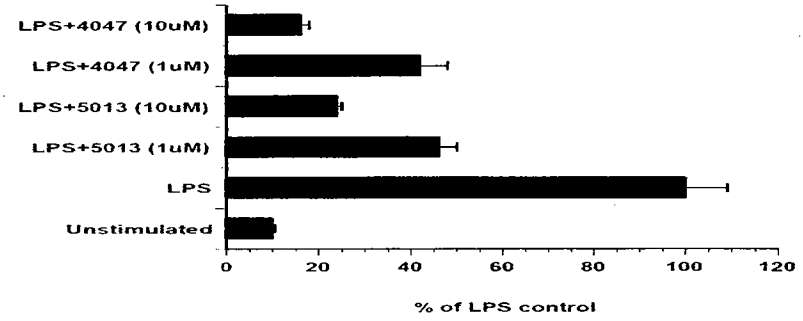
A and B. PBMC were pretreated with CC- 5013 for one hour, stimulated with LPS (10 ng/ml) in the absence or presence of neutralizing antibody (TNF-α, IL-1ß, IL-10, or isotype control) for 24 hours and cell lysates were harvested for Western blot (A) or Northern blot (B) analysis.



(Excerpted from the sponsor's submission)

CC-5013 inhibits the synthesis of prostaglandin E2 (PGE2).

PBMC from healthy donors were pretreated with CC-5013 and CC-4047 at either 1 μM or 10 μM for one hour and stimulated with LPS (10 ng/ml) in the presence of 20 μM arachidonic acid for 3 hours. The supernatants were tested for the presence of PGE2 by ELISA.



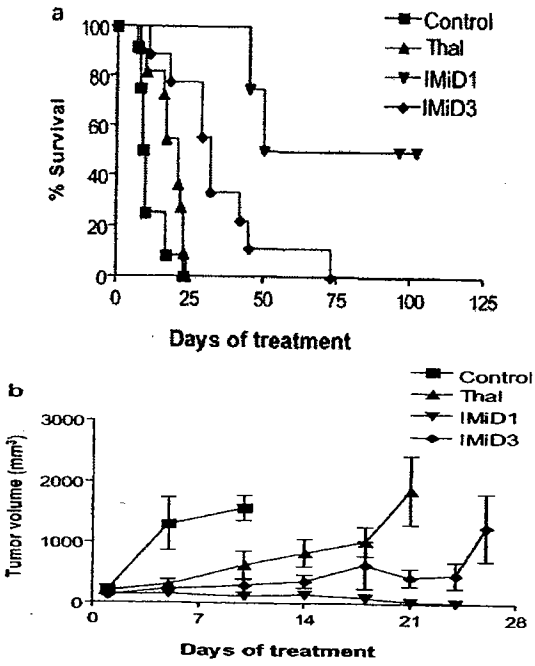
(Excerpted from the sponsor's submission)

10. Immunomodulatory analogs of thalidomide inhibit growth of Hs Sultan cells and angiogenesis in vivo. Lentzsch et al, Leukemia 2003, 17:41-44.

- Daily treatment of beige-nude-xid (BNX) mouse with IMiD1 and IMiD3 (CC-5013) were significantly more potent in this model than thalidomide in suppressing tumor growth (tumor volume), increasing the survival time, and mediating anti-angiogenic effects (decreased microvessel density, MVD).

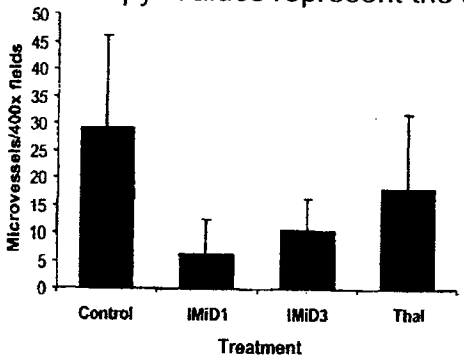
Effect of thalidomide and IMiDs on *in vivo* growth of established tumors.

BNX mice were inoculated with 3 x 10^7 Hs Sultan tumor cells, and daily i.p, treatment with either thalidomide, IMiD1, IMID3 (all 50 mg/kg) or methylcellulose control was started 1 day after the development of measurable tumor.
(a) Survival of animals is calculated from the start of treatment; mice were killed if tumor was ≥2 cm or necrotic.
(b) Tumor volume (mean ± s.e.m. mm^3) was calculated from the start of treatment.



Effect of thalidomide and IMiDs on *in vivo* angiogenesis.

Resected tumors from mice treated with thalidomide, IMiD1, IMiD3 (all 50 mg/kg) or methylcellulose (control) were stained for CD31 expression, and MVD was determined by enumerating vessels in five high power (x400) magnification fields per slide using light microscopy. Values represent the average ± s.d.



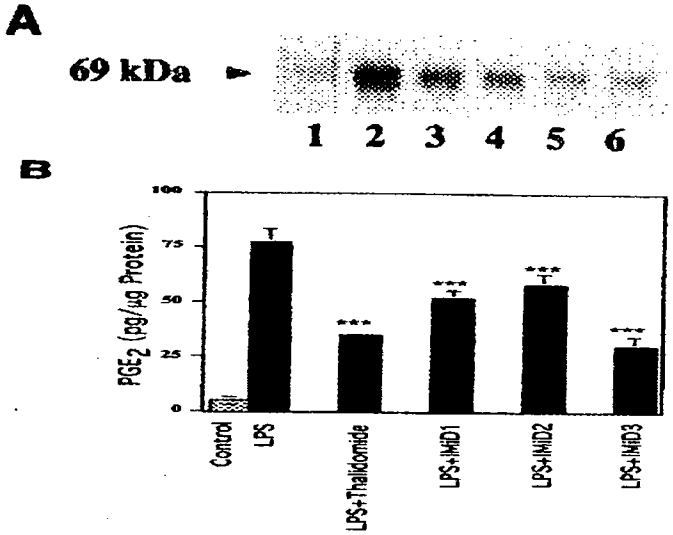
(Excerpted from the sponsor's submission)

11. **Thalidomide and its analogues inhibit lipopolysaccharide-mediated induction of cyclooxygenase-2.** Fujita et al., Clin Cancer Res 2001; 7:3349-3355.

- Thalidomide,IMiD1, IMiD2 and IMiD3 (CC-5013) caused a dose dependent inhibition of lipopolysaccharide (LPS) mediated induction of cyclooxygenase-2 (Cox 2) and prostaglandin E_2 (PGE_2) synthesis in RAW 264.7 cells (murine macrophage cells).

Analogues of thalidomide inhibit LPS-mediated induction of COX-2 and PGE2 production.

Cells were treated with vehicle (Lane I), LPS (2 ng/ml; Lane 2) or LPS plus 100 μM thalidomide (Lane 3), IMiD I (Lane 4), IMiD2 (Lane 5), and IMiD3 (Lane 6) for 16 h.
A. Cellular lysate protein (25 μg/lane) was loaded onto a 10% SDS-polyacrylamide gel, electrophoresed, and subsequently transferred onto nitrocellulose. The immunoblot was probed with antibody specific for COX-2.
B. Culture medium was assayed for spontaneous production of PGE2 with an enzyme immunoassay.



(Excerpted from the sponsor's submission)

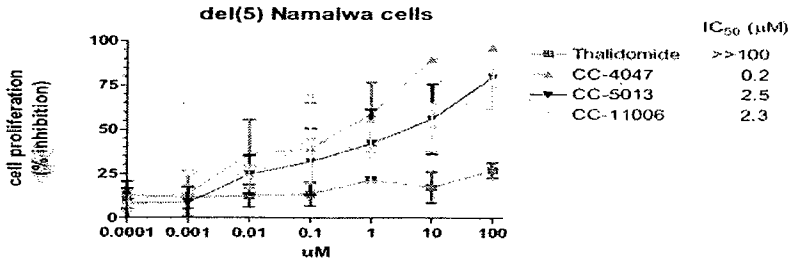
12. **Anti-proliferative activity and mechanism of action of thalidomide, CC-4047, CC-5013 and CC-11006 in chromosome 5 deleted cells Namalwa and KG-1 and control cell lines MUTZ-5 and UT-7 in vitro.** Study Number: 5232- 59- 76 (REVISED)

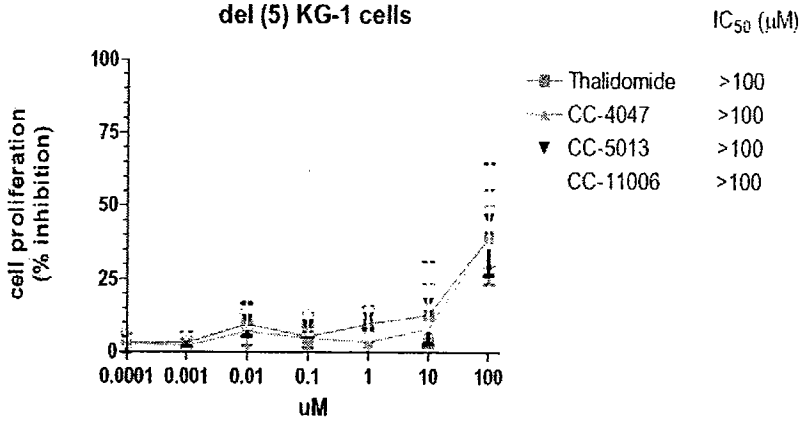
- CC-5013 induced anti-proliferative activity in Namalwa and KG-1 cells. No activity was seen in either UT-7 (AML, deletion at 5q13) or MUTZ-5 (B cell precursor, all cell line, normal 5q chromosomes).
- CC-5013 inhibited Epo-induced (10 U/mL) Akt phosphorylation (Ser473 and Thr308) in Namalwa cells.

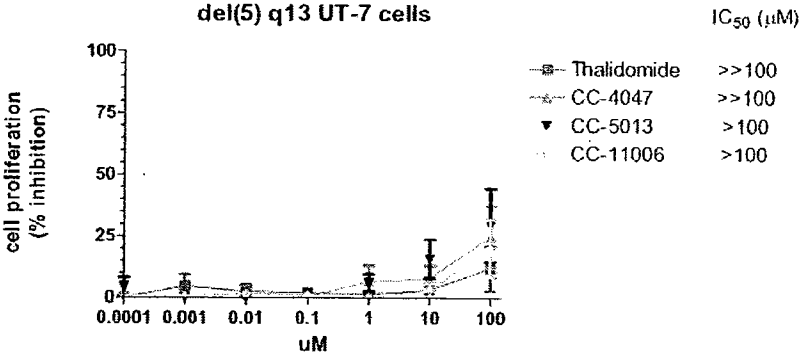
Results:

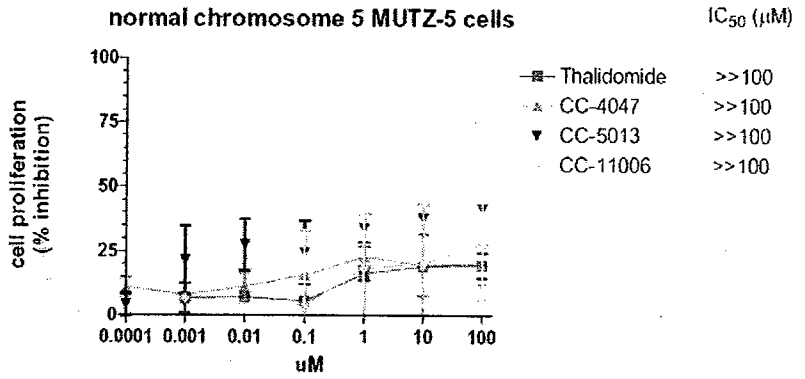
Inhibition of cell proliferation

Cells were plated in 96- well plates, pre- treated with compounds, ^{3}H-thymidine added to each well, incubated for 6 hours and ^{3}H- thymidine incorporation measured.









(Excerpted from the sponsor's submission)

Summary:

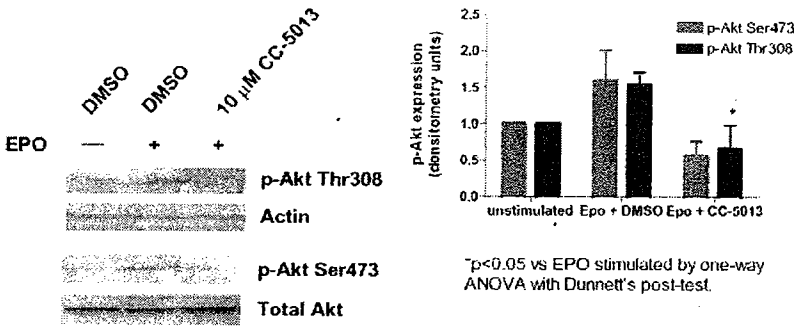
Inhibition of cell proliferation.

Cell line	IC_{50} (μM)			
	Thalidomide	CC-4047	CC-5013	CC-11006
del(5) Namalwa	>>100	0.2	2.5	2.3
Del(5)KG-1	>100	>100	>100	>100
Del(5)q13 UT-7	>>100	>>100	>100	>100
Normal chromosome 5 MUTZ-5	>>100	>>100	>>100	>>100

Namalwa cells: B-cell, Burkitt's Lymphoma cell line, del(5)
KG-1 cells: AML, del(5)
UT-7 cells: AML, deletion at 5q13 as a control for KG-1
MUTZ-5 cells: B-cell precursor, ALL cell line, normal 5q chromosome as a control for Namalwa

Inhibition of Epo-induced Akt phosphorylation in Namalwa cells.

Namalwa cells were treated with DMSO or 10 μM CC-5013 for 1 hour and stimulated with Epo (10 U/ml) for 30 minutes. Cell lysates were separated, probed with phospho-Akt Ser473 and Thr308 Abs and quantitated using — The average of three experiments is shown. Control Akt and actin Abs indicate equal protein loading (data not shown).



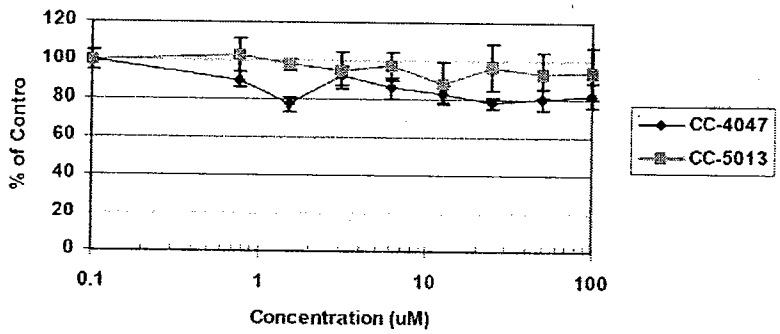
(Excerpted from the sponsor's submission)

13. **Effects of IMiDs on proliferation of breast cancer, NSCLC, CML and NHL cell lines in vitro.** Study Number: PD385.

- CC-5013 and CC-4047 did not affect K562 (leukemic cell line) cell proliferation *in vitro.*
- CC-5013 in combination with anti-CD20 antibodies B1 or Rituxan had a slight effect on Raji cells (Burkitt's lymphoma cell line).
- CC-5013 and CC-4047 did not affect breast cancer cells and NSCLC proliferation.

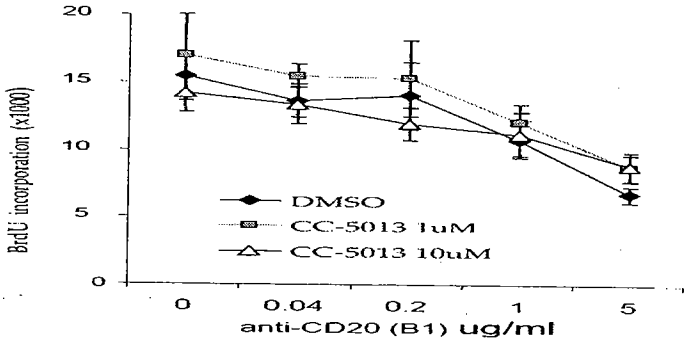
Results:

Effect of CC-5013 and CC-4047 in K562 cell proliferation



(Excerpted from the sponsor's submission)

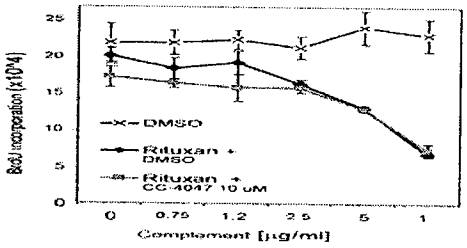
Combination treatment of Raji cells with CC-5013 and anti-CD20 (B1) antibody.



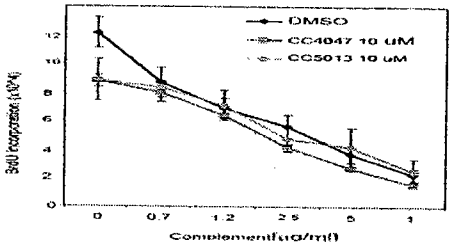
(Excerpted from the sponsor's submission)

Combination treatment of Raji cells with 10 µM CC-5013 or CC-4047 and Rituxan (10 µg/ml) in the presence of complement

A.



B.



(Excerpted from the sponsor's submission)

IC50 (µM) of CC-5013 and CC-4047 in solid tumor proliferation assay

Cell Line	CC-5013	CC-4047
NCI-H460	>100	>100
A549	>100	>100
SKBR3	>100	>100
ZR-75-1	>100	>100
MDA-MB-231	>100	>100
MDA-MB-468	40.40, 43.64	38.55, 17.98
MCF-7	69.15, 50.42	94.09, 96.22
BT-474	>100	>100

(Excerpted from the sponsor's submission)

14. **Anti-proliferative activity of CC-4047, CC-5013, CC-5079, and CC-10004 against the non-Hodgkin's B lymphoma cell line Farage in vitro.** Study Number: 5196-141-155.

Anti- proliferative activity was observed in Farage NHL cells as shown below.

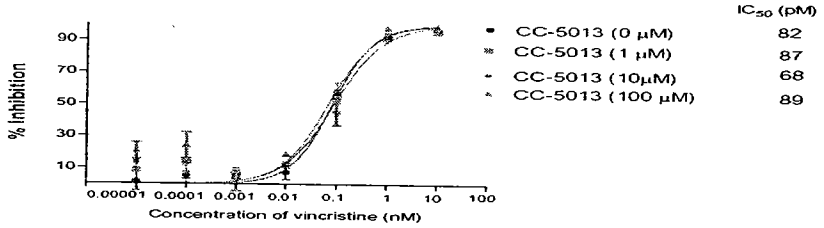
CC-	Farage NHL cell proliferation IC_{50} (µM)
4047	0.36
5013	24
5079	0.0023
10004	>100 (540)

(Excerpted from the sponsor's submission)

15. **Effect of CC-10004 and CC-5013 on proliferation of the mouse CLL line LNC, alone and in combination with vincristine.** Study Number: 5228-024-13.

- CC-5013 (1-100 µM) had no significant effect on mouse chronic lymphocytic leukemia LNC cell proliferation alone or in combination with vincristine.

Effect of CC-5013 on vincristine-induced inhibition of LNC cell proliferation

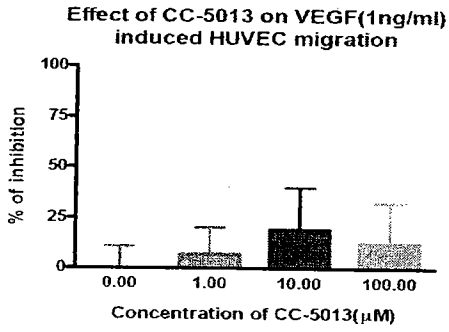


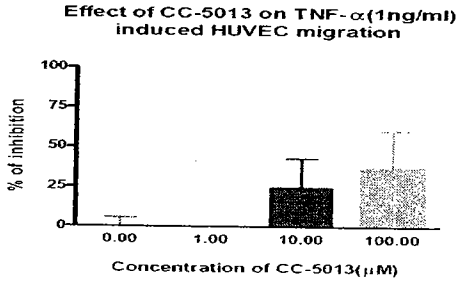
(Excerpted from the sponsor's submission)

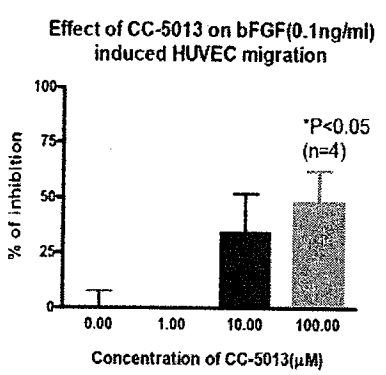
16. **Inhibition of endothelial cell migration by thalidomide, CC-4047, and CC-5013.** Study Number: 5239-92-5239-188.

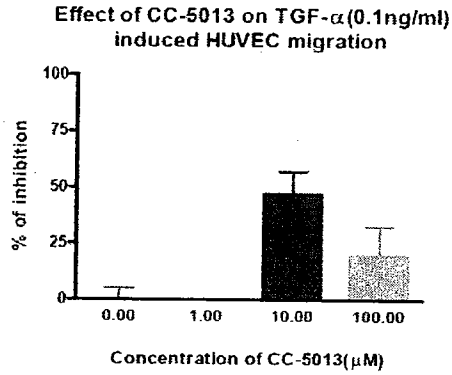
- CC-5013 at higher concentration (10-100 µM) inhibited the human umbilical vein endothelial cells (HUVEC) migration through the fibronectin-coated membrane towards 1 ng/ml of VEGF.
- CC-5013 at 100 µM also significantly inhibited 1 ng/ml TNF-α induced cell migration.
- CC-5013 at lower concentration (1.0 µM) did not show significant effect on VEGF, TNF-α, bFGF and TGF-α (0.1 ng/ml) induced HUVEC migration.

Inhibition of endothelial cell migration by CC- 5013: Data were compiled from three independent experiments with HUVECs from 3 separate donors, each tested in duplicate.







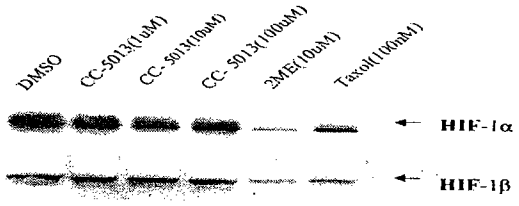


(Excerpted from the sponsor's submission)

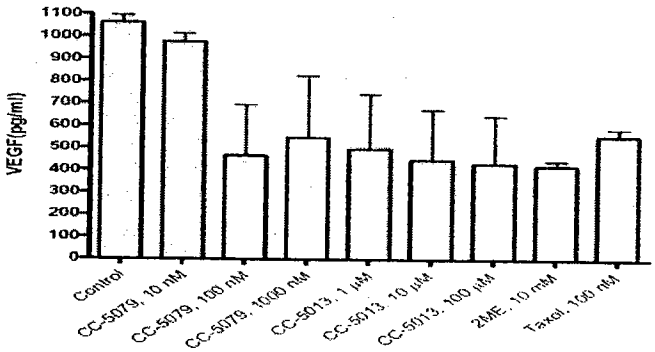
17. **Effect of CC-5013 on HIF-1 alpha expression and VEGF production in PC-3 cells.** Study Number: 5228- 026.

- CC- 5013 inhibited the expression of HIF-1α in PC-3 human prostate cancer cells and reduced the expression level of VEGF of these cells as shown below.

Effect of lenalidomide on HIF-1α expression in PC-3 cells



Effect of lenalidomide on VEGF expression in PC3 prostate cancer cells



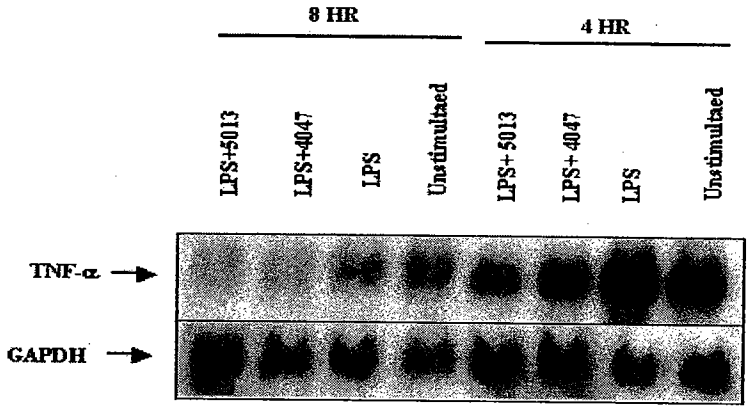
(Excerpted from the sponsor's submission)

18. **Lenalidomide inhibits angiogenesis in vitro and reduces lung metastasis of mouse melanoma cells in an animal model.** Study Number: 5071-180.

- Lenalidomide inhibited PBMC TNF-α mRNA expression by LPS.
- Lenalidomide inhibited the association of β-catenin with cadherin-5 (endothelial tube formation) in a dose-dependent manner.
- Lenalidomide inhibited both migration and invasion of HUVECs
- Lenalidomide blocked metastasis of lung tumor cells in BDF mice.

Effects of CC- 5013 on expression of TNF-α mRNA by PBMC stimulated with LPS.

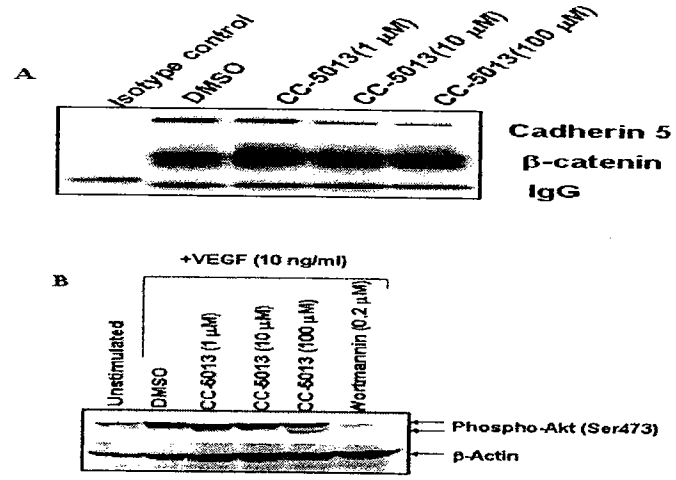
PBMC from healthy donors were pretreated with 1 μM CC-5013 for 1 hour, stimulated with LPS (10 ng/ml), total RNA harvested at different time points and 20 μg of each sample subjected to Northern blot analysis.



(Excerpted from the sponsor's submission)

The effects of lenalidomide on the adherens junction proteins (cadherin-5 and β-catenin) and phosphorylation of Akt- 1:

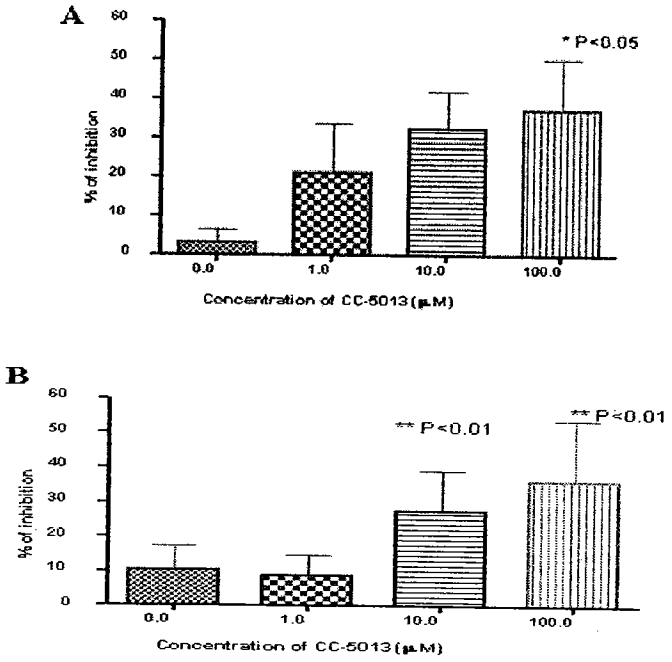
Human umbilical vein endothelial cells (HUVEC) were pretreated with either lenalidomide (1, 10 and 100 μM) or DMSO (0.1%) and stimulated with VEGF (10ng/ml).
A. Immunoprecipitation of ß-catenin was performed, and associated proteins were analyzed for cadherin 5 by Western blot.
B. The treated cells were lysed and the cell lysates were used in Western blot to determine the effects of lenalidomide on the phosphorylation of Akt. Results are shown below.



(Excerpted from the sponsor's submission)

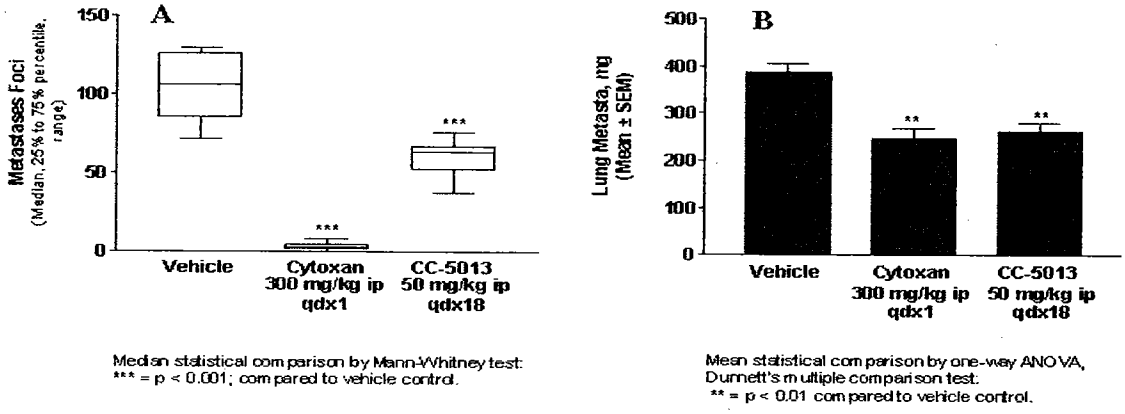
Effects of lenalidomide on the migration and invasion of HUVEC cells:

A. HUVEC cells were pretreated with either lenalidomide (1, 10 and 100 μM) or DMSO control (0.1%) and incubated for 22 hours. The migrations of the cells (through uncoated membranes) were measured.
B. Similar treatments, the effect of compound were tested on the invasion of the HUVEC cells (through fibronectin-coated membranes). Data shown are the average of three separate experiments.



(Excerpted from the sponsor's submission)

Effect of lenalidomide on the metastasis of B16F10 mouse melanoma into lung: B16-F10 melanoma cells were injected into BDF-1 mice. The drug treatments were started on day 1 and continued for day 18. Results are shown below.



Median statistical comparison by Mann-Whitney test:
*** = p < 0.001; compared to vehicle control.

Mean statistical comparison by one-way ANOVA, Dunnett's multiple comparison test:
** = p < 0.01 compared to vehicle control.

(Excerpted from the sponsor's submission)

19. Novel Thalidomide Analogues Display Anti- Angiogenic Activity Independently of Immunomodulatory Effects. Dredge et al., B J Cancer 2002; 87:1166-1172.

Both IMiDs (Immunomodulatory Drugs) and SelCIDs (selective cytokine inhibitory drugs, SelCIDs^{TM}) tested showed more anti-angiogenic activity than thalidomide as shown below. IMiD-1 is CC-5013 (REVIMID^{TM}, Revlimid®, lenalidomide).

Compound	PDE4 activity IC_{50} (μM)	TNF-α activity IC_{50} (μM)	HUVEC inhibition ~IC_{50} ($\mu g\ ml^{-1}$)		Anti-angiogenesis	
			bFGF	VEGF	Rat aorta	Human model
IMiD-1	>100	0.1	>50	>50	3	3
IMiD-2	>100	0.02	>50	48	2	2
Thalidomide	>100	190	>50	>50	0	1
SelCID-1	0.08	0.19	>50	>50	3	3
SelCID-2	2.98	1.06	20	30	2	2
SelCID-3	9.42	12.60	25	45	3	N/A

Note: approximate figures are described for IC_{50} values in proliferation studies. Also, angiogenesis data is ranked where 0=no effect, 1=non significant inhibition, 2=significant inhibition at 10 $\mu g\ ml^{-1}$, 3=significant inhibition at 1 $\mu g\ ml^{-1}$. N/A=compound was not available at time of testing.

(Excerpted from the publication)

2.6.2.3 Secondary pharmacodynamics

1. **Addition of Immunomodulatory Drugs CC5013 or CC4047 to Rituximab Enhances Anti- Tumor Activity in a Severe Combined Immunodeficiency (SCID) Mouse Lymphoma Model.** Hernandez et al., Am Soc Hematology 45th Annual Meeting 2003, Abstract # 235.

Concurrent administration of CC-4047 or CC-5013 with rituximab enhanced anti-tumor activity and prolonged survival of severe combined immunodeficiency mice than rituximab monotherapy alone.

2. **Use of ImiD3, a thalidomide analog, as an adjunct to therapy for experimental tuberculous meningitis.** Tsenova et al., Antimicrob Agents Chemother 2002; 46:1887-1895.

Treatment of experimental tuberculous meningitis in rabbits with anti-tuberculous drugs in combination with IMiD3 (lenalidomide) enhanced the survival to day 28 (73%) as compared to treatment with anti-tuberculous drugs or with anti-tuberculous drug plus thalidomide (50%) as shown below.

Treatment	No. of survivors/ total no. of rabbits	No. of rabbits with clinical score of:				
		0	1	2	3	4
None (control)	0/10	0	0	0	0	0
Antibotics	5/9	3	0	1	1	0
Antibiotics + thalidomide	4/10	3	1	0	0	0
Antibiotics + IMiD3	8/11	7	0	1	0	0

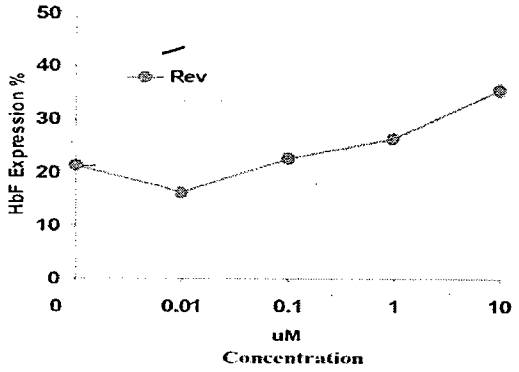
(Excerpted from the publication)

3. **Thalidomide and its Analogs Overcome Drug Resistance of Human Multiple Myeloma Cells to Conventional Therapy.** Hideshima et al, Blood 2000; 96:2943-2950. See Appendix A for details.

- Thalidomide and its analogues act directly on tumor cells via inducing apoptosis or G1 growth arrest.
- These agents also enhance the anti-multiple myeloma (MM) activity of dexamethasone.

4. **IMiDs augment fetal hemoglobin synthesis and can be used for the treatment of hemoglobin disorders like Sickle cell anemia and ß-thalassemia.** Study Number: 9516172999.

- CC-5013 (Revlimid) increased fetal hemoglobin expression during erythroid differentiation in CD34 cells cultured in a dose dependent manner.



5. **Thalidomide and Thalidomide analogue drug costimulate virus- specific CD8$^+$ T cells in vitro.** Haslett et al., J Infect Dis 2003; 187:946-955.

- Thalidomide and CC- 5013 costimulated virus-specific responses in CD^{8+} T cell from patients chronically infected with HIV and CMV.

Thalidomide and CC-5013 enhanced the virus-specific activities of CD8$^+$ T cell isolated from 6 chronically HIV-infected patients as shown below.

Patient	Duration of HIV infection, years	HIV antigen	Baseline frequency of HIV-specific IFN-γ–positive T cells, %	Increase in virus-specific CD8$^+$ IFN-γ–positive cells, compared with DMSO control, *n*-fold			
				HIV		CMV pp65	
				Thalidomide	CC-5013	Thalidomide	CC-5013
3	14	Pol	0.17	4.1	ND	ND	ND
11	14	Gag	0.19	1.2	1.7	2.2	1.6
30	8	Env	0.04	0.6	1.9	4.9	9.3
14	10	Pol	0.09	4.5	21.5	13.0	153.0
12	12	Pol	0.03	2.3	3.8	4.7	23.2
28	13	Nef	0.05	2.0	5.1	0.8	2.0
				2.0[a,b]	3.8[a,b]	4.7[a,c]	9.3[a,c]

NOTE. CMV, cytomegalovirus; DMSO, dimethyl sulfoxide; IFN-γ, interferon-γ; ND, not done.
[a] Median *n*-fold increase in virus-specific CD8$^+$ IFN-g–positive cells.
[b] $P < .01$ (Freidman test).
[c] $P < .05$ (Freidman test).

(Excerpted from the publication)

2.6.2.4 Safety pharmacology

Neurological effects:

1. CC-5013: Effects on General Activity and Behaviour in the Rat Following Oral Administration. Study Number: 1398/ 437. See Appendix B for details.

   Oral administration of single dose of lenalidomide at dose levels of 500, 1000 and 2000 mg/kg did not produce physiological changes in male rats.

Cardiovascular effects:

1. Effects of CC- 5013 on Cloned hERG Channels Expressed in Mammalian Cells. Study Number: 031205. DFN. See Appendix B

   Lenalidomide (787 µM) and terfenandine (60 nM) inhibited hERG current by 8% (n=3) and 79% (n=2), respectively.

2. CC- 5013: Cardiovascular and Respiratory Effects in the Anaesthetised Dog Following Intravenous Administration. Study Number: 1398.131. See Appendix A.

   Single intravenous administration of lenalidomide at 2, 10 and 20 mg/kg did not cause major effects on the cardiovascular and respiratory system of the anesthetized dogs.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Study type	Test system	Effect of lenalidomide
Modulation of cytokines	LPS-stimulated PBMC and / or whole blood	↓ (TNF-α, IL-1β, IL-6, IL-12, MCP-1α),↑ (IL-10, Rantes)
	Anti-CD3 stimulated CD4+T cells	↑ (IFN-γ, IL-2, Rantes), ↓ (IL-10)
	CD4 and $CD8^+$ cells	↑ IL-2
	Con A stimulated whole blood	Human IL-2 ↑ No effect on rat IL-2
Innate and adaptive immunity	DC/PBMC and DC/CD8 co cultures	↑ $CD8^+$ T cell cytokine and cytotoxicity
	Anti-CD3 stimulated T cells	↑ T-cell activation
Hematopoietic stem cell differentiation	Human CD34+ progenitor cells	↑ Fetal hemoglobin expression
COX-2 protein expression	LPS-stimulated PBMC	↓ COX-2; no effect on COX-1
	Murine macrophage-like cell line	↓ COX2 ↓ PGE2
Hematopoietic tumor cell proliferation	Mouse chronic lymphocytic leukemia cell line	No effect on proliferation
	Human MM cell lines	Overcome drug resistance to conventional therapy
	Human Non-Hodgkin's lymphoma cell line	↓ Farage NHL cell proliferation
	Human Burkitt's lymphoma, acute myeloid leukemia, and acute lymphoblastic leukemia cell lines	↓proliferative activity in del(5) Namalwa cells
Angiogenesis	Human prostate cancer cells HUVEC proliferation and HUVEC migration Rat aortic model	↓VEGF ↓VEGF-induced HUVEC migration
	Beige-nude-xid (BNX) mouse	↓ Angiogenic effects (↓ microvessel density)
Solid tumor cell proliferation	Breast cancer cell lines and non-small cell lung cancer cell lines	No effect on breast cancer and NSCLC proliferation.

Con- A: Concanavalin A
DC: Dendritic cell
HIF- 1: Hypoxia- Inducible Factor- 1 Alpha
LPS: Lipopolysaccharide
NK: natural killer
VEGF: Vascular Endothelial Growth Factor
COX: Cyclooxygenase
HEK: Human Embryonic Kidney
HUVEC: Human umbilical vein endothelial cell
MM: Multiple Myeloma
PBMC: Peripheral Blood Mononuclear Cell

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Lenalidomide is structurally similar to thalidomide. The metabolic data available on lenalidomide and thalidomide indicate that both readily undergo *in vivo* chemical degradation (hydrolysis) rather than phase 1 oxidative metabolism.

Oral (gavage) administration of CC- 5013 to male and female cynomolgus monkeys for 52 weeks at dose levels of up to 2 mg/ kg/ day (24 mg/m^2/day) did not effect hepatic, microsomal protein and cytochrome P450 concentrations nor the activities of CYP1A, CYP2B, CYP2C, CYP2E, CYP3A and CYP4A. There was no *in vitro* metabolism of (^{14}C)-CC-5013 in human liver microsomes and cDNA Supersomes^{TM}, indicating that CC- 5013 may be resistant to phase 1 metabolism.

No metabolism of (^{14}C)-CC-5013 was observed during phase 2 metabolism in suspension of human liver hepatocytes. These data indicate that (^{14}C)-CC-5013 may be metabolically stable in humans.

During quantitative whole-body autoradiography following single oral administration to the rat, radioactivity in fetal tissues was generally lower than those of the dam. However, the concentrations of radioactivity in the fetal brain were higher than those in the maternal brain. This may be due to generally more permeable blood/ brain barrier in fetuses.

2.6.4.2 Methods of Analysis

[See under individual study reviews]

2.6.4.3 Absorption

1. [^{14}C] CC- 5013: A study of absorption and excretion following oral and intravenous administration to the rat.

Key study findings:

- After oral administration, radioactivity was eliminated in almost equal proportions in urine and feces.
- Following intravenous administration, radioactivity was excreted mainly in the urine.
- The absolute bioavailability of radioactivity was 64% in males and 75% in females.
- There was no sex difference in the pharmacokinetics of CC- 5013 or in the excretion radioactivity.
- It is not clear from this study whether the products produced are metabolites or degradation products.

Study no.: 1398/302
Volume #, and page #: Electronic module
Conducting laboratory and location:

Date of study initiation: 20 February 2002
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: CC-5013, batch # CFQ12756 & CFQ12755, specific radioactivity ~212 µCi/mg.

Methods

Doses: 20 mg/kg [^{14}C] CC-5013 by intravenous administration
150 mg/kg [^{14}C] CC-5013 by oral administration
Species/strain: Sprague Dawley - CD (SD)IGSBR] rats
Route, formulation, volume, and infusion rate: oral by gavage or intravenous via a lateral tail vein
Age: 6 weeks
Weight: 163-279 g

PK: Blood samples collected at 0.083, 0.167, 0.33, 0.5, 1, 2, 4, 6, 12, 24, and 48 hours after dosing. Radioactivity determined in both blood and plasma.
Excretion: Urine, feces and expired air samples were collected at 24 h intervals until 168 h after dose administration and radioactivity in the samples determined

Metabolites: At 1.5, 3.5 and 8 hours after dosing, animals were exsanguinated. Plasma samples were used for metabolite profiling and identification.

Results

Mortality: None
Clinical signs: None

Plasma Pharmacokinetic Parameters

Group, sex and dose level	Group A Male 20 mg/kg	Group A Female 20 mg/kg	Group B Male 150 mg/kg	Group B Female 150 mg/kg
	Intravenous dose		Oral dose	
C_{max} (µg equiv./mL)	17.02	19.00	24.52	31.29
T_{max} (h)	NA	NA	1.3	1.7
$T_{½elim}$ (h)	4.771	5.951	2.132	2.252
$AUC_{(0-t)}$ (µg equiv.h/mL)	16.58	14.25	99.08	107.2
AUC (µg equiv.h/mL)	16.90	14.70	102.2	111.2
Vd (L/kg)	6.672	8.718	NA	NA
Cl (L/h/kg)	0.945	1.048	NA	NA
Bioavailability	NA	NA	64%	75%

NA – Not Applicable

Blood Pharmacokinetic Parameters

Group, sex and dose level	Group A Male 20 mg/kg	Group A Female 20 mg/kg	Group B Male 150 mg/kg	Group B Female 150 mg/kg
	Intravenous dose		Oral dose	
C_{max} (µg equiv./g)	16.55	17.03	24.29	30.13
T_{max} (h)	NA	NA	1.7	1.7
$T_{½elim}$ (h)	16.72	41.31	15.67	32.76
$AUC_{(0-t)}$ (µg equiv.h/g)	18.49	16.38	131.2	122.4
AUC (µg equiv.h/g)	19.22	19.44	144.6	147.5

NA – Not Applicable

(Excerpted from the sponsor's submission)

Excretion Balance Investigation

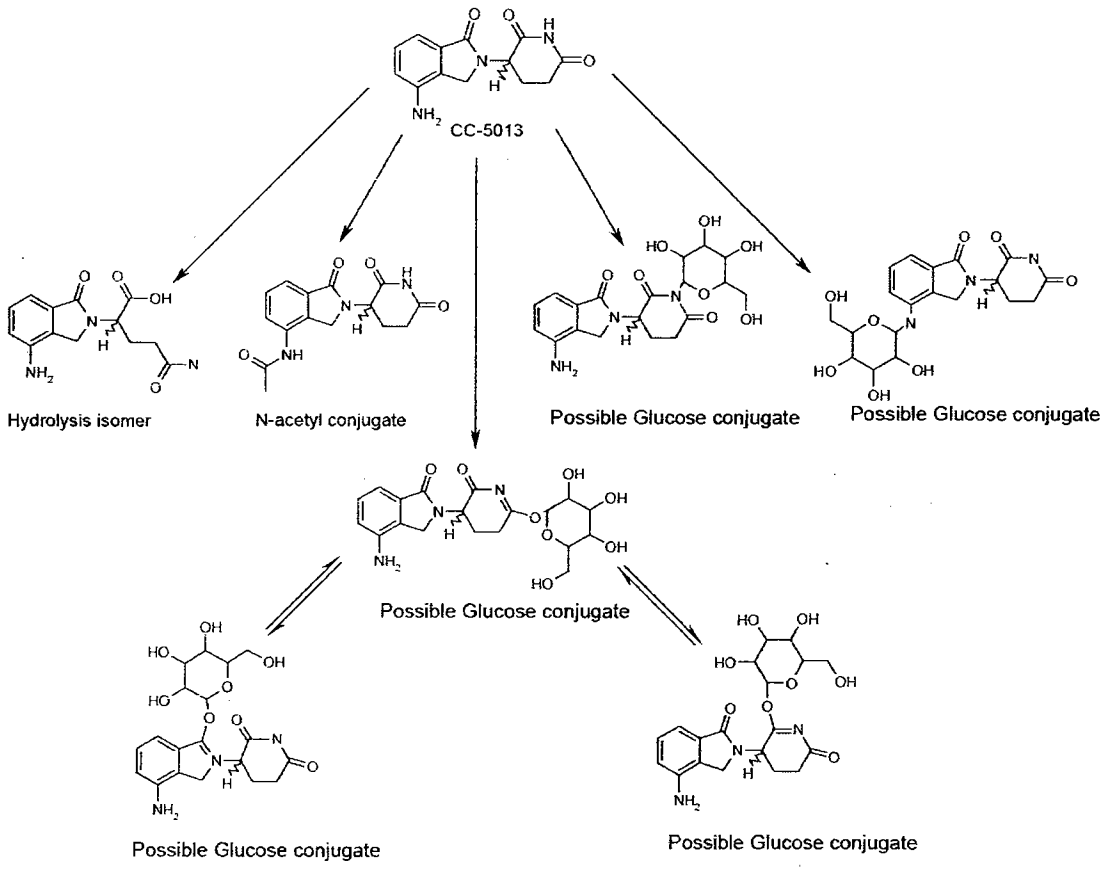
Tissue	Per cent of administered dose			
	Group C Male 20 mg/kg	Group C Female 20 mg/kg	Group D Male 150 mg/kg	Group D Female 150 mg/kg
	Intravenous dose		Oral dose	
Urine	86.76	79.94	53.42	42.54
Faeces	8.281	9.035	37.19	47.00
Cage wash*	1.812	4.991	4.815	4.860
Cage debris	BLOQ	0.005	BLOQ	0.017
Carcass	0.654	0.535	0.152	0.166
Expired air	BLOQ	BLOQ	BLOQ	BLOQ
Total	97.51	94.50	95.58	94.58

*Including final cage wash BLOQ – Below limit of quantification

(Excerpted from the sponsor's submission)

The major component (~50%) in urine and feces was parent compound. CC- 5013 was rapidly metabolized and/or degraded into isomers of hydrolysis metabolites (~20%), N-acetyl conjugate isomers (~2%) and glucose conjugate isomers(~1%). The remaining (~27%) radioactivity was not identified.

Metabolites of CC-5013 following a single oral or intravenous administration to rats at nominal dose levels of 20 or 150 mg/kg body weight



2. [^{14}C] CC- 5013: A study of absorption, excretion and metabolism following oral and intravenous administration to the cynomolgus monkey.

Key study findings:

- Following intravenous administration, excretion was rapid and occurred principally via the urine.
- The route of excretion following oral administration appeared to be via the urine and feces.
- The principal product produced for [^{14}C] CC-5013 in the cynomolgus monkey was hydrolysis of the piperidine dione ring.
- Retention of radioactivity in tissues one week after oral dosing was minimal.
- There were no apparent sex/dose/route-related differences in the pharmacokinetics, excretion or metabolism of CC-5013.

Study no.: 1398/ 301.
Volume #, and page #: Electronic module
Conducting laboratory and location:

Date of study initiation: 22 February 2002
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: CC-5013, batch # CFQ12755 & CFQ12756, specific activity ~55.5 mCi/mmol, and radiochemical purity —

Methods

Doses: Oral 100 mg/kg; intravenous 10 mg/kg
Species/strain: Cynomolgus monkey
Number/sex/group or time point (main study): 3
Age: 26-30 months
Weight: 2.65-3.95 kg

Results

Mortality: None Clinical signs: None

Excretion

Sample	Recovery (mean % of administered radioactivity)					
	Intravenous		Oral*			
	Males	Females	Males		Females	
Urine	65.47	74.30	42.30	32.90	45.51	36.94
Faeces	3.001	3.206	20.60	33.31	15.25	33.02
Cage wash	19.53	10.84	5.912	5.304	4.775	5.902
Cage debris	2.207	5.167	5.247	0.937	3.244	0.837
Swabs	0.814	0.237	0.177	8.880	0.045	0.259
Total (0-168 h)	91.02	93.75	74.24	81.34	68.83	76.95

*: experiment repeated due to low total recoveries

Tissue distribution

Tissue	Radioactivity*	
	501M	502F
Thyroid	0.97	1.27
Skin	0.42	0.50
Uveal tract	2.59	2.34
Urinary bladder contents	low	moderate
Stomach contents	low	low
Small intestine contents	low	low
Caecum contents	moderate	moderate
Large intestine contents	moderate	moderate
Rectum contents	moderate	moderate

*: where numerical data are shown, the units are µg eq./g. For tissues for which full quantification was not possible, assessment of the relative levels of radioactivity in the autoradiograms was made by visual inspection

Metabolism and/or degradation

Plasma

Dose route	Region	Mean RT (min)	Concentration (µg CC-5013 eq./g)						Identification
			Intravenous			Oral			
			1 h	3 h	6 h	1 h	2 h	6 h	
Males	P4	33.5	4.03	1.15	0.23	25.60	8.77	1.88	ring-hydrolysed
	P5	35.7	0.02	<0.01	ND	0.19	0.02	0.01	-
	P6	36.4	1.77	0.51	0.09	11.04	3.68	0.80	ring-hydrolysed
Females	P4	33.5	3.46	0.96	0.23	24.63	9.08	1.77	ring-hydrolysed
	P5	35.7	ND	0.01	ND	0.07	0.06	ND	-
	P6	36.4	1.58	0.43	0.08	10.01	4.10	0.82	ring-hydrolysed

ND: not detected

Urine

Region	Mean RT (min)	% Administered dose				Identification
		Intravenous (0-12 h)		Oral (0-48 h)		
		Male	Female	Male	Female	
U1	30.7	ND	0.12	ND	0.68	-
U2	31.8	ND	0.30	ND	ND	-
U3	32.6	ND	0.18	ND	ND	-
U4	33.5	26.79	11.03	4.49	6.37	ring-hydrolysed
U5	35.2	0.07	ND	0.90	1.44	-
U6	36.3	11.49	4.64	0.80	ND	ring-hydrolysed
U7	37.9	22.80	52.85	25.19	30.85	CC-5013
U8	40.3	0.07	0.33	0.08	0.28	

ND: not detected

Faeces

Region	Mean RT (min)	% Administered dose				Identification
		Intravenous (24-48 h)		Oral (12-72 h)		
		Male	Female	Male	Female	
F1	31.5	0.05	0.06	0.04	0.01	glucose conjugates
F4	33.9	0.42	0.43	1.51	2.51	ring-hydrolysed
F5	36.0	0.21	0.17	0.93	0.73	-
F6	37.2	0.04	0.04	1.63	1.48	ring-hydrolysed
F7	38.2	0.85	0.77	28.36	27.07	CC-5013
F8	40.7	0.19	0.09	0.45	0.34	N-acetyl
F9	43.2	ND	0.01	ND	0.01	-

ND: not detected

(Excerpted from the sponsor's submission)

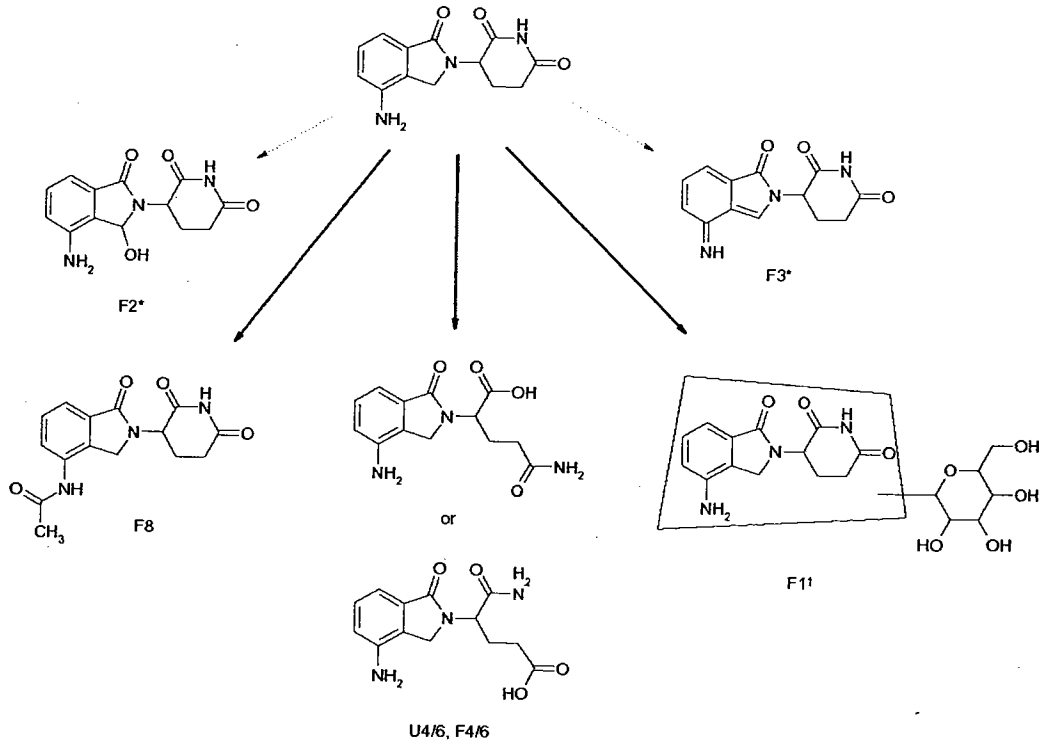
Pharmacokinetics

Parameter (units)	Mean values*							
	Intravenous				Oral			
	Plasma		Blood		Plasma		Blood	
	Males	Females	Males	Females	Males	Females	Males	Females
$C_{(t)}$ (µg eq./g)	14.23	14.70	13.96	14.08	NA	NA	NA	NA
C_{max} (µg eq./g)	NA	NA	NA	NA	37.08	37.43	35.25	35.85
T_{max} (h)	NA	NA	NA	NA	0.75	1.17	0.75	1.17
$t_{½ elim}$ (h)	47.0	42.0	34.1	42.3	11.6	11.9	20.5†	9.9‡
$AUC_{(0-t)}$ (h.µg eq./g)	21.0	19.3	21.2	19.6	98.4	124.8	101.7	141.7
$AUC_{(0-8)}$ (h.µg eq./g)	22.4	20.6	22.7	22.1	104.4	128.8	105.4†	115.3‡
Vd (kg/kg)	31.6	28.3	22.5	27.2	NA	NA	NA	NA
Cl (g/min/kg)	7.8	8.1	7.7	7.4	NA	NA	NA	NA
F (%)	NA	NA	NA	NA	50.1	62.0	53.5§	68.7§

NA: not applicable
*: n = 3, except † (n = 2) and ‡ (n = 1)
§: estimated from $AUC_{(0-t)}$ for animals where $AUC_{(0-8)}$ not calculated

(Excerpted from the sponsor's submission)

Proposed metabolism of CC-5013 in the cynomolgus monkey



*: minor metabolites, not observed in metabolite profiling phase.
†: the test article contains five potential sites of glucosylation; two isomers were detected in faeces by LC-MS, however it was not possible to determine the positions of conjugation.

3. **CC- 5013: In vitro Binding to Plasma Proteins in Rat, Rabbit, Monkey and Human. Study Number: 1398/ 295. See Appendix B.**

4. **CC- 5013: A Study to Determine the Oral Bioavailability in the Rat, Dog and Monkey. Study Number: 1398/ 124. See Appendix A**

5. **CC-1088, CC-4047, CC-5013 and CC-7025: Comparative Absorption by the Caco-2 cell line. Study Number 1398/109. See Appendix A.**

2.6.4.4 Distribution:

1. [^{14}C] CC- 5013: Quantitative whole- body autoradiography following a single oral administration (150 mg/kg) to the rat.

Key study findings:

- Highest concentrations of radioactivity were present in the kidney (cortex and medulla), and liver at 1 hour post-dose.
- Transfer of radioactivity across the blood/ brain barrier occurred (data not provided).
- Radioactivity in pregnant females was generally lower than comparable tissues in males (data not provided.
- Fetal brain showed more radioactivity than maternal brain (maternal brain radioactivity was below LOQ).

Study no. 1398/ 300
Volume #, and page #: Electronic module
Conducting laboratory and location:

Date of study initiation:
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: [^{14}C]CC-5013, batch # CFQ12756 and CFQ12755 , purity.
Specific activity R-isomer (55 mCi/mmol, 211 µCi/mg) and S-isomer (56 mCi/mmol, 214 µCi/mg)

Methods

Doses: 150 mg/kg (~142 µCi/kg, ~37 µCi/animal)
Species/strain: SD-albino and Lister-Hooded pigmented rats
Number/sex/group or time point (main study): Male (Albino) – total - 18
Male (pigmented) – 6, and time-mated pregnant rats - 3
Route: Oral
Satellite groups used for toxicokinetics or recovery: None
Age: 5-6 weeks

Sampling time:

Dose Group	Animal sex/strain	Sampling times post-dose
A	Male albino	1, 4 and 8 hours and 1, 3 and 7 days (3 animals per time point)
B	Male pigmented	1, 3, 7, 14 and 35 days (single animal per time point)
C	Pregnant albino*	1 and 8 hours and 1 and 3 days (single animal per time point)

*calculated to be in day 17 of gestation when dosed

(Excerpted from the sponsor's submission)

Results

Mortality: None
Clinical signs: None

Tissue distribution of radioactivity

	µg equivalents of CC-5013/g of tissue					
Tissue Kill Time	1 hour	4 hours	8 hours	1 day	3 days	7 days
Plasma	23.85	7.50	3.85	0.08	ND	ND
Kidney cortex	92.51	34.20	20.66	BLQ	BLQ	BLQ
Kidney medulla	80.00	30.49	13.90	BLQ	BLQ	BLQ
Liver	40.64	12.46	6.21	BLQ	BLQ	BLQ
Spleen	32.79	8.10	4.28	BLQ	BLQ	BLQ
Pancreas	28.98	13.89	4.56	BLQ	BLQ	BLQ
Aorta	28.68	11.55	4.15	BLQ	BLQ	BLQ
Non-pigmented skin	28.58	21.30	8.99	1.81	1.67	2.24
Adrenal	28.00	7.43	3.66	BLQ	BLQ	BLQ
Bulbo-urethral gland	25.74	2.75	4.33	BLQ	BLQ	BLQ
Lung	25.44	6.78	4.47	BLQ	BLQ	BLQ
Blood	25.30	6.76	3.17	BLQ	BLQ	BLQ
Myocardium	25.24	BLQ	BLQ	BLQ	BLQ	BLQ
Tongue	24.14	7.56	3.24	BLQ	BLQ	BLQ
Salivary glands	23.90	6.23	2.93	BLQ	BLQ	BLQ

BLQ – Tissue radioactive concentration below lower limit of quantification
ND - Radioactivity not detected

(Excerpted from the sponsor's submission)

Quantifiable activity (1.37 µg equiv/g) in the spinal cord was present at 1 hour only.

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Concentrations of radioactivity in the foetal tissues of pregnant albino rats (calculated to be in day 17 of gestation at dosing) after a single oral administration of [^{14}C]CC-5013 at a nominal dose level of 150 mg/kg body weight (Group C)

		µg equivaleuts of CC-5013/g of tissue			
Animal number and sex		311F	307F	309F	389F
Tissue	Kill time	1 hour	8 hour	1 day	3 days
Foetal blood		4.89	BLQ	BLQ	BLQ
Foetal brain		3.80	BLQ	BLQ	BLQ
Foetal heart		10.14	BLQ	BLQ	BLQ
Foetal liver		5.69	BLQ	BLQ	BLQ
Foetal kidney		4.17	NS	BLQ	BLQ
Placenta		11.54	1.63	BLQ	BLQ
Upper limit of quantification =					
Lower limit of quantification =					

[1] For plasma (radioactivity determined by liquid scintillation counting methods).
Limit of detection = µg equiv/g ND - radioactivity not detected
BLQ - Tissue radioactive concentration below lower limit of detection
NS - Tissue not sectioned

(Excerpted from the sponsor's submission)

Radioactivity in the tissues of pregnant dams was generally lower than those of the males at comparable sampling times. Some activity was present in the fetal tissues at 1 and 8 hours post dosing. Fetal brain showed higher activity than maternal brain (it was below the level of quantification). This may be due to undeveloped blood brain barrier in fetal as compared to mother.

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ON ORIGINAL

2.6.4.5 Metabolism

1. [^{14}C] CC- 5013: A study of absorption, excretion and metabolism following oral and intravenous administration to the cynomolgus monkey. Study Number: 1398/ 301.

See under absorption.

2. Metabolism of (^{14}C)-CC-5013 in Isolated Human Hepatocytes. Study Number 1398/348.

See appendix B

2.6.4.6 Excretion

1. [^{14}C] CC- 5013: A study of absorption, excretion and metabolism following oral and intravenous administration to the cynomolgus monkey. Study Number: 1398/ 301.

See under absorption.

2. [^{14}C] CC- 5013: A study of absorption and excretion following oral and intravenous administration to the rat. Study Number: 1398/ 302.

See under absorption.

2.6.4.7 Pharmacokinetic drug interactions

1. CC- 5013: Effect on cytochrome P450 and related parameters in the male and female Sprague Dawley rat following oral (gavage) administration at 0, 75, 150 and 300 mg/kg/day for 26 weeks.

Key study findings:

- Oral administration of CC-5013 up to 300 mg/kg/day to SD rats for 26 weeks did not induce or inhibit CYP1A, CYP2B, CYP2C, CYP2E, CYP3A, and CYP4A enzymes.

Study no.:	1398/310
Volume #, and page #:	Electronic module
Conducting laboratory and location:	/
Date of study initiation:	/
GLP compliance:	Yes

QA report: yes (X) no ( )
Drug, lot #, and % purity: CC-5013, lot # 61457-06, ~ , purity

Methods

Doses:

Group #	Group description	Dose level (mg/kg/day)	Animal #	
			♂	♀
1	Control	0	1-4	101-104
2	Low	75	31-34	131-134
3	Intermediate	150	51-54	151-154
4	High	300	71-74	171-174

Control animals received vehicle only [1% w/v aqueous carboxymethylcellulose]

Species/strain: SD rats
Number/sex/group or time point (main study): 4
Route: Oral gavage

Results

Hepatic microsomal P450 activities (pmoles/min/mg protein, Mean ±S.D.) following oral administration of lenalidomide to rats for 26 weeks

Enzyme (pmoles/min/mg protein)	Marker	Dose Level (mg/kg/day)							
		0		75		150		300	
		♂	♀	♂	♀	♂	♀	♂	♀
Total CYP450 (nmoles/mg protein)		0.77± 0.10	0.36± 0.03	0.59± 0.15	0.43± 0.13	0.60± 0.18	0.47± 0.07	0.55 ±0.05	0.32 ±0.04
7-Ethoxyresorufin *O*-deethylase	CYP1A	18.8± 9.8	43± 13	26.0± 7.1	39± 13	19.3± 4.6	39± 10	15.5± 4.0	42± 10
Testosterone 6α-hydroxylase	CYP3A	49± 16		74± 12		72±9		80± 11	
Testosterone 6β-hydroxylase	CYP3A	898± 182	106± 28	760± 73	104± 7	588± 33	129± 17	802± 126	16± 12
Testosterone 16α-hydroxylase	CYP2C	2851± 813		2408 ±101		2107± 294		2478 ±609	
Testosterone 2α-hydroxylase	CYP2C	1949± 494		1699 ±48		1484± 232		1741 ±446	
Testosterone 7α-hydroxylase	CYP2A	222± 58	396± 53	246± 118	412± 53	233± 62	462± 29	239± 57	272± 66
Testosterone 17β-dehydrogenase	CYP2C	1480± 395	63± 12	1248 ±49	92± 82	1113± 135	89± 29	1218 ±246	12± 17
Lauric acid 11-hydroxylase	CYP2E	804± 142	813± 123	703± 55	1092 ±168	648± 158	857± 60	533± 88	721± 252
Lauric acid 12-hydroxylase	CYP4A	703± 226	628± 28	546± 94	841± 45	457± 74	621± 121	386± 124	574± 246

2. CC- 5013: Effect on cytochrome P450 and related parameters in the male and female cynomolgus monkey following oral (gavage) administration at 0, 1 and 2 mg/ kg/ day for 52 weeks.

Key study findings:

- CC-5013 did not affect total cytochrome P450 concentrations, EROD, lauric acid hydroxylase, testosterone hydroxylase or dehydrogenase activities of cynomolgus monkeys.

Study no.: 1398/ 311
Volume #, and page #: Electronic module
Conducting laboratory and location:

Date of study initiation: Sept. 16, 2002
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: CC-5013, batch # 61457-06, ~ purity (from study # 1398/243)

Methods

Doses:

Group number	Group description	Dose level (mg/kg/day)	Animal ID number Male	Female
1	Control#	0	1-4	25-28
2	Low	1	7-10	31-34
3	Intermediate (I)	2	11-14	35-38

#Control animals received dose vehicle only (1% [w/v] aqueous carboxymethylcellulose)

(Excerpted from the sponsor's submission)

Species/strain: Cynomolgus monkey
Number/sex/group or time point (main study): 4
Route, formulation, volume, and infusion rate: Oral gavage

Results

Hepatic microsomal P450 activities (pmoles/min/mg protein, Mean±S.D.) following oral administration of lenalidomide to monkeys for 52 weeks

Enzyme (pmoles/min/mg protein)	Marker	Dose Level (mg/kg/day)					
		0		1		2	
		♂	♀	♂	♀	♂	♀
Total CYP450 (nmoles/mg protein)		1.1± 0.12	1.1± 0.06	0.99± 0.05	1.13+ 0.15	1.07± 0.22	1.15± 0.18
7-Ethoxyresorufin *0*-deethylase	CYP1A1/2	558± 123	533± 237	571± 143	573± 106	446± 130	586± 79
Testosterone 2 β – hydroxylase	CYP3A8	0.67± 0.15	0.53± 0.05	0.67± 0.05	0.49± 0.1	0.65± 0.22	0.49± 0.07
Testosterone 6β-hydroxylase	CYP3A8	6.6± 1.7	4.8± 0.4	6.6± 0.5	4.4± 0.9	6.9± 1.1	4.3± 0.7
Testosterone 16 β - hydroxylase	CYP2B17	0.41± 0.06	0.34± 0.1	0.36± 0.03	0.37± 0.08	0.37± 0.1	0.34± 0.06
Testosterone 2α-hydroxylase	CYP2C	0.16± 0.05	0.11± 0.04	0.13± 0.03	0.11± 0.03	0.18± 0.05	0.12± 0.03
Testosterone 7α-hydroxylase			0.07± 0.02		0.08± 0.02		0.08± 0.02
Testosterone 17β-dehydrogenase	CYP2C20	0.27± 0.02	0.20± 0.05	0.24± 0.03	0.26± 0.05	0.27± 0.05	0.24± 0.03
Lauric acid 11-hydroxylase	CYP2E1	742± 42	807± 67	764± 66	848± 105	790± 28	740± 109
Lauric acid 12-hydroxylase	CYP4A	2270± 586	2537± 172	2314± 260	2547± 137	2340± 295	2400± 283

3. Identification of the cytochrome P450 enzymes responsible for the *in- vitro* metabolism of (^{14}C)-CC- 5013 in human liver microsomes.

Key study findings:

- CC- 5013 was not metabolized in either human liver microsomes or cDNA Supersomes™.

Study no.: 1398/335.
Volume #, and page #: Electronic module
Conducting laboratory and location:

GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: – purity,
specific radioactivity ~ 55.5 mCi/mmol
Tissue source: Human liver microsomes

(Lot 0210198, 46 donors)
Supersomes™ (microsomes obtained from insect cells over-expressing cDNA for human CYP450 isozymes)

Results

In vitro metabolism of (^{14}C)-CC-5013 (10 µM) was not observed in human liver microsomes and cDNA Supersomes™ (CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 or CYP3A4 at 100 pmol P450/ mL) after 60 minutes incubation.

4. Identification of human P450 isozymes involved in the metabolism of CC-1088 and CC- 5013.

Key study findings: Both CC-1088 and CC-5013 were resistant to in vitro microsomal metabolism.

Study no.: 1398/ 208
Volume #, and page #: Electronic module
Conducting laboratory and location:

Date of study initiation: 28 September 2000
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: CC-5013 – lot # 61246-04, — purity
CC-1088 – lot # 61063-01, — purity
Microsome source: lot #0010079, 16 donors
Supersome™ source:

Results

As shown below, concentrations of CC-5013 in all samples after 30 minute incubation were lower as compared to non-incubated control and test samples. This may be due to the degradation of test compound under the assay conditions.

Concentrations of CC-5013 in Human Supersomal™ Supernatant and Microsomal Supernatant (μM)

Sample name	0 min	30 min
CYP1A2	12.0 (	10.5 (
CYP2C9	13.0(	11.4 (
CYP2C19	11.9 (	11.1
CYP2D6	12.4 (	9.9
CYP3A4	13.0	10.8
CYP2A6	12.3	10.8 (
CYP2E1	12.2	10.2 (
CYP2C8	13.3 (	9.9 (
CYP2B6	12.2 (	9.9
Buffer	<1.0 (	12.4
HLM (+)	12.9 (	9.8
HLM (-)	12.4 (	9.8 (
Control (+)	13.4 (	10.2 (
Control (-)	13.4 (	10.0

Values are expressed as the mean of 2 determinations, individual values are in parenthesis
HLM (+) Human liver microsomes incubated in the presence of β-NADPH
HLM (-) Human liver microsomes incubated in the absence of β-NADPH
Control (+) cDNA expressed human Supersomes™ incubated in the presence of β-NADPH
Control (-) cDNA expressed human Supersomes™ incubated in the absence of β-NADPH

(Excerpted from the sponsor's submission)

No significant metabolism of CC-1088 and CC-5013 (see above table) was detected in the human liver microsomes or cDNA expressed human P450 enzymes incubations.

5. Effects of CC-1088 and CC-5013 on selected cytochrome P450 activities in human liver microsomes: Prediction of drug interactions.

Key study findings: CC-5013 and CC-1088 did not inhibit P450 activities in human liver microsomes.

Study no.: 1398/199
Volume #, and page #: Electronic module

Conducting laboratory and location:

Date of study initiation: 9 June 2000
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: CC-1088 – batch # 61063-01, — purity.
CC-5013 – batch # 60832-01, — purity.
Tissue source: — , lot # 9910092, 16 donors

Results

CC-5013 and CC-1088 did not inhibit or induce CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1 or CYP3A4 in human liver microsomes.

6. Metabolism of (^{14}C)- CC- 5013 in Isolated Human Hepatocytes. Study Number: 1398/ 348.

See appendix B

7. Comparison of chemical degradation pathways of lenalidomide and thalidomide.

Key study findings:

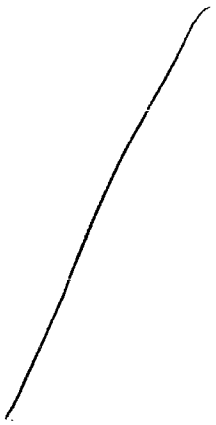
- The degradation (hydrolysis) products from lenalidomide and thalidomide are different based on anticipated metabolites projected from analysis by software modeling.

Study no.: 001
Volume #, and page #: Electronic module
Conducting laboratory and location: Celgene Corporation
7Powder Horn Drive, Warren, NJ
Date of study initiation: July 2004
GLP compliance: No
QA report: yes ( ) no (X)

Methods: The degradation products were drawn using ISIS Draw program version 2.4 (MDL Information Systems, San Leandro, CA).

Results:

Lenalidomide degradation



Thalidomide degradation

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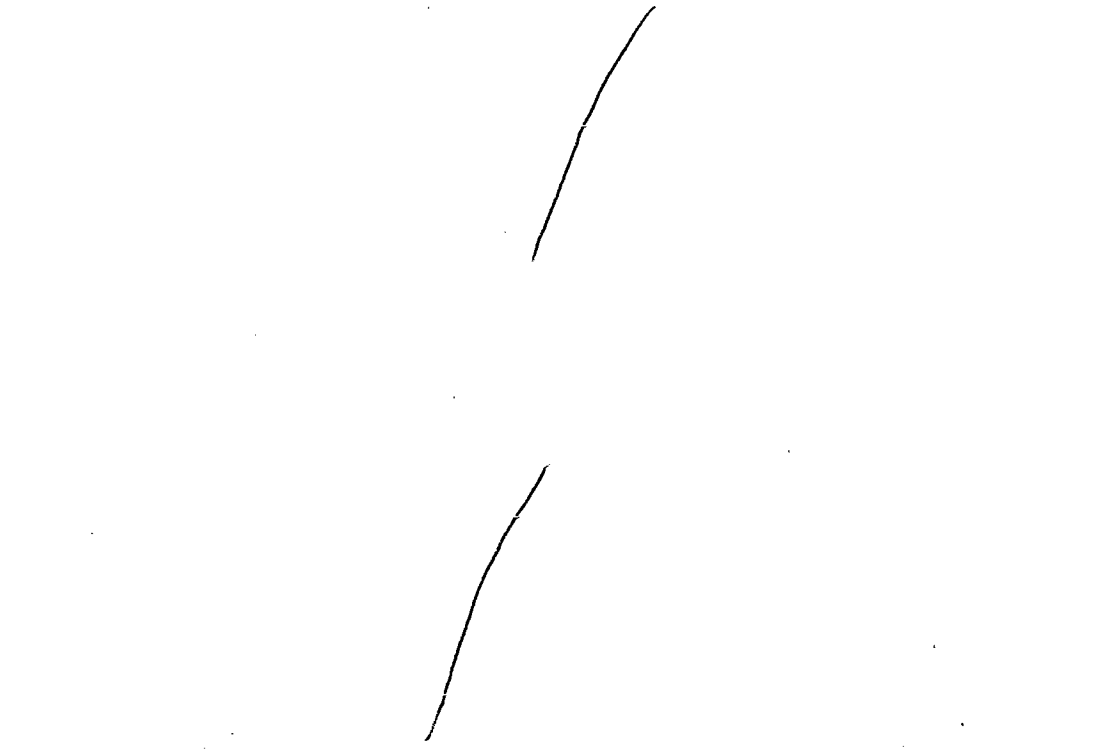

(Excerpted from the sponsor's submission)

2.6.4.8 **Other Pharmacokinetic Studies**
None

2.6.4.9 Discussion and Conclusions

The pharmacokinetic studies of lenalidomide were carried out in rodents (rats) and non rodents (dogs and monkeys). The drug was rapidly absorbed after oral administration in animals. The highest concentrations were found in the kidney (cortex and medulla), and liver. In pregnant rats, fetal tissue concentrations were generally lower than in comparable tissues in the dams, except in the brain. This may be due to the immature blood/brain barrier in the fetus and an indication to be careful during pregnancy. Lenalidomide did not affect major CYP 450 enzymes. The major component in both urine and feces was the parent compound. No metabolism of lenalidomide was seen in isolated human hepatocytes. Degradation products were observed. The bioavailability of lenalidomide was 88, 68 and 50% for dog, rat and monkey, respectively. The oral $t_{1/2}$ was the highest in the monkey (13 h) when compared to dog (6 h) and rat (2 h).

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2.6.4.10 Tables and figures to include comparative TK summary:
See Pharmacokinetics Tabulated Summary below.

APPEARS THIS WAY
ON ORIGINAL

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Lenalidomide was rapidly absorbed and eliminated in rats, dogs, and monkeys as shown below.

Single dose pharmacokinetics of lenalidomide

Species	Rat		Dog		Monkey	
Dose and Route of Administration	IV 10 mg/kg	Oral 100 mg/kg	IV 10 mg/kg	Oral 80 mg/kg	IV 10 mg/kg	Oral 100 mg/kg
C_{max} (µg/mL)	18.14	26.02	28.27 ± 9.16	45.11 ± 13.52	28.73 ± 5.44	21.58 ± 4.32
T_{max} (hour)	0.03	0.5	0.03 ± 0.0	1.3 ± 0.5	0.03 ± 0.0	1.5 ± 0.6
$AUC_{(0\text{-}48h)}$ (µg•h/mL)	12.22	82.83	32.14 ± 2.33	226.25 ± 94.16	21.04 ± 2.92	105.35 ± 16.68
$T_{½}$ (h)	3.0	2.3	2.1± 0.6	6.4 ± 4.2	1.3 ± 0.1	13.4 ± 9.2
CL_{tot} (mL/min/kg)	13.6	--	5.2 ± 0.4	--	8.0 ± 1.2	--
Vd (L/kg)	3.6	--	1.0 (0.3)	--	0.9 ± 0.1	--
F (%)	--	68	--	88	--	50

(Excerpted from the sponsor's submission)

The bioavailability for lenalidomide was the highest in the dog (88%) when compared to rat (68%) and monkey (50%). Plasma clearance was the highest in rat.

Comparison of PK parameters in rats, dogs, monkeys and humans is shown below.

Pharmacokinetics comparison across species after oral administration

	Study Reference	AUC (ng·hr/ml)	C_{max} (ng/ml)	$T_{½}$ (hours)	Safety Factor (Human: Animal AUC)
Humans (10 mg)	CDC-501-001[1]	922	178	3	NA
Rat (300 mg/kg)[2]	1398/206[3]	118,028[4]	22,340[4]	1.5[4]	128
Dog (80 mg/kg)	1398/124[5]	226,254	45,111	1.3	NA
Monkey (1 mg/kg)[2]	1398/243[6]	2187[7]	620[7]	1-2[7]	2.4

[1]Phase II study in multiple myeloma patients
[2]Well tolerated or NOAEL dose
[3]26 week toxicity study in rat
[4]Mean of males and females, 26 week study
[5]Bioavailability study after a single dose
[6]52 week toxicity study in monkey
[7]Mean of males and females on Week 52

(Excerpted from the sponsor's submission)

APPEARS THIS WAY ON ORIGINAL

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: The general toxicity of lenalidomide has been examined in rodents and non-rodents. Single dose oral administration of lenalidomide up to 6,000 mg/m^2 in mice and 12000 mg/m^2 in rats did not produced any mortality or macroscopic abnormalities. Daily oral administration of CC-5013 at 6000 mg/m^2/day to **rats** for 28 days was associated with moderate to severe tubular nephropathy/nephritis, which was attributed to precipitation of the test article in the kidney. Reduced body weight gain, food consumption and reduced red blood cell indices in high dose animals are considered secondary effects to the kidney changes. There were no generalized effects on blood parameters suggestive of myelotoxicity. Daily administration at dose levels of 600 and 1800 mg/m^2/day for 28 days was well tolerated. There was no accumulation of CC-5013 in the rats over the period of study.

26 Week Oral (Gavage) Administration Toxicity Study in the Rat with a 4 Week Treatment- Free Period: Oral administration of CC-5013 to male and female rats at doses of 75, 150, and 300 mg/kg/day for 26 weeks was generally well tolerated. There were no treatment-related mortalities or clinical signs observed. Body weight

gain of high dose males (300 mg/kg/day) was approximately 12 % lower than control animals. There were no treatment-related hematological or macroscopic changes. Pelvic mineralization in the kidney seen in treated animals did not affect the kidney functions. The focal deposit of basophilic amorphous was not seen on the completion of the treatment-free period. The $AUC_{0\text{-}24h}$ and C_{max} values increased with dose for male and female animals on day 1 and week 27. AUC and C_{max} were generally similar for females between day 1 and week 27 indicating no induction/inhibition of liver metabolizing enzymes.

In **monkeys**, oral administration of CC-5013 at 24 mg/m^2/day for 28 days did not show significant difference in AUC $_{0\text{-}24h}$ and C_{max} values between Day 1 and Day 28. In a second study in monkeys, the gavage administration of CC-5013 at 240 mg/m^2/day for 28 days adversely affected lymphoreticular organs (thymus and spleen), altered hemopoiesis in the bone marrow and evidence of nephrotoxicity.

52 Week Oral (Gavage) Administration Toxicity Study in the Monkey with a 7 Week Treatment free Period: Male and female cynomolgus monkeys were supposed to be dosed at 0, 12, 24, 48, and 72 mg/m^2/day for 52 weeks to assess the toxicity of CC-5013. Nine monkeys (1 ♂ & 2 ♀ at 48 mg/m^2/day and 4 ♂ & 1 ♀ at 72 mg/m^2/day and one replacement male at 48 mg/m^2/day) were killed prior to week 20 due to adverse toxicity. The remaining animals in groups 4 and 5 were terminated in week 20 except 2 animals in 72 mg/m^2 /day group were sacrificed on completion of the 7 week treatment-free period. Clinical signs associated with decent animals were thin appearance, hunched posture, soft/liquid feces, sluggish movement and dehydration. Clinical signs observed in animals treated with 12 and 24 mg/m^2/day were comparable to control. No effects on body weights, clinical chemistry, urinalysis, organ weight, macroscopic and microscopic findings were noted in groups 2 and 3 animals. Hematology showed some effect on platelets, red blood cells, and an effect on myelopoietic cells in bone marrow in decedent animals at 48 and 72 mg/m^2/day. Microscopic finding from decedent animals consisted of hemorrhage in multiple organs, gastrointestinal tract inflammation and thymus and bone marrow atrophy. Atrophy of the thymus, was also seen in 12 or 24 mg/m^2/day terminal sacrifice animals. Reversals of macroscopic and microscopic findings were seen in treatment-free sacrifice.

Genetic toxicology: Lenalidomide did not induce mutation in the Ames test, chromosome aberrations in cultured human peripheral blood lymphocytes, or mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells. Lenalidomide did not induce micronuclei in the polychromatic erythrocytes of the bone marrow of male rats. No P450 metabolism was seen in other assays, so the addition of microsomes to the genotoxicity assays provides limited information.

Carcinogenicity: Carcinogenicity studies have not been conducted.

Reproductive toxicology: Lenalidomide was studied in the rat and rabbit for reproductive toxicology. In a fertility and early embryonic study in the rat, lenalidomide was not parentally toxic and did not have significant impact on fertility or early development. Higher doses of lenalidomide could have been tested to ensure that adequate parental exposure was achieved. Pre- and post-natal development was examined in rats, with the dams dosed during organogenesis and lactation with lenalidomide. Minimal effects of prenatal exposure of lenalidomide were seen on the post-natal development of the offspring. Slightly delayed sexual maturation was seen in the male offspring. The female offspring, when mated, had significantly fewer embryos than control offspring and gained less weight during gestation. As with the fertility study, the maternal toxicity in the treated rats was very slight. This study also should have included doses high enough to achieve sufficient maternal toxicity to ensure adequate exposure to lenalidomide. The embryo-fetal development effects of lenalidomide were studied in the rat and rabbit. The rat study had very minimal toxicity in the treated rats and little impact of drug treatment on the gestational and developmental parameters. Historical data shows that the rat is not an adequate model for the full assessment of the teratogenic effects of thalidomide. Given the similar structure of lenalidomide to thalidomide, the rat model is not adequate for the full assessment of the embryo-fetal developmental effects of lenalidomide. The rabbit study, while an adequate animal model for embryo-fetal development of lenalidomide, was confounded by inappetence of several rabbits. Additionally, the dosing levels in this study were not sufficient to ensure adequate maternal exposure.

Special toxicology: Not studied

2.6.6.2 Single-dose toxicity

See Appendix A.

2.6.6.3 Repeat-dose toxicity

1. CC- 5013: 7 Day Oral (Gavage) Range- finding Toxicity Study in the Rat. Study Number: 1398/ 105.

   See Appendix A.

2. CC- 5013: 28 Day Oral (Gavage Administration) Toxicity Study in the Rat. Study Number: 1398/ 107.

   See Appendix A.

3. **CC- 5013: 13 Week Oral (Gavage Administration) Toxicity Study in the Rat. Study Number: 1398/ 206.**

Key study findings:

- Oral administration of CC-5013 at 75, 150, and 300 mg/kg/day for 13 weeks to male and female rats did not result in systemic toxicity.
- Crystals were present in the urine of mid and high dose animals.
- $AUC_{0\text{-}24h}$ and C_{max} values of CC-5013 increased in a dose-dependent manner.

See Appendix B for details.

4. CC- 5013: 26 Week Oral (Gavage) Administration Toxicity Study in the Rat with a 4 Week Treatment- Free Period. Study Number: 1398/ 244.

Key study findings:

- Body weight gains for HD males were reduced (12% ↓ than control).
- Reversible pelvic mineralization in the kidneys was seen in a treatment-related manner.
- C_{max} and $AUC_{(0\text{-}24h)}$ were decreased between day 1 and week 27 for males only.

Study no.: 1398/244
Volume #, and page #: Electronic module
Conducting laboratory and location:

Date of study initiation: 18 September 2001
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: CC-5013, lot # 61457-06, — purity.

Methods

Doses:

Group number	Group description	Group Code	Dose level (mg/kg/day)	Animals/group			
				Main study#		Satellite study##	
				Male	Female	Male	Female
1	Control	Buff	0	30	30	3	3
2	Low	Green	75	20	20	12	12
3	Intermediate	Blue	150	20	20	12	12
4	High	Pink	300	30	30	12	12

ten males and ten females with the highest numbers from the control and high dose groups were maintained for a four-week treatment-free period after completion of the 26-week treatment period.
animals for toxicokinetic analysis only. Body weight and food consumption data were recorded but not reported. Animals were killed and discarded without necropsy following completion of their blood sampling schedule.

(Excerpted from the sponsor's submission)

Species/strain: — CD ® (SD) IGSBR rats
Number/sex/group or time point (main study): 20
Route and volume: Oral by gavage, 10 mL/kg
Satellite groups used for toxicokinetics or recovery: 12
Age: 6 weeks
Weight: ♂ 117-206 g, ♀ 127-188 g

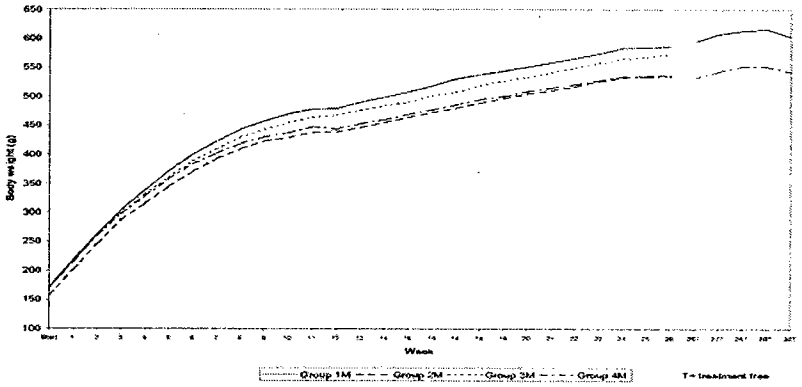
Observations and times:

Mortality: Twice daily
Clinical signs: Daily
Body weights: Weekly and before necropsy

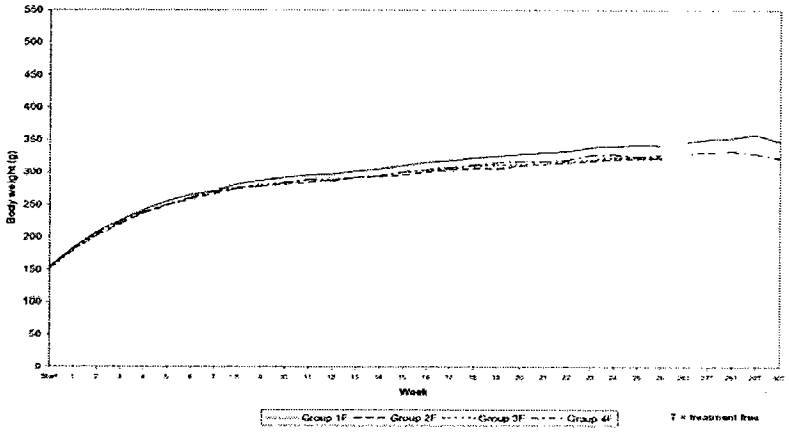
Food consumption: Weekly
Ophthalmoscopy: Pre-treatment and in week 25
EKG: Not conducted
Hematology: Weeks 12, 26, and 31
Clinical chemistry: Weeks 12, 26, and 31
Urinalysis: Weeks 12 and 25
Gross pathology: At necropsy
Organ weights: See histopathology inventory for NDA 21-880
Histopathology: See histopathology inventory
Toxicokinetics: Day 1 and in week 27 at 0.0, 0.5, 1, 2, 4, 8, 12, and 24 hours after dosing

Results:

Mortality: Group 1 – 1 ♀ Group 3 – 1 ♀ Group 4 – 1 ♂
Deaths not related to lenalidomide exposure.
Clinical signs: No drug-related clinical signs
Body weights: Group mean body weights - males



Group mean body weights - females



(Excerpted from the sponsor's submission)

Group 4 – Weight gain - ♂ 12 %↓ vs. control ♀ 8 % ↓ vs. control.
Recovered during the non-dosing phase.

Food consumption: HD ♂ - 5 % ↓ for ♂ vs. control group
Ophthalmoscopy: Unremarkable
EKG: N/A
Hematology: No dose-related changes
Clinical chemistry: HD males – ↑Bilirubin (39% vs. control at week 26)
HD females – ↑Globulin (16% vs. control at week 26)
No difference at week 31
Urinalysis: Unaffected
Gross pathology: No macroscopic findings due to treatment.
Organ weights: HD males – liver organ to body weight (9 % ↓ vs. control)

Histopathology:

Terminal Sacrifice

Group incidence of selected microscopic findings

		Males				Females			
		1M	2M	3M	4M	1F	2F	3F	4F
Tissue and finding	Level (mg/kg/day)	0	75	150	300	0	75	150	300
Liver	No. examined:	20	5	2	19	20	2	1	20
hepatocyte vacuolation		11	2	2	5	13	2	0	11
Kidney	No. examined:	20	20	20	19	20	20	19	20
pelvic mineralisation		1	3	2	5	1	7	9	15
pyelitis		1	4	7	5	0	0	1	3

(Excerpted from the sponsor's submission)

Microscopic findings in the eye, skin, sciatic nerve, spleen, pancreas, stomach, colon, adrenal, testes, epididymis, ovary, urinary bladder, thymus, lung, heart, trachea, esophagus, thyroid, pituitary, and seminal vesicle of treated animals were similar to the usual pattern of findings seen in control animals of this strain and age. There were no microscopic findings in these tissues due to test article administration.

Treatment free sacrifice: No residual treatment-related effects.

Toxicokinetics:

Dose (mg/kg/day)	Dose Ratio#	Group	Sex (n=3)	C_{max} (ng/mL)				T_{max} (hour)	
				Day 1	Day 1 ratios#	Week 27	Week 27 ratios#	Day 1	Week 27
75	1	2	M	12183.7	1	8638.6	1	1.0	1.0
150	2	3		28140.3	2.3	12281.7	1.4	2.0	1.0
300	4	4		50861.0	4.2	12311.7	1.4	4.0	2.0
75	1	2	F	15854.1	1	13923.1	1	2.0	1.0
150	2	3		20850.0	1.3	18812.8	1.4	0.5	1.0
300	4	4		30224.0	1.9	32367.6	2.3	4.0	1.0

normalised to 75mg/kg/day dose

Dose (mg/kg/day)	Dose Ratio#	Group	Sex (n=3)	$AUC_{(0-24h)}$ (ng.h/mL)			
				Day 1	Day 1 ratios#	Week 27	Week 27 ratios#
75	1	2	M	55915.3	1	55244.3	1
150	2	3		125772.5	2.2	61234.8	1.1
300	4	4		231477.5	4.1	71112.6	1.3
75	1	2	F	57804.8	1	58689.5	1
150	2	3		107566.3	1.9	76735.5	1.3
300	4	4		177129.6	3.1	164944.6	2.8

normalised to 75mg/kg/day dose

(Excerpted from the sponsor's submission)

- On day 1, C_{max} and AUC_{0-24h} were generally higher for males than females.
- On week 27, AUC_{0-24h} and C_{max} were higher for females than males.
- CC-13013 and CC-6011, the enantiomers of CC-5013, were detected in all dosed animals. The enantiomeric stability for the time the samples were stored (5 months) is not known.

Effect on cytochrome P450 enzymes: No effect (see study # 1398/311 in PK section for effect on cytochrome P450 enzymes).

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5. CC- 5013: 28 Day Oral (Gavage Administration) Toxicity Study in the Monkey. Study Number: 1398/ 108.

See Appendix A.

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6. **CC- 4047 & CC- 5013: 28 Day Oral (Gavage Administration) Toxicity Study in the Monkey. Study Number: 1398/ 126.**

See appendix A.

7. **CC- 5013: 13 Week Oral (Gavage Administration) Toxicity Study in the Monkey. Study Number: 1398/ 191.**

See appendix B

8. **CC- 5013: 52 Week Oral (Gavage) Administration Toxicity Study in the Monkey with a 7 Week Treatment free Period. Study Number: 1398/ 243.**

See Appendix B

Histopathology inventory:

Study	1398/244	1398/243
Species	Rat	Monkey
Adrenals	X*	X*
Aorta	X	X
Bone Marrow smear	X	X
Bone (femur)	X	X
Brain	X*	X*
Cecum	X	X
Cervix		
Colon	X	X
Duodenum	X	X
Epididymis	X*	X*
Esophagus	X	X
Eye	X	X
Fallopian tube		
Gall bladder		X
Gross lesions	X	X
Harderian gland	X	
Heart	X*	X*
Ileum	X	X
Injection site		
Jejunum	X	X
Kidneys	X*	X*
Lachrymal gland	X	X
Larynx	X	
Liver	X*	X*
Lungs	X	X
Lymph nodes, cervical		
Lymph nodes mandibular	X	X
Lymph nodes, mesenteric	X	X
Mammary Gland	X	X
Nasal cavity	X	
Optic nerves	X	X
Ovaries	X*	X*
Pancreas	X	X
Parathyroid	X*	X*
Peripheral nerve		

Study	1398/244	1398/243
Species	Rat	Monkey
Pharynx		
Pituitary	X*	X*
Prostate	X*	X*
Rectum	X	X
Salivary gland	X	X
Sciatic nerve	X	X
Seminal vesicles	X	X
Skeletal muscle		
Skin	X	X
Spinal cord	X	X
Spleen	X*	X*
Sternum	X	X
Stomach	X	X
Testes	X*	X*
Thymus	X	X
Thyroid	X*	X*
Tongue	X	X
Trachea	X	X
Urinary bladder	X	X
Uterus	X	X*
Vagina	X	X
Zymbal gland	X	

X, histopathology performed
*, organ weight obtained

6.6.6.4 Genetic toxicology

1. CC-5013: Reverse Mutation in four Histidine-requiring strains of Salmonella typhimurium and two Tryptophan- requiring strains of Escherichia coli. Study Number: 1398/100. See Appendix A.

   Lenalidomide did not induce mutation in Ames test at concentration up to 5,000 µg/plate with or without metabolic activation (S-9).

2. CC- 5013: Mutation at the Thymidine Kinase (tk) Locus of Mouse Lymphoma L5178Y Cells (MLA) using the — Technique. Study Number: 1398/ 95. See Appendix A.

   Lenalidomide did not increase mutant frequency at the thymidine kinase locus of mouse lymphoma cells in the presence or absence of S-9.

3. CC- 5013: Induction of Chromosome Aberrations in Cultured Human Peripheral Blood Lymphocytes. Study Number: 1398/ 118. See Appendix A.

   Lenalidomide (10 mM) did not induce chromosome aberrations in cultured human peripheral blood lymphocytes in the presence or absence of S-9.

4. CC- 5013: induction of micronuclei in the bone marrow of treated rats. Study Number: 1398/ 187. See Appendix B.

   Lenalidomide up to 2000 mg/kg/day did not induce micronuclei in the polychromatic erythrocytes of the bone marrow of treated male rats.

2.6.6.5 Carcinogenicity

Not done

2.6.6.6 Reproductive and developmental toxicology

Reviewed by: Kimberly A. Benson, Ph.D.

Fertility and early embryonic development

Study title: CCI-5013: Oral (gavage) study of fertility and early embryonic development in the rat (Segment 1).

Key study findings:

- No mortality or clinical signs related to drug treatment
- MD and HD dams showed slight increases in weight gain and food consumption in the period after dosing ceased, GD 6 –10. A significant dose-relationship in these parameters was noted.
- No significant effects on fertility/fecundity parameters
- No significant effects on sperm count, motility or incidence of abnormal sperm
- Significant decreases in sperm velocity parameters in the CC-5013 treated rats, though with no dose-relationship evident.
- No effects of treatment on testicular staging
- No effects of treatment on histopathology of the testes, epididymis, prostate or ovaries

Study no.:	Report 1398/245-D6154
Volume #, and page #:	Module 4.2.3.5.1
Conducting laboratory and location:	
Date of study initiation:	17 December 2001
GLP compliance:	Letter included and signed
QA reports:	yes (X) no ( )
Drug, lot #, and % purity:	CC-5013, batch # 61457-06

Methods

Doses:	0, 100, 300, and 500 mg/kg/day
Species/strain:	Rat; CD(SD)IGSBR
Number/sex/group:	24/sex/dose
Route, formulation, volume, and infusion rate:	Oral gavage, 1% w/v aqueous carboxymethyl cellulose; 5 mL/kg/day
Satellite groups used for toxicokinetics:	None
Study design:	Males -Dosed for two weeks prior to pairing and until euthanized during

	Week 9. Females – Dosed for two weeks prior to mating, through mating and until GD 6
Parameters and endpoints evaluated:	Mean # of estrous cycles, median pre-coital time, mating index, fertility and fecundity indices, # of corpora lutea, # of implantations, pre-implantation loss, post-implantation loss
Dose justification:	The doses were selected based on the 13-week toxicology study where 300 mg/kg was the NOEL. Additionally, the range-finding embryo-fetal study in the rat (Report 1398/236-D6154) showed doses of 300 and 500 mg/kg produced only slight reductions in mean body weight gain. The HD was expected to produce slight adult toxicity and the LD was expected to be the NOAEL.

Results

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Mortality:
None

Clinical signs:
None

Body weight:
No significant changes in body weights during the two-week treatment period, prior to mating.

During gestation, the MD and HD dams gained slightly more weight after the cessation of dosing than did the control rats and a significant dose relationship was seen between GD 6 and 10.

Group Mean Body Weight Gains During Gestation
CC-5013 Treatment Prior to Breeding and Continuing Until GD 6

Gestational Days	Control	LD 100 mg/kg	MD 300 mg/kg	HD 500 mg/kg
0-3	15.5	16.1	13.5	14.0
3-6	13.4	12.5	12.6	13.8
6-10&	16.9	16.3	19.0	20.1
10-13	18.5	17.0	18.5	17.3

& - Significant Dose Response Test for this parameter – $p<0.05$ – Trend toward increased weight gain with increased CC-5013 doses.

Food consumption:
No significant changes in food consumption were seen prior to mating.

During gestation, once dosing ceased the MD and HD females ate slightly more food than the control dams and a significant dose-related increase in food intake was seen between GD 6 and 10.

Group Mean Food Intake (g/animal/day) During Gestation CC-5013 Treatment Prior to Breeding and Continuing Until GD 6				
Gestational Days	**Control**	**LD 100 mg/kg**	**MD 300 mg/kg**	**HD 500 mg/kg**
0-3	24.2	24.2	23.9	23.7
3-6	27.0	27.1	26.9	27.3
6-10&	27.2	27.4	28.1	28.9
10-13	29.8	29.4	30.0	30.7

& - Significant Dose Response Test for this parameter– $p<0.05$ – Trend toward increased food intake with increased CC-5013 doses.

Toxicokinetics:
Not conducted

Necropsy:
No treatment related effects were seen upon necropsy

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):
Mating data – Mean number of estrous cycles during the two weeks of dosing prior to mating did not differ significantly from control.

Median precoital time – No impact of drug treatment, all rats successfully mated during the first estrous cycle after pairing.

Fertility/fecundity – Two females in the MD and HD groups were positive for copulation, based on the presence of a vaginal plug *in situ* or sperm in the vaginal washings, and later were determined to not be pregnant. The control group had no animals that were positive for mating but then not pregnant. This is not what is usually seen and the report notes that from 6 recent studies in the contract laboratory that the rate in the control group ranged from 0 to 4 females not being pregnant despite positive evidence of mating. This is not evidence of a drug-related effect on the fertility of fecundity of the test rats. The sponsor's data is recreated in the following table.

Mating Parameters Following Two Weeks Of CC-5013 Treatment To Male And Female Rats

	Control	LD 100 mg/kg	MD 300 mg/kg	HD 500 mg/kg
Number females mated	24	24	24	24
Number positive for mating	23	22	24	23
Non-pregnant with positive smear			2	2
Number actually pregnant	24	24	22	22
Number of males inducing pregnancy	24	24	22	22
Number of pregnant females	24	24	22	22
Fertility and Fecundity Index – males or females	100.0	100.0	91.7	91.7
Mating Index	100.0	100.0	100.0	95.8

Uterine/implantation data – No effect on number of corpora lutea. Significant decrease in number of implantation sites in the MD and HD rats, resulting in a significant increase in the pre-implantation loss for these groups. The sponsor notes that both the LD and control rats had higher implantation numbers and therefore lower pre-implantation loss than would normally be expected. The sponsor's data are recreated in the table below.

Uterine/Implantation Parameters Following Two Weeks Of CC-5013 Treatment To Male And Female Rats

	Control	LD 100 mg/kg	MD 300 mg/kg	HD 500 mg/kg
Number females with live embryos on GD 13	23	22	22	21
Mean number of corpora lutea/female	16.7	17.2	16.1	16.3
Mean number of implantations/female&	15.9	16.1	14.5	14.3
Pre-implantation loss&				
Mean %	4.6	6.0	10.1	12.0
Number of dams affected	13	8	14	16
Early intrauterine deaths				
Mean %	0.8	0.5	0.3	0.6
Number of dams affected	12	6	7	8
Late intrauterine deaths				
Mean %	0.3	0.2	0.2	0.3
Number of dams affected	6	4	5	3
Post-implantation loss				
Mean %	7.4	3.8	3.8	5.8
Number of dams affected	13	9	9	10
Mean number of embryos/female	14.7	15.5	14.0	13.5

& - Significant Dose Response Test for this parameter, $p<0.05$ – Trend to decreased mean # of implantations and increased pre-implantation loss with increased CC-5013 doses.

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ON ORIGINAL

Seminology data – No treatment-related effects were seen on sperm count, sperm motility, or the incidence of morphologically abnormal sperm. The velocities of the sperm from the treated males were significantly slower than that of controls, but with no dose-relationship. The sponsor indicates that the velocity in the control males was higher than expected from historical data. The sponsor's results are recreated in the table below.

Seminology Parameters Following Two Weeks Of CC-5013 Treatment To Male Rats

	Control	LD 100 mg/kg	MD 300 mg/kg	HD 500 mg/kg
Number of males examined	24	24	24	24
Mean total sperm count (10^6/mL)	25.5	22.1	25.5	24.5
Mean % motile	92	90	91	92
Mean VAP (µm/s)	190.4	171.9***	174.7*	171.3**
Mean VSL (µm/s)	136.4	122.7**	124.6**	123.0**
Mean VCL (µm/s)	336.4	304.7***	309.1*	303.0**
Mean STR (%)	70	71	70	71
Mean abnormal sperm (%)	1.5	---	---	1.1

* - Significant effect, $p<0.05$, **$p<0.01$, ***$p<0.001$
VAP – average path velocity VSL – straight line velocity VCL – curvilinear velocity
STR – straightness (VSL:VAP)

Testis staging – There were no treatment-related findings on testicular staging in the testes of the 5 control and 5 HD male rats examined.

Necropsy – No significant treatment-related findings in the histopathology of the testes, epididymis, prostate or ovaries.

Conclusions:

- This study is not acceptable. Should an indication be sought for this drug where this study was required, this study would need to be redone with appropriate dosing levels.

Embryofetal development

Study title: Oral pilot study for effects on embryo-fetal and postnatal development in the rat.

Key study findings:

- Preliminary dose-range finding study
- No mortality or drug-related clinical signs
- No effects of drug treatment on pregnancy rate, corrected body weight gains, or food intake
- Decreased mean gravid uterus weights as the CC-5013 doses increased
- Decreased corpora lutea and implantation sites in MD and HD dams
- Slightly higher percentage of post-implantation loss in HD group, due to skewing of data by one dam with 2 pups in her litter, one which was an early intrauterine death
- Smaller litter weights in the HD group also due to the rat with 1 viable pup in the litter as well as a dam with only 5 pups in the litter
- Minimal effects seen in this study were likely attributable to the small group numbers and a couple of dams whose data skewed the results

Study no.:	1398/236-D6154
Volume #, and page #:	Module 4.2.3.5.2
Conducting laboratory and location:	
Date of study initiation:	11 June 2001
GLP compliance:	Letter included and signed
QA reports:	Yes (X) no ( )
Drug, lot #, and % purity:	CC-5013-5013, batch # 61246-04,

Methods

Doses:	0, 100, 300, and 500 mg/kg/day
Species/strain:	Rat: – CD(SD)IGSBR
Number/sex/group:	7 females/dose (control = 6 females)
Route, formulation, volume, and infusion rate:	Oral gavage, 1% w/v aqueous carboxymethyl cellulose; 5 mL/kg/day
Satellite groups used for toxicokinetics:	None
Study design:	Females were mated by the supplier prior to delivery to the laboratory on GD 3. Rats were dosed on GD 6-17, inclusive, then euthanized on GD 20.
Parameters and endpoints evaluated:	Mortality and clinical signs, maternal body weights and food consumption, pregnancy rate, gravid uterus weight, #

of corpora lutea, pre- and post-implantation loss, resorptions, # of live and dead fetuses, early and late intrauterine deaths, pup sex, pup body weights, placental weight litter size, external fetal exams

Dose justification: The doses were selected based on the 13-week toxicology study where 300 mg/kg was the NOEL.

Results

Mortality (dams):
No mortality

Clinical signs (dams):
No treatment-related clinical signs

Body weight (dams):
There was a significant decrease in the body weights of the MD and HD dams from the start of drug administration until the end of the study on GD 20. However when the body weights were corrected for the weight of the gravid uterus, the groups are similar to control. The significant decrease was, therefore, due to the smaller gravid uteri weights in the MD and HD groups.

Gestational Body Weight Parameters In Gravid Rats Treated With CC-5013

	Control	LD 100 mg/kg	MD 300 mg/kg	HD 500 mg/kg
Mean body weight (g) - GD 6	224.6	226.7	226.7	226.8
Mean body weight (g) – GD 20	355.4	360.8	345.1	337.1
Mean body weight change – GD 6-20	130.8	134.1	118.4	110.3
Percent change from control in mean body weight gains GD 6-20	--	+2.5	-9.5	-15.7
Mean gravid uterus weight (g) GD 20	77.4	73.6	68.3	57.3
Mean corrected body weight (g)	278.0	287.2	276.8	279.8
% Body weight change (corrected) GD 6-20	24.6	26.8	22.2	23.5

Food consumption (dams):
No treatment-related effects seen on food consumption.

Toxicokinetics:
Not conducted

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):
No statistically significant treatment-related effects were seen on the caesarian and fetal parameters examined in this study. The mean number of corpora lutea, and therefore

implantations, was lower in the HD dams than they were in the other groups. As the animals were dosed beginning on GD 6 and implantation occurs on GD 5, the sponsor postulates that this can not be a drug-related effect. The ICH S5A document, however, states that implantation can be assumed to occur on GD 6-7, which would not eliminate a possible effect of CC-5013 on this parameter. An increased percentage of post-implantation loss is seen in the HD dams also, due primarily to one dam that had only two implantations and one was an early intrauterine death. This animal's 50% post-implantation loss skews the data, especially given the small sample size used in this preliminary study. Mean litter weights are also impacted by this one HD rat with only one viable pup, and another HD dam had only 5 viable pups in her litter, thus making the mean litter weight in that group appear much lower. Again, the very small group sizes in this study would have impacted that. The small group sizes may also have caused the differences seen in the percent of fetuses that were male, as there is no dose-relationship noted, just random differences among groups. The sponsor's data is recreated in the table below.

Caesarian And Fetal Parameters For Gravid Rats Dosed With CC-5013 From GD 6-17

Parameter	Dose			
	Control	100 mg/kg/day	300 mg/kg/day	500 mg/kg/day
Number of females with live fetuses$	6	7	6	6
Mean # of corpora lutea/female	15.5	13.3	13.2	10.8
Mean # of implantations/female	14.0	12.7	12.7	10.0
Pre-implantation loss				
Mean %	8.5	3.9	4.0	8.0
Number of dams affected	3	3	2	2
Early intrauterine deaths				
Mean %	1.0	0.6	1.0	0.5
Number of dams affected	4	3	4	3
Late intrauterine deaths				
Mean %	0.2	0.0	0.2	0.0
Number of dams affected	1	0	1	0
Post-implantation loss				
Mean %	8.2	4.8	9.9	12.4
Number of dams affected	5	3	4	3
Dead fetuses				
Mean %	0.0	0.0	0.0	0.0
Number of dams affected	0	0	0	0
Mean # of fetuses/female	12.8	12.1	11.5	9.5
Mean # of male fetuses	39	38	38	25
Mean # of female fetuses	38	47	31	32
% of fetuses that were male	50.7	44.9	55.6	48.1
Mean litter weight (g)	50.10	46.86	45.44	36.37
Mean placental weight (g)	0.52	0.55	0.50	0.63
Mean fetal weight (g)	3.93	3.86	3.96	3.93
Mean fetal weight – males (g)	4.05	4.02	4.06	3.99
Mean fetal weight – females (g)	3.81	3.71	3.81	3.80

$ - Only 6 rats were in the control group and 1 rat each in the MD and HD groups were not pregnant despite positive signs of mating

Offspring (malformations, variations, etc.):
External exams only were conducted. No abnormalities were noted upon exam.

Conclusions:
- ❖ No maternal toxicity noted – body weight gains, when corrected for the gravid uterus, did not show a clear impact of CC-5013 administration.
- ❖ No clear evidence of embryo-fetal effects of CC-5013 as the numbers are low and two HD litters with very small litter numbers skew some of the parameters.
- ❖ Sponsor claims no maternal or embryo-fetal toxicity at 500 mg/kg, making it a suitable HD level for the pivotal rat study. In fact, a higher dose for the HD level would be preferable, one that exhibited sufficient toxicity to ensure that a potential drug effect is not being missed due to insufficient dosing.

Study title: CC-5013: Oral (gavage) study of embryo-fetal development in the rat.

Key study findings:
- Minimal maternal toxicity seen – food intake and body weights lower in the first couple days of dosing only
- Significant decreased placental weights seen in treatment groups, though with no dose-relationship
- No significant treatment effects on pregnancy or caesarian parameters
- No treatment-related skeletal or visceral malformations

Study no.:	1398/237-D6154
Volume #, and page #:	Module 4.2.3.5.2
Conducting laboratory and location:	
Date of study initiation:	29 January 2002
GLP compliance:	Letter included and signed
QA reports:	yes (X) no ( )
Drug, lot #, and % purity:	CC-5013, batch # 61457-06, [illegible]

Methods

Doses:	0, 100, 300, and 500 mg/kg/day
Species/strain:	Rat; [illegible]CD(SD)IGSBR
Number/sex/group:	24 females/dose
Route, formulation, volume, and infusion rate:	Oral gavage, 1% w/v aqueous carboxymethyl cellulose; 5 mL/kg/day
Satellite groups used for toxicokinetics:	None
Study design:	Females were mated by the supplier prior to delivery to the laboratory on GD

	3. Rats were dosed on GD 6-17, inclusive, then euthanized on GD 20.
Parameters and endpoints evaluated:	Mortality and clinical signs, maternal body weights and food consumption, pregnancy rate, gravid uterus weight, # of corpora lutea, pre- and post-implantation loss, resorptions, # of live and dead fetuses, early and late intrauterine deaths, pup sex, pup body weights, placental weight litter size, external fetal exams
Dose justification:	The doses were selected based on the 13-week toxicology study where 300 mg/kg was the NOEL. Additionally, the range-finding embryo-fetal study in the rat (Report 1398/236-D6154) showed doses of 300 and 500 mg/kg produced only slight reductions in mean body weight gain. The HD was expected to produce slight adult toxicity and the LD was expected to be the NOAEL.

Results

Mortality (dams):
1 LD rat found dead on GD 9
1 LD rat euthanized moribund on GD 14 – labored respiration noted
Necropsy showed that both rats were likely dosed incorrectly

No other morbidity or mortality

Clinical signs (dams):
No treatment-related maternal clinical signs

Body weight (dams):
There was a slight dose-related decrease in body weight gain on the first day of CC-5013 dosing, GD 6-7, with statistical significance reached with the HD compared to control. The lower body weight gains in the HD dams was also significantly different from control between GD 8 and 9.

Gestational Body Weight Parameters In Gravid Rats Treated With CC-5013.

	Control	LD 100 mg/kg	MD 300 mg/kg	HD 500 mg/kg
Body weight changes GD 6-7	6.1	5.4	4.1	3.2*
Body weight changes GD 8-9	8.0	5.5	6.2	4.1**
Mean body weight (g) - GD 6	261.9	262.3	261.8	264.4
Mean body weight (g) – GD 20	390.0	381.8	383.8	383.4
% body weight change – GD 6-20	49.3	45.8	47.0	45.2
Mean gravid uterus weight (g) GD 20	74.7	76.9	74.5	70.9
Mean corrected body weight (g)	315.3	304.9	309.3	312.5
% Body weight change (corrected) GD 6-20	20.7	16.5	18.5	18.5

Food consumption (dams):
There was a significant dose-related decrease in food consumption over the first day of dosing only. During all other time points the food intake was comparable across groups.

Food Intake (g/rat/day)

	Control	LD	MD	HD
Day 6-7&	25.7	25.3	24.6	24.0

& - Significant Dose Response for this parameter– $p<0.05$ – Trend to decreased food intake with increased CC-5013 doses.

Toxicokinetics:
Not conducted in this study, but the data exist from general toxicology studies.

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Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

The sponsor's data is recreated in the table below. The only significant effect seen was a decrease in the placental weights. There was no dose-relationship with this effect and the differences, though significant, were small enough to likely be of no consequence to the fetuses, especially given that no significant effect was seen on fetal weights, though the HD mean fetal weight was slightly lower than control. The LD dams had a significant increase in pre-implantation loss, but the lack of any dose-relationship indicates that this was unlikely to be due to the drug administration.

Caesarian And Fetal Parameters For Gravid Rats Dosed With CC-5013 From GD 6-17

Parameter	Dose			
	Control	100 mg/kg/day	300 mg/kg/day	500 mg/kg/day
Number of females with live fetuses	24	22	24	23
Mean # of corpora lutea/female	15.2	16.5	16.9	15.1
Mean # of implantations/female	13.4	13.7	13.8	12.7
Pre-implantation loss				
Mean %	12.1	15.7	16.0	16.9
Number of dams affected	14	19*	18	18
Early intrauterine deaths				
Mean %	0.8	0.2	0.8	0.3
Number of dams affected	13	4	11	6
Late intrauterine deaths				
Mean %	0.0	0.1	0.0	0.0
Number of dams affected	0	2	1	0
Post-implantation loss				
Mean %	6.6	2.3	5.5	2.3
Number of dams affected	13	6	11	6
Dead fetuses				
Mean %	0.0	0.0	0.0	0.0
Number of dams affected	0	0	0	0
Mean # of fetuses/female	12.5	13.4	13.0	12.4
Mean # of male fetuses	164	154	157	149
Mean # of female fetuses	137	140	155	137
% of fetuses that were male	53.0	52.6	50.5	52.9
Mean litter weight (g)	47.92	50.89	49.54	46.67
Mean placental weight (g)	0.54	0.50*	0.49***	0.52*
Mean fetal weight (g)	3.83	3.82	3.82	3.78
Mean fetal weight – males (g)	3.94	3.94	3.94	3.89
Mean fetal weight – females (g)	3.72	3.69	3.71	3.65

* - Statistically different from control - * $p<0.05$, ** $p<0.01$, *** $p<0.001$

Offspring (malformations, variations, etc.):

The sponsor's data is recreated in the table below, showing the incidence of external/visceral and skeletal malformations and variations. No significant effects of drug treatment were seen on the number of litters affected or the percent of fetuses affected.

Caesarian And Fetal Parameters For Gravid Rats Dosed With CC-5013 From GD 6-17				
Parameter	**Dose**			
	Control	100 mg/kg/day	300 mg/kg/day	500 mg/kg/day
External/Visceral Defects				
Number of fetuses examined	301	294	312	286
Number of litters examined	24	22	24	23
Number showing malformations	3	2	2	2
Mean % of fetuses affected	1.0	0.6	0.5	0.7
Number of litters affected	3	2	2	2
Number showing variations	26	34	36	20
Mean % of fetuses affected	9.3	11.9	11.9	9.1
Number of litters affected	18	18	17	15
Skeletal Defects				
Number of fetuses examined	150	147	156	142
Number of litters examined	24	22	24	23
Number showing malformations	3	1	1	0
Mean % of fetuses affected	1.8	0.5	0.5	0.0
Number of litters affected	3	1	1	0
Number showing variations	139	134	145	133
Mean % of fetuses affected	92.9	90.9	91.8	94.2
Number of litters affected	24	22	24	23
Total Malformations				
Number showing malformations	5	2	2	2
Mean % of fetuses affected	1.7	0.7	0.6	0.7
Number of litters affected	5	2	2	2

The sponsor's table showing the malformations that were noted in each group is recreated below. No significant drug effect was seen on the malformations in the offspring.

Fetal Malformations Noted In Rats After Maternal Exposure To CC-5013	
Dam/Fetus #	**Defect**
Control	
4/R6	Small kidney
15/L5	Small eye
20/R2	Cleft, fused and misshapen sternebra(e)
23/R2	Lumbar vertebral arch displaced
24/L9	Small kidneys, cleft palate, shortened tibias, fibulas and femurs, malrotated hindlimbs, pelvic girdle structures disorganized, absent palatines, fused sternebrae, umbilical hernia, shortened trunk, splayed lumbar vertebral arches, absent vertebra
LD – 100 mg/kg/day	
34/L6	Abnormal texture of lens of eye
48/R7	Shortened tail, non-patent anus, absent caudal vertebrae, misshapen lumbar vertebral arch, absent lumbar vertebral centrum, absent lumbar vertebrae, absent sacral vertebrae
MD – 300 mg/kg/day	
50/R2	Abnormal pathway of ascending aorta/aortic arch, cleft sternebrae
59/L5	Abnormal texture of lens of eye
HD – 500 mg/kg/day	
89/R1	Distended ureter, hydronephrosis
92/L8	Small testis

Conclusions:

- Slight transient maternal effects of CC-5013 on body weight gains and food intake, seen only in the first couple of days of dosing.
- Decreased placental weights in all CC-5013 dose groups, though no dose-response relationship and no significant effects seen on fetal weights. There does not appear to be any toxicological significance of the placental effects.
- No other embryo-fetal effects of CC-5013 treatment.
- No effects on malformations seen.
- The fact that historical data tells us that the rat model is not sensitive to the full range of thalidomide's teratogenic effects makes this an inadequate model to examine the full range of CC-5013's developmental effects.

Study title: CC-5013: Oral (Gavage) range-finding study of embryo-fetal development in the rabbit.

Key study findings:

- Morbidity/mortality seen at all dosing levels of CC-5013.
- At the highest dose tested, 150 mg/kg/day, no live litters were available by GD 29 for evaluation.
- Only 1 litter for evaluation in MD group and 2 litters in the LD group.
- Premature deaths/euthanized rabbits not examined for effects.
- Drug treatment increased maternal toxicity as evidenced by decreased body weights and food intake and by lethality and increased aborted litters.
- The lack of sufficient dams that survived through dosing until GD 29 with live litters for evaluation indicates that all doses tested in this study are too high for use in the pivotal rabbit embryo/fetal development study.

Study no.:	1398/225-D6154
Volume #, and page #:	Module 4.2.3.5.2
Conducting laboratory and location:	
Date of study initiation:	13 May 2001
GLP compliance:	GLP letter included and signed
QA reports:	yes (X) no ( )
Drug, lot #, and % purity:	CC-5013, batch # 61246-04, purity

Methods

Doses:	50, 100, 150 mg/kg/day
Species/strain:	Rabbit/New Zealand White (NZW/Kbl BR)
Number/sex/group:	7 females/dose
Route, formulation, volume, and infusion rate:	Oral gavage, carboxymethyl cellulose formulation, 5 mL/kg volume
Satellite groups used for toxicokinetics:	None
Study design:	Dosing on GD 7-19 inclusive, euthanized on GD 29
Parameters and endpoints evaluated:	Clinical signs, morbidity/mortality, body weight, food consumption, pregnancy status, gravid uterus weight, # of corpora lutea, number and intrauterine position of implantations by subgroups: live fetuses, early intrauterine deaths, late intrauterine deaths, and dead fetuses.

Dose justification: Based on a preliminary study (Report 1398/224-D1654) not presented here where non-pregnant rabbits were dosed and 300 mg/kg produced marked toxicity in terms of body weight loss and 150 mg/kg led to a slight reduction in body weight gain. This dose was chosen as the HD for the range-finding study in pregnant rabbits.

Results

Mortality (dams):
Control – 0/7 deaths
50 mg/kg – 1/7 (14%) – found dead on GD 29
100 mg/kg – 1/7 (14%) – euthanized moribund on GD 28
150 mg/kg – 3/7 (43%) – 1 euthanized moribund on GD 26, 2 found dead GD 27 and 29

Aborted litters:
Control – 0/6 aborted litters
50 mg/kg – 2/5 (40%) – including the animal found dead, both on Day 29
100 mg/kg – 3/5 (60%) – GD 20, 27 and 28
150 mg/kg – 3/6 (50%) – GD 27 and 29

Pregnancy Parameters in New Zealand White Rabbits Following Treatment With CC-5013

	Control	LD 50 mg/kg	MD 100 mg/kg	HD 150 mg/kg
Total # per dose	7	7	7	7
Not pregnant	1	2	2	1
Pregnant (%)	6 (86)	5 (71)	5 (71)	6 (86)
Died/euthanized moribund	0	0	1	3
Died and aborted litter	0	1	0	0
Aborted & euthanized	0	1	3	3
With total fetal/embryo loss	0	1	0	0
With evaluable litters (%)	6 (86)	2 (40)	1 (20)	0 (0)

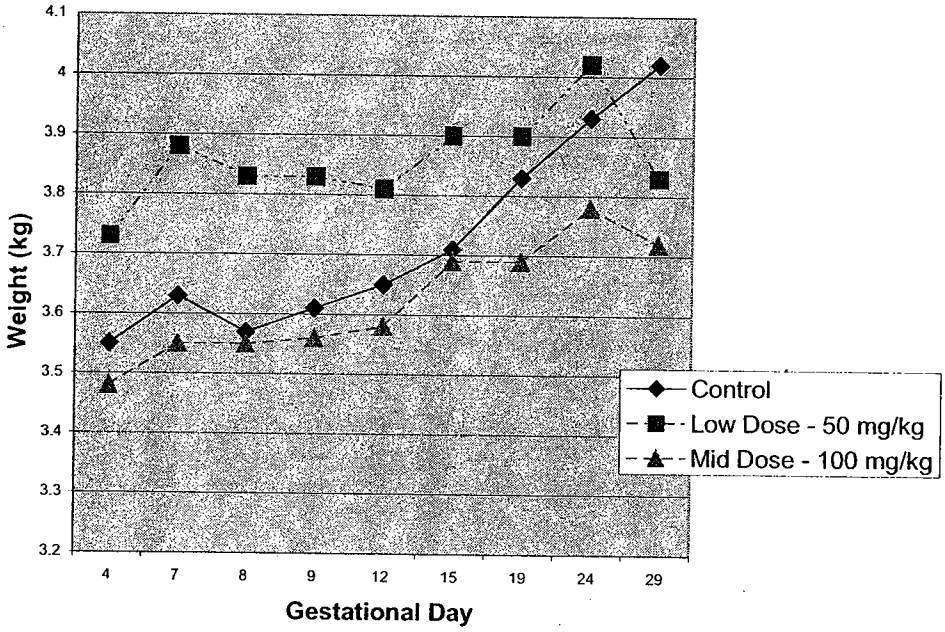
Clinical signs (dams):

No treatment-related clinical signs were seen in the dams that survived with evaluable litters.

Body weight (dams):

The graph below shows the body weights of the pregnant does that survived with evaluable litters. The two treated groups show less weight gain in the latter part of gestation than the control group, though it should be noted that the 100 mg/day group consists of just one rabbit.

Body Weights of Gravid Rabbits With CC-5013 Administration on GD 7-19 Inclusive



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When body weights were corrected for the gravid uterus weight, a significant difference in maternal body weights was still noted in the LD and MD rabbits. The following table, reconstructed from the sponsor's information, compares the body weights, body weight changes and corrected body weights of the dams in the control, LD and MD groups. The two rabbits in the LD group were equally affected in the body weight change (corrected) parameter, as one had a loss of 18.0 grams and the other lost 15.2 grams. Again, note that the MD group consists of one rabbit that survived to GD 29 with an evaluable litter.

Gestational Body Weight Parameters In Gravid Rabbits Treated With CC-5013.

	Control	LD 50 mg/kg	MD 100 mg/kg
Mean body weight - GD 7	3.63	3.89	3.55
Mean body weight – GD 29	4.02	3.84	3.72
% Body weight change – GD 7 - 29	10.7	-1.2	4.8
Mean gravid uterus weight on GD 29	0.47	0.59	0.62
Mean corrected body weight	3.56	3.25	3.10
% Body weight change (corrected)	-2.2	-16.6	-12.7

Food consumption (dams):

Food intake was similar in all groups during treatment. Once the treatment period ended the LD and MD dams ate less food than the controls. The mean intake of food for the two CC-5013 treated groups during the period of GD 19-29 was 37-40% less than the intake in the control rabbits.

Food Intake in Gravid Rabbits Treated with CC-5013.

	Control	LD 50 mg/kg	MD 100 mg/kg
GD 4-7	144	190	123
GD 7-8	141	142	149
GD 8-9	141	161	155
GD 9-12	144	135	143
GD 12-15	126	137	137
GD 15-19	155	136	124
GD 19-24	175	132	127
GD 24-29	147	70	67
Mean intake (g/rabbit/day) GD 4-29	150	130	119
Mean intake (g/rabbit/day) GD 7-19	143	139	137
Mean intake (g/rabbit/day) GD 19-29	161	101	97

Toxicokinetics:
Not conducted

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

Caesarian and fetal data for the dams and litters that were available for evaluation by the termination day of the study are recreated from the sponsor's data below. No litters were alive for evaluation in the HD group. The two remaining groups had only 3 litters with live fetuses for evaluation by the end of the study. The data show that although there were fetal losses in the control group (pre- and post-implantation loss as well as intrauterine deaths) there were considerably more post-implantation losses noted in the 2 LD litters.

Uterine/Implantation Data For Gravid Female Rabbits Treated With CC-5013

	Control	LD 50 mg/kg	MD 100 mg/kg	HD 150 mg/kg
Number of females with live fetuses at GD 29	6	2	1	0
Mean # corpora lutea/female	9.8	15.5	13^	
Mean # implantations/female	8.2	12.5	13^	
Pre-implantation loss				
Mean %	17.2	19.8	---	
Dams affected	5	2	0	
Early intrauterine deaths				
Mean #	0.3	1.0	5^	
Dams affected	2	2	1	
Late intrauterine deaths				
Mean #	0.3	0.5	1^	
Dams affected	1	1	1	
Dead fetuses				
Mean #	0.0	1.5	5^	
Dams affected	0	1	1	
Post-implantation loss				
Mean %	8.8	28.4	84.6^	
Dams affected	3	2	1	
Mean # fetuses/female	7.5	9.5	2^	

^ - Not a mean, as the N=1, actual number from that litter

Fetal Data For Litters of Gravid Female Rabbits Treated With CC-5013

	Control	LD 50 mg/kg	MD 100 mg/kg	HD 150 mg/kg
Number of male fetuses	25	10	2	---
Number of female fetuses	20	9	0	---
% male fetuses	53.8	55.0	100.0	---
Mean litter weight (g)	318.3	303.0	71.8	---
Mean placental weight (g)	6.15	6.39	6.99	---
Mean fetal weight (g)	43.4	32.7	35.9	---
Mean fetal weight – males (g)	44.2	32.4	35.9	---
Mean fetal weight – females (g)	42.8	33.1	---	---

Offspring (malformations, variations, etc.):
While the offspring were only examined for gross malformations, none were noted that were considered treatment related. There were only 3 litters of live fetuses in the drug-treatment groups, however, and the only MD litter had only 2 offspring.

Conclusions:
- ❖ This dose range-finding study utilized doses that were too high to support a pregnancy to parturition.
- ❖ Mortality was seen at all doses, as well as aborted litters. Animals that aborted were excluded from further analysis, though necropsy data was collected.
- ❖ Food intake was only affected after the drug administration concluded. Decreased food intake for the 2 LD rabbits and 1 MD rabbit during the GD 19-29 time period. Food intake during the GD 7-19 time period is comparable in all dose groups.
- ❖ Too few animals with viable litters to get a clear effect of CC-5013 on pregnancy, uterine/implantation and fetal data.
- ❖ No NOAEL dose established in this study.

Study title: CC-5013: Oral (Gavage) study of embryo-fetal development in the rabbit.

Key study findings:
- CC-5013 was detectable in plasma samples of all dose groups.
- Several deaths were seen in the study, but did not have a CC-5013 relationship.
- Body weights affected by the MD and HD CC-5013 treatment and to a greater magnitude by thalidomide treatment.
- Food consumption was affected by MD and HD CC-5013 treatment as well as by thalidomide.
- Structural malformations were clearly present in the thalidomide group and were indicative of that treatment, confirming the positive control for this assay.
- Similar limb malformations were NOT seen in the CC-5013 treated groups.
- While there does appear to be a trend toward increased structural malformations in the CC-5013 treated groups, this is not statistically significant and no statistically significant dose-response relationship was seen.
- Increased variations seen in the CC-5013 treated groups, though overall this wasn't statistically significant and it was due to a number of minor defects, some of which were significant.

Study no.: 1398/226-D6154
Volume #, and page #: Module 4.2.3.5.2
Conducting laboratory and location:

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Date of study initiation:	16 July 2001
GLP compliance:	GLP letter included and signed
QA reports:	yes (X) no ( )
Drug, lot #, and % purity:	CC-5013, batch # 61246-04, purity Thalidomide, batch #574-574-00-013, purity
Methods	
Doses:	5, 15, and 25mg/kg/day 250 mg/kg/day thalidomide – positive control
Species/strain:	Rabbit/New Zealand White :NZW/Kbl BR)
Number/sex/group:	24 females/dose 5 females/dose of thalidomide
Route, formulation, volume, and infusion rate:	Oral gavage, carboxymethyl cellulose formulation, 5 mL/kg volume
Satellite groups used for toxicokinetics:	Toxicokinetics conducted on study rabbits
Study design:	Dosing on GD 7-19 inclusive, euthanized on GD 29
Parameters and endpoints evaluated:	Clinical signs, morbidity/mortality, body weight, food consumption, pregnancy status, gravid uterus weight, # of corpora lutea, number and intrauterine position of implantations by subgroups: live fetuses, early intrauterine deaths, late intrauterine deaths, dead fetuses, fetal examinations for skeletal and visceral malformations.
Dose justification:	Dose levels were selected based on the range finding study (Report 1398/225-D6154) reviewed above. The sponsor chose 25 mg/kg as the HD in this study due to the abortions late in gestation seen with the LD of 50 mg/kg in the range-finding study. The thalidomide dose was chosen, as it is known to cause teratogenic effects when administered to rabbits during organogenesis.

Results

Mortality (dams):
Control – 4/24 deaths
- one found dead, inappetence and weight loss, not pregnant. Necropsy showed small spleen, firm and mottled liver, no cause of death determined.
- one found dead, GD 17, necropsy indicated rabbit was misdosed
- one died, GD 14, while under anesthetic to remove part of feeding catheter that had been bitten off and swallowed
- euthanized on GD21 after continued inappetence and severe weight loss

5 mg/kg – 1/24– found dead on GD 17, necropsy indicated rabbit was dosed incorrectly
15 mg/kg – 1/24 – found dead on GD 29, stopped eating, weight loss, no cause of death
25 mg/kg – 0/24
Thalidomide – 0/5

Aborted litters:
Control – 0/22 aborted litters
5 mg/kg – 1/23 (4.3%) – on GD 14
15 mg/kg – 0/24
25 mg/kg – 1/22 (4.5%) – on GD 29
Thalidomide – 0/5

Pregnancy Parameters in New Zealand White Rabbits Following Treatment With CC-5013

	Control	LD 5 mg/kg	MD 15 mg/kg	HD 25 mg/kg	Thalid. 250 mg/kg
Total # per dose	23*	24	24	24	5
Not pregnant	1**	1	0	2	0
Pregnant (%)	22 (95.7)	23 (95.8)	24 (100.0)	22 (91.7)	5 (100.0)
Died/euthanized moribund	3	1	1	0	0
Aborted & euthanized	0	1	0	1***	0
With total fetal/embryo loss	0	0	2	3	0
With evaluable litters (%)	19 (86.4)	21 (91.3)	21 (87.5)	18 (81.8)	5 (100)

* - One animal with fractured tooth, not eating, euthanized before dosing began
** - Euthanized moribund
*** - Euthanized on GD 29

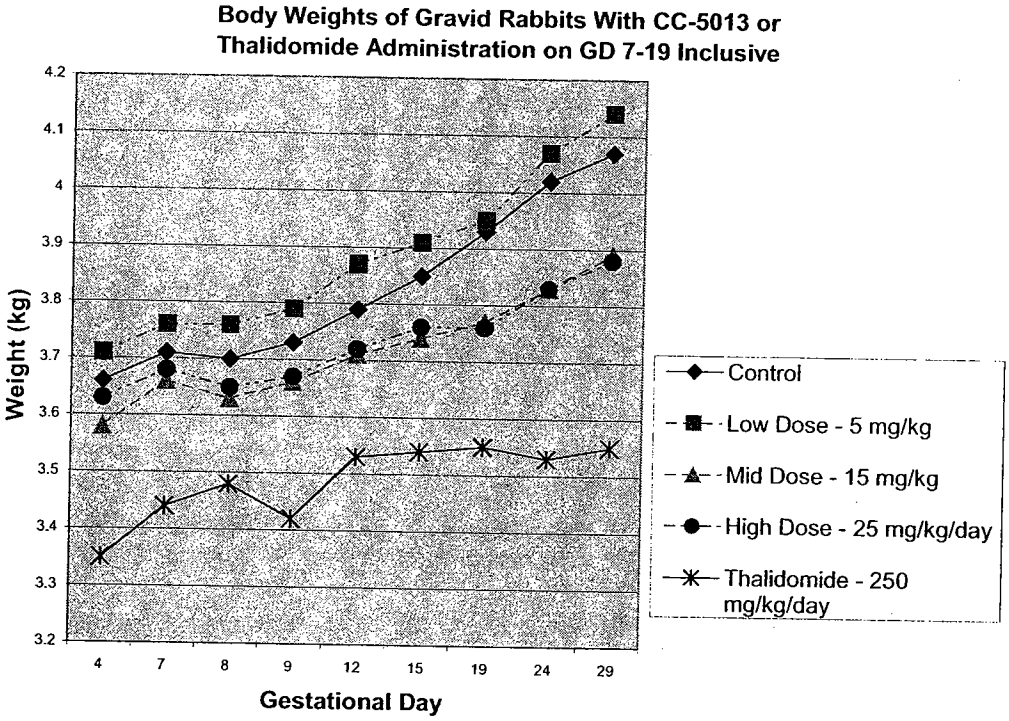
Clinical signs (dams):

The only clinical sign seen in the treated groups with more frequency than the control was few or no feces. This occurred in 1/23 control rabbits, 4/24 in both the LD and MD group, 2/24 in the HD group and 3/5 in the thalidomide. As constipation is a known toxicity of thalidomide, it is not surprising that during treatment over half of these rabbits

had little or no feces. The majority of all the noted instances occurred during the drug treatment period. The lack of a dose-response relationship and the lower incidence in the CC-5013 groups makes this clinical sign of little significance.

Body weight (dams):

The graph below shows the body weights of the pregnant does that survived until the end of the study with evaluable litters. The thalidomide rabbits began the study weighing significantly less than the control rabbits. The change in their weights in the period of GD 8-9 and again during GD 19-24 were significantly different from control ($p < 0.001$ and $p < 0.01$, respectively). The HD CC-5013 group body weight change was different from control during the period of GD 15-19, $p < 0.01$. A statistical test for a dose response relationship with the three dose groups of CC-5013 compared to control was significant, $p < 0.05$.



When body weights were corrected for the gravid uterus weight, the thalidomide group was significantly different from control, $p<0.05$. The following table, reconstructed from the sponsor's information, compares the body weights, body weight changes and corrected body weights of the dams with evaluable litters at GD 29. The mean gravid uterus weights of the thalidomide rabbits was slightly lower than the control, while the CC-5013 groups mean gravid uterus weights were not affected by treatment. While the MD and HD CC-5013 group dams do show an effect of drug treatment on body weights, % body weight change and corrected body weight, it is not quite the same magnitude as that seen with thalidomide.

Gestational Body Weight Parameters In Gravid Rabbits Treated With CC-5013 or Thalidomide.

	Control	LD 5 mg/kg	MD 15 mg/kg	HD 25 mg/kg	Thalid. 250 mg/kg
	19	21	21	18	5
Mean body weight - GD 7	3.71	3.76	3.66	3.68	3.43
Mean body weight – GD 29	4.07	4.14	3.89	3.88	3.55
% Body weight change – GD 7 – 29	10.0	10.4	6.5	5.8	3.4
Mean gravid uterus weight on GD 29	0.52	0.55	0.48	0.52	0.43
Mean corrected body weight	3.56	3.59	3.41	3.37	3.12*
% Body weight change (corrected)	-3.9	-4.4	-6.8	-8.3	-9.1

* - Significantly different from Control, $p< 0.05$

Food consumption (dams):

It is important to note that food intake was severely affected in several rabbits before the study began. Two control, 3 LD, 4 MD and 3 HD rabbits all did not eat for the first week after arriving in the laboratory. They were fed a diet paste to encourage eating. These rabbits all had some form of adverse outcome in the study and the contribution of the lack of eating can not be discounted.

Outcome For Rabbits Who Did Not Eat For First Week in the Laboratory - Prior To Study

	Control	LD 5 mg/kg	MD 15 mg/kg	HD 25 mg/kg
Number that did not eat	2	3	4	3
Euthanized due to weight loss	2	1		
Aborted litter		1		1
Resumed eating, but total fetal loss at term			2	1
High levels of post-implantation loss		1	2	1

The table below recreates the sponsor data. Both thalidomide and CC-5013 administration adversely affected food intake in the rabbits with surviving litters on GD 29. Significantly less food was consumed in the MD and HD CC-5013 groups between GD 15 and 24. Regression analysis showed that even during the GD 8-15 and GD 24-

29 periods, though the food intake was not significantly different from control in the CC-5013 groups, there was a significant decreasing dose-response. The thalidomide rabbits consumed significantly less food than control from GD7-12 and then again from GD 19-24. The thalidomide impact on food intake is somewhat different than the CC-5013 effect. Thalidomide affects food intake as early as the first day of dosing and the impact is seen to a greater magnitude in the 5 days after dosing has concluded. This is also evident when looking at the mean food intake from the end of treatment until the study termination, GD 19-29. The mean food intake during the dosing period, GD 7-19, shows a greater impact of CC-5013 than thalidomide. This is the same effect that was seen in the range-finding study. In that study, the decreased food intake was primarily seen after the end of the drug administration period.

Food Intake in Gravid Rabbits Treated with CC-5013.

	Control	LD 5 mg/kg	MD 15 mg/kg	HD 25 mg/kg	Thalid. 250 mg/kg
GD 4-7	162	151	145	140	142
GD 7-8	158	147	134	126	93*
GD 8-9&	174	166	144	142	109**
GD 9-12&	175	160	154	140	143*
GD 12-15&	152	131	119	116	161
GD 15-19	166	137	128*	107**	152
GD 19-24	152	147	126*	113**	97**
GD 24-29&	111	116	100	92	92
Mean intake (g/rabbit/day) GD 4-29	151	140	127	116	124
Mean intake (g/rabbit/day) GD 7-19	165	145	134	122	143
Mean intake (g/rabbit/day) GD 19-29	132	131	113	103	94

* - $p<0.05$

** - $p<0.01$

& - Significant dose response test for this parameter, control group vs. LD, MD, HD – $p<0.05$ – Trend to decreased food intake with increased CC-5013 doses.

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Toxicokinetics:

Blood was obtained from four rabbits in each of the treatment groups of GD 7 and GD 19 at the 0, 1, 2, 4, 8 and 24 hr time points. CC-5013 was measurable in all treatment groups on both sampling days. Given the plasma half-life of CC-5013 seen in other studies (3 hrs in humans, 1-2 hrs in rat, dog, and monkey) there is no reason to think that any drug would accumulate and the levels would be significantly high at GD 29. Toxicokinetic parameters are presented in the sponsor's table below.

CC-5013

Dose group	Dose (mg/kg/day)	C_{max} (ng/mL)		$AUC_{(0-24h)}$ (ng.h/mL)		T_{max} (hour)	
		Day 7	Day 19	Day 7	Day 19	Day 7	Day 19
2	5	1114.7	751.0	-	-	1.0	1.0
3	15	3873.3	2042.8	9103.2	6468.7	1.0	1.0
4	25	7832.5	3750.9	19240.9	18590.6	1.0	1.0

- insufficient data to calulate $AUC_{(0-24h)}$ values

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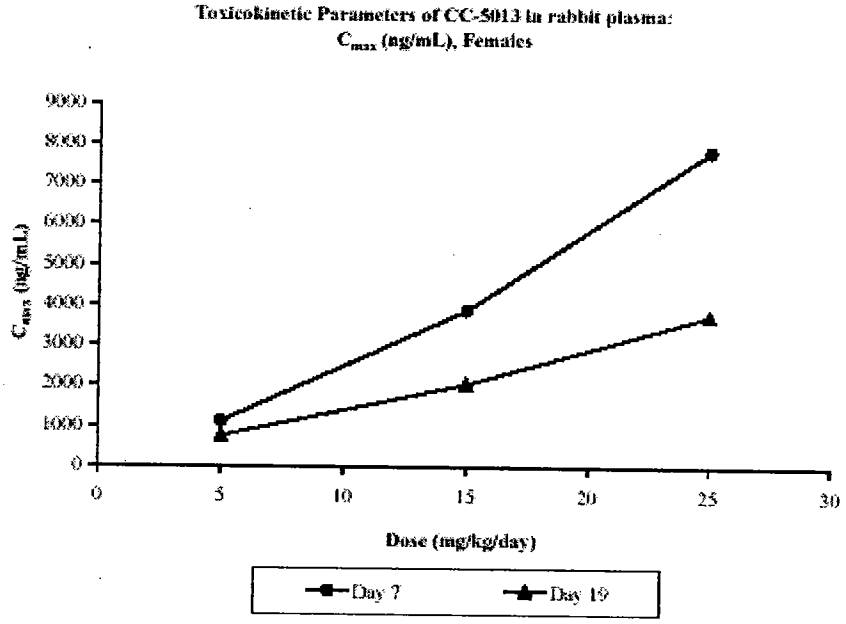
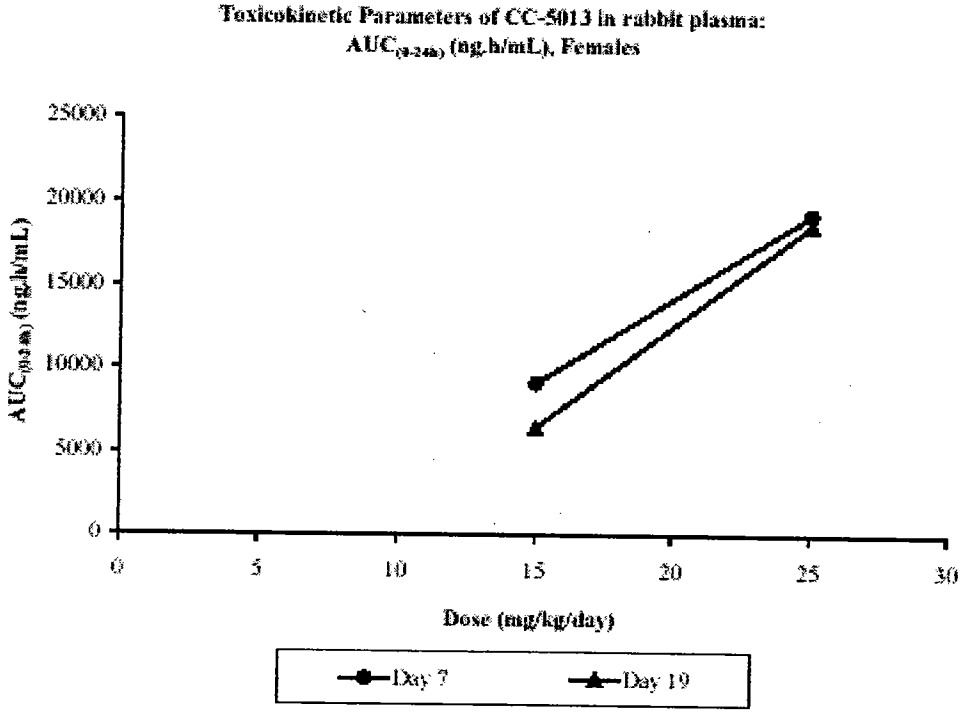
The sponsor's graphs below show that the Cmax and AUC of CC-5013 increased approximately proportionally as the dose of CC-5013 increased. Drug accumulation did not appear to occur, as AUC and Cmax were lower on GD 19 than GD 7.





Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

Caesarian and fetal data for the dams and litters that were available for evaluation by the termination day of the study are recreated from the sponsor's data below. There was a significantly higher incidence of dams with early intrauterine deaths in the thalidomide group than in the control group, $p<0.05$, as 100% of the thalidomide litters had early intrauterine deaths and 47% of the control litters had early intrauterine deaths. This parameter also had a significant dose-response relationship for the CC-5013 groups compared to control, $p<0.05$. These were the only statistically significant effects of either drug on the uterine/implantation parameters presented in the table. There was a slight increase in the mean number of post-implantation losses in the CC-5013 groups, due to early and late intrauterine deaths and dead fetuses. Though not listed in this table, note that two MD females and three HD females had complete embryo/fetal loss.

Uterine/Implantation Data For Gravid Female Rabbits Treated With CC-5013 or Thalidomide

	Control	LD 5 mg/kg	MD 15 mg/kg	HD 25 mg/kg	Thalid. 250 mg/kg
Number of females with live fetuses at GD 29	19	21	21	18	5
Mean # corpora lutea/female	11.8	13.5	11.4	11.3	10.6
Mean # implantations/female	10.3	11.2	9.4	10.6	10.2
Pre-implantation loss					
Mean #	14.2	16.9	16.7	5.9	4.4
Dams affected	13	18	15	9	2
Early intrauterine deaths					
Mean #	0.7	0.9	0.7	1.5	2.2
Dams affected&	9	9	11	14	5*
Late intrauterine deaths					
Mean #	0.7	0.5	0.9	0.7	0.6
Dams affected	9	9	8	7	2
Dead fetuses					
Mean #	0.0	0.0	0.1	0.2	0.0
Dams affected	0	1	2	2	0
Post-implantation loss					
Mean #	12.8	13.3	18.0	21.5	27.4
Dams affected	15	16	14	15	5
Mean # fetuses/female	8.8	9.8	7.7	8.2	7.4

* - $p<0.05$

& - Significant dose response test for this parameter control group vs. LD, MD, HD – $p<0.05$ – Trend to increased dams with early intrauterine deaths with increased CC-5013 doses.

Fetal data for the litters of the dams treated with either CC-5013 or thalidomide showed no statistically significant differences. There was a significant dose-response effect

seen on mean placental weights, with the CC-5013 groups showing a dose-related increase in mean placental weights. Mean fetal weights of the thalidomide litters is slightly lower than the other groups, though within the range of fetal weights in New Zealand White rabbits from control groups in other studies conducted by — . The slight difference may be due to the smaller N in the thalidomide group than the other groups.

Fetal Data For Litters of Gravid Female Rabbits Treated With CC-5013 or Thalidomide					
	Control	**LD 5 mg/kg**	**MD 15 mg/kg**	**HD 25 mg/kg**	**Thalid. 250 mg/kg**
Number of male fetuses	91	98	76	77	18
Number of female fetuses	77	107	85	70	19
% male fetuses	53.8	48.6	47.2	49.8	48.6
Mean litter weight (g)	339.0	365.2	291.2	312.9	261.8
Mean placental weight (g)&	5.26	5.30	5.72	5.83	5.28
Mean fetal weight (g)	39.9	37.7	37.7	38.2	36.1
Mean fetal weight – males (g)	40.6	38.1	38.2	38.8	38.6
Mean fetal weight – females (g)	38.9	37.5	36.7	37.4	33.4

& - Significant dose response test for this parameter, control group vs. LD, MD, HD – $p<0.05$ – Trend to increased placental weights with increased CC-5013 doses.

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Offspring (malformations, variations, etc.):

Malformations

Structural malformations in offspring of control, CC-5013, and thalidomide New Zealand White Rabbits		
Control Dam #	Fetus	Malformation
6	R3	Shortened forelimb digits; flexed ankle joints; umbilical hernia; incomplete diaphragm; displaced kidneys
9	L4	Malrotated wrist joint
	L6	Malrotated knee joints, extended ankle joints
	R2	Thoracic vertebral hemicentrum fused to lumbar hemicentrum
15	R2	Retro-oesophageal subclavian artery, right, arising from descending aorta
17	L2	Displaced left testis; cleft cartilage between sternebrae; umbilical hernia
	R9	Absent lung lobe; absent right thoracic vertebral centrum; fused ribs
	R7	Additional rib; branched rib; fused rib arches; additional thoracic vertebral arches
	R10	Forelimb digit(s) absent or shortened or misaligned; hindlimb phalanges absent; cleft palate; partially open eye
19	L1	Fused sternebrae; shortened premaxillae

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Low Dose Dam #	Fetus	Malformation
29	R1	Fused sternebrae
	R3	Fused sternebrae; umbilical hernia
30	R2	Retro-oesophageal subclavian artery, right, arising from descending aorta
33	L2	Additional thoracic vertebral hemicentrum, additional rib.
35	R3	Cleft palate; diaphragmatic hernia; fused lung lobes; dilated aortic arch; fused sternebrae; cleft and fused sternebra(e); free blood in thoracic cavity
38	L2	Small pituitary
	L5	Fused lung lobes
	R1	Retro-oesophageal subclavian artery, right, arising from descending aorta
42	L1	Right-sided aortic arch; retro-oesophageal subclavian artery; absent ductus arteriosus; common carotid artery arising from pulmonary trunk; fused sternebrae; additional rib; fused ribs; additional structures in cervical vertebra(e); fused and misaligned thoracic vertebral arches; additional thoracic vertebral centrum;
42	L1	hemicentrum present; lateral curvature of the vertebral column
45	L1	Fused ribs; thoracic vertebral arches and centra fused; thoracic vertebral centrum hemicentric; lateral curvature of vertebral column
	R1	Fused sternebrae; absent rib; branched rib; thoracic hemivertebrum present; lateral curvature of the vertebral column
46	L3	Fused sternebrae

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Mid Dose Dam #	Fetus	Malformation
49	L1	Bent and branched rib(s)
52	L3	Retro-oesophageal subclavian artery, right, arising from descending aorta
	R1	Absent rib; thoracic vertebral arches fused; thoracic vertebral centrum misaligned and hemicentric; lateral curvature of the vertebral column
53	L4	Retro-oesophageal subclavian artery, right, arising from descending aorta; fused lung lobes; absent sternebrum
55	L1	Interrupted aortic arch,
	L2	Retro-oesophageal subclavian artery, right, arising from descending aorta;
	R4	Retro-oesophageal subclavian artery, right, arising from descending aorta;
56	R3	Retro-oesophageal subclavian artery, right, arising from descending aorta;
63	L2	Fused nasal bones
64	L5	Absent common carotid artery
65	L2	Retro-oesophageal subclavian artery, right, arising from descending aorta
66	L2	Absent digits
70	R1	Branched rib

High Dose Dam #	Fetus	Malformation
79	R3	Retro-oesophageal subclavian artery, right, arising from descending aorta; free blood in thoracic cavity
80	R1	Absent areas of olfactory lobe of brain; lateral ventricular of brain dilated
84	L2	Retro-oesophageal subclavian artery, right, arising from descending aorta
85	R1	Flexed wrist joint
86	R1	Retro-oesophageal subclavian artery, right, arising from descending aorta
	R2	Displaced kidney
	R5	Displaced kidney
93	L4	Retro-oesophageal subclavian artery, right, arising from descending aorta
94	R2	Diaphragmatic hernia
95	L1	Fused sternebrae
96	R6	Cleft cervical vertebral centrum

Thalidomide Dam #	Fetus	Malformation
97	L2	Cleft palate; enlarged fontanelle; flexed wrist joints; absent tail; non-patent anal opening; interrupted aortic arch; incomplete ventricular septum
	L4	Malrotated ankle joint; additional hindlimb digit
98	L3	Diaphragmatic hernia; shortened oesophagus
	R1	Diaphragmatic hernia;
	R5	Malrotated ankle joint; absent forelimb digit
	R7	Malrotated wrist and ankle joints; narrowed ductus arteriosus; incomplete interventricular septum; enlarged left heart ventricle; absent kidney; misshapen ovaries; absent ureter
	R9	Malrotated ankle joint; small thyroid
99	L1	Malrotated ankle joints
	L2	Malrotated ankle joints
	L3	Displaced and blind-ended larynx; absent thyroid; malrotated ankle joints
	L4	Malrotated ankle joints; diaphragmatic hernia; blind-ended and displaced larynx; flattened trachea; absent thyroid
	L5	Interrupted aortic arch,
	R2	Malrotated ankle joints
	R4	Folded retina; enlarged eye; fused lung lobes; malrotated ankle joints
100	L1	Misaligned forelimb digit; flexed wrist joint; malrotated ankle joint; fused lung lobes; distended and blind-ended ureter, absent kidney
	L2	Flexed wrist joint; absent forelimb digit; malrotated ankle joints; flattened trachea; widened oesophagus
	L3	Displaced and blind-ended larynx; flattened trachea; absent thyroid; interrupted aortic arch
	L4	Flexed wrist joint; additional hindlimb digit; forelimb digit malformed
	L5	Forelimb digit malformed
	L7	Diaphragmatic hernia
	R1	Flexed wrist joint; malrotated ankle joint; fused lung lobes
	R2	Shortened forelimb digit; flexed ankle joints; umbilical hernia; incomplete diaphragm; displaced kidneys
	R3	Shortened forelimb digit; umbilical hernia
	R4	Flexed wrist joint; absent forelimb digit
	R5	Shortened forelimb digit; diaphragmatic hernia; dilated aortic arch; narrowed pulmonary trunk; fused lung lobes
101	L1	Flexed wrist joint; absent forelimb digits; misaligned hindlimb digit; malrotated ankle joints; fused lung lobes
	L2	Malrotated ankle joint; fused lung lobes
	L3	Flexed and malrotated wrist joints; malrotated ankle joints; absent fore and hindlimb digit(s); shortened hindlimbs; absent thyroid; interrupted aortic arch; forelimb digit malformed
	R1	Malrotated ankle joints; absent left thyroid; enlarged right thyroid; fused lung lobes
	R3	Flexed wrist joints; malrotated ankle joints; fused lung lobes; forelimb digit malformed

The following table summarizes the incidence of structural malformations, both the number of fetuses affected as well as the number of litters.

Structural Malformations in Litters Prenatally Exposed to CC-5013 or Thalidomide				
Group	**Fetuses**		**Litters**	
	# Affected	%	# Affected	%
Control	10/168	6	5/19	26
LD	12/205	6	8/21	38
MD	13/161	8	10/21	48
HD	11/147	7	9/18	50
Thalidomide	30/37	81	5/5	100

The thalidomide fetuses were not examined for skeletal malformations as the sponsor felt that the malformations noted were indicative of thalidomide reproductive toxicity and had served the purpose of validating the test system.

While the number of fetuses exhibiting structural malformations in the CC-5013 groups do not differ from the control group, there does appear to be a trend towards increased numbers of litters affected in the CC-5013 groups. The sponsor notes that these malformations are ones that spontaneously occur in this strain of rabbit based on control groups in previous studies conducted by — This does not account for the apparent trend in increased litter frequency across the CC-5013 dose groups. The sponsor's table below shows that there was not a significant dose effect on the number of litters with external/visceral malformations or variations or the number of litters with skeletal malformations or variations, or the total litters with any type of malformation.

Group mean caesarian data

Test article	Control	CC-5013			Thalidomide
Group	1	2	3	4	5
Level (mg/kg/day)	0	5	15	25	250

6.3 Foetal defect data

	Group 1	Group 2	Group 3	Group 4	Group 5	Statistics
EXTERNAL/VISCERAL DEFECTS						
Number of foetuses examined	169	205	161	147	37	
Number of litters examined	19	21	21	18	5	
Number showing malformations	7	7	8	9	30	
Mean % of foetuses examined	4.9	4.0	5.5	11.0	80.0	
Number of litters affected	4	5	6	7	5**	F+,f+
Number showing variations	56	95	84	72	34	
Mean % of foetuses examined	33.4	48.8	54.9	51.7	92.5	
Number of litters affected	18	21	21	17	5	F+,f+
SKELETAL DEFECTS						
Number of foetuses examined	168	205	161	147	0	
Number of litters examined	19	21	21	18	0	
Number showing malformations	5	8	6	2		
Mean % of foetuses examined	2.8	5.5	4.1	1.3		
Number of litters affected	3	6	6	2		F+
Number showing variations	149	193	154	136		
Mean % of foetuses examined	86.9	95.0	96.2	93.1		
Number of litters affected	19	21	21	18		F+
Total number of foetuses showing malformations	10	12	13	11		
% of foetuses examined	6.0	5.9	8.1	7.5		
Number of litters affected	5	8	10	9		F+

F+ = Cochran-Armitage and Fisher's Exact (upper tail) (Group 1 vs 2,3,4)
f+ = Fisher's Exact (upper tail) (Group 1 vs 5)

* P<0.05
** P<0.01
*** P<0.001

Clearly the thalidomide group exhibits far more structural malformations than the other treatment or control groups. The above table shows that the number of litters with external/visceral malformations was significantly different from control, $p<0.01$. The incidence of several fore- and hind limb defects are statistically significant compared to control. Other significant occurrences of malformations include absent kidney, aortic arch interrupted, herniated diaphragm, incomplete interventricular septum in the heart, blind-ended or displaced larynx, severely fused lungs, absent thyroid gland, and flattened trachea.

External/Visceral Variations

There was a slightly higher incidence of variations seen in the CC-5013 dose groups, though this was not statistically significant. This was due to a number of different variations, several of which were significant. The MD and HD CC-5013 groups had higher incidences of mottled, discolored liver, absent intermediate lobe of the lung (HD), skin petechiae on the trunk (HD), supernumerary cervical vertebrae (LD, MD, HD) and innominate/left common carotid arteries with a common origin from the aortic arch (MD). The sponsor states that the increased incidence of minor variations is incidental and is not evidence of an adverse event. It is possible that with adequate dosing more information would be gained about the increased minor variations. There were several other significant increases in variations that were noted in the thalidomide group only.

Conclusions:

- ❖ The lack of eating seen in the twelve rabbits, spread out among all dose groups, confounds this study and makes analysis difficult.
- ❖ Decreased body weight gains and food intake in the MD and HD rabbits, but the magnitude of this effect may not have been great enough to ensure sufficient dosing.
- ❖ Expected structural malformations seen in the thalidomide group, but no limb malformations seen in the CC-5013 groups.
- ❖ The inappetence along with possible insufficient dosing negates the results of this study. Sponsor has been advised of this and has conducted another rabbit study. This additional report is not included in the NDA.

Prenatal and postnatal development

Study title: CC-5013: Oral (gavage) study of pre- and postnatal development in the rat.

Key study findings:

- HD F_0 females gained slightly less weight during gestation, but no significant difference, indicative of very minimal maternal toxicity in this study
- Significant dose response relationship seen in percentage of offspring that were male, with CC-5013 treatment leading to a lower percentage of males
- No significant effects of CC-5013 treatment on physical development and functional tests
- Delayed sexual maturation seen in the MD and HD male offspring of CC-5013 treated dams, but not in the female offspring
- A trend to increased activity in the CC-5013 treated offspring during maturation, but no clear dose response relationship seen
- No significant effects of CC-5013 on learning and memory in the offspring
- CC-5013 F_1 females gained less weight during the early stage of gestation, GD 0-3, and the HD F_1 females gained less weight later, GD 6-10
- CC-5013 F_1 females had fewer embryos than control, with a significant dose response relationship
- CC-5013 F_1 females also had more early intrauterine deaths with their litters, but with no clear dose response seen, questioning its toxicological relevance

Study no.: 1398/246-D6154
Volume #, and page #: 4.2.3.5.3
Conducting laboratory and location:

Date of study initiation: 28 January 2002
GLP compliance: Letter included and signed
QA reports: yes (X) no ( )
Drug, lot #, and % purity: CC-5013, batch # 61457-06,

Methods

Doses:	0, 100, 300, and 500 mg/kg/day
Species/strain:	Rat: ~ CD(SD)IGSBR
Number/sex/group:	24 females/dose in P generation
Route, formulation, volume, and infusion rate:	Oral gavage, 1% w/v aqueous carboxymethyl cellulose; 5 mL/kg/day
Satellite groups used for toxicokinetics:	None
Study design:	Females were mated by the supplier prior to delivery to the laboratory on GD 3. Rats were dosed on GD 6 until PND 21, inclusive. Rats delivered and weaned their litters and 20 offspring of each sex were left untreated for 12 weeks post-weaning and then used to form the F_1 generation. Mated F_1 females euthanized on GD 13 and F_1 males during Week 17
Parameters and endpoints evaluated:	F_0 generation – mortality, clinical signs, body weights, food consumption, litter data (number of live/dead pups, litter size and sexes, daily clinical observations, individual pup weights, necropsy findings of dead and culled pups), necropsy data F_1 generation – developmental parameters (pinna detachment, incisor eruption, eye opening, surface righting, air righting, grip strength, pupillary reflex, auditory response, visual placing response), physical development (vaginal opening and balano-preputial separation), learning (water maze – control and HD only), motor activity (Control and HD only), mating data and caesarian data (pregnancy rate, # of corpora lutea, # of implantations, # of live embryos, # early intrauterine deaths, # of late intrauterine deaths, necropsy data
Dose justification:	The doses were selected based on the 13-week toxicology study where 300 mg/kg was the NOEL. Additionally, the range-finding embryo-fetal study in the rat (Report 1398/236-D6154) showed

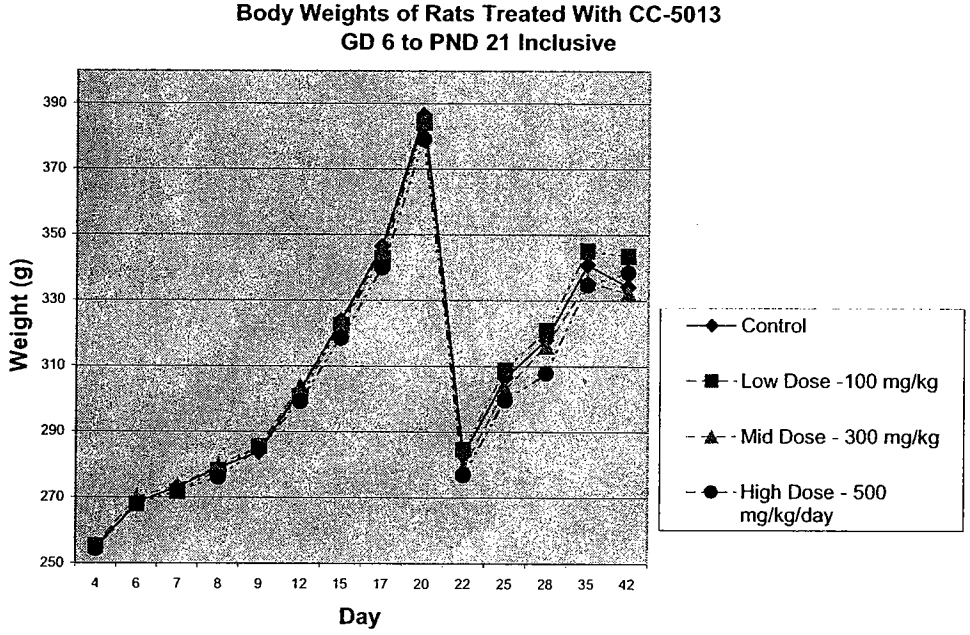
doses of 300 and 500 mg/kg produced only slight reductions in mean body weight gain. The HD was expected to produce slight adult toxicity and the LD was expected to be the NOAEL.

Results

F_0 in-life:

- No mortality
- Slightly higher incidence of thinning fur in the MD and HD animals, no other treatment-related clinical signs
- During gestation, HD dams gained slightly less weight than controls, but no statistical significance. During lactation, HD dams gained weight during the final week while the control, LD and MD dams lost weight. Difference from control was statistically significant.

A graph of the body weights of the F_0 dams during gestation and lactation is presented below.



- No treatment-related effects on food intake
- No treatment-related effects on pregnancy rate
- Clinical signs – thinning hair more prevalent in the treated dams during pregnancy and lactation, though not dose-related

- Pregnancy parameters are presented below, from the sponsor's table, no significant effects of treatment were seen.

Pregnancy Parameters Of The F_0 Generation Treated with CC-5013-5013 From GD 6 – PND 21				
	Control	LD 100 mg/kg/day	MD 300 mg/kg/day	HD 500 mg/kg/day
Number of rats	24	24	24	24
Number not pregnant	1	1	2	2
Number pregnant (%)	23 (95.8)	23 (95.8)	22 (91.7)	22 (91.7)
Number died/euthanized	0	0	0	0
Number with total embryo/fetal loss	0	0	0	0
Gestation index	100.0	100.0	100.0	100.0
Number with total litter loss	0	0	0	0
Number with live pups at PND 21	23	23	22	22

- Litter and neonatal parameters are presented below, from the sponsor's tables. A significant dose response relationship was seen on percent of pups that were male, with fewer males seen with increasing doses of CC-5013. A significantly lower post-implantation survival index was seen in the LD group, but not in the MD or HD groups. The lack of a dose relationship makes this result unlikely to be a drug effect.

Litter And Neonatal Parameters Of The F_0 Generation Treated With CC-5013 From GD 6 – PND 21				
	Control	LD 100 mg/kg/day	MD 300 mg/kg/day	HD 500 mg/kg/day
Number rats with live pups at PND 21	23	23	22	22
Mean duration of gestation (days)	22.3	22.2	22.3	22.4
Mean # of implantation sites	13.0	13.9	14.4	13.7
Mean # of pups born	12.7	12.5	13.4	12.9
Mean number of pups alive at PND 1	12.7	12.4	13.2	12.8
Mean % male pups PND 1&	54.4	53.0	48.3	43.7
Mean # pups alive PND 4 before culling	12.6	12.2	12.8	12.7
Mean # pups culled PND 4	4.8	4.3	4.9	4.9
Mean # pups alive PND 4 after culling	7.8	8.0	8.0	7.8
Mean # pups alive PND 7	7.8	8.0	8.0	7.8
Mean # pups alive PND 14	7.7	7.8	7.8	7.6
Mean # pups alive PND 21	7.7	7.8	7.7	7.6
Post-implantation survival index	96.6	90.0***	92.6	94.0
Live birth index %	100.0	99.3	98.8	99.3

& - Significant dose response test for this parameter $p<0.05$ - Trend to decreased percent of pups that were male with increased CC-5013 doses.
*** - $p<0.001$

- The litter weights are presented below, taken from the sponsor's table. No significant effects were seen.

Litter Weights Of The F_1 Generation From An F_0 Generation Treated With CC-5013 From GD 6 – PND 21				
	Control	LD 100 mg/kg/day	MD 300 mg/kg/day	HD 500 mg/kg/day
Mean weight (g) Day 1				
Males	6.7	6.4	6.5	6.5
Females	6.3	6.0	6.1	6.2
Combined	6.5	6.2	6.3	6.3
Mean weight Day 4				
Males	9.4	9.4	9.3	9.3
Females	9.1	8.9	8.7	8.9
Combined	9.3	9.2	9.0	9.1
Mean weight Day 7				
Males	15.7	15.5	15.4	15.0
Females	15.2	14.6	14.7	14.6
Combined	15.5	15.1	15.1	14.9
Mean weight Day 14				
Males	33.8	33.8	32.7	33.2
Females	32.4	31.9	32.0	32.1
Combined	33.1	33.0	32.4	32.7
Mean weight Day 21				
Males	54.5	55.0	53.5	55.2
Females	52.4	52.0	52.1	53.0
Combined	53.5	53.7	52.9	54.0
Percent weight change – Days 1-21				
Combined	724.4	764.3	742.6	762.2

F_0 necropsy:

- No treatment-related effects were seen on necropsy of the F_0 female rats

F_1 physical development:

- No treatment-related effects on pinna detachment, incisor eruption or eye opening. There was a significantly lower percentage of pups with pinna detachment on PND 2 in the LD group, but with no dose relationship, this is unlikely to be a treatment effect.
- No treatment-related effects on surface righting reflex, air righting reflex, grip strength, pupillary reflex, auditory response and visual placing response

- The mean day for balano-preputial separation slightly later in the MD and HD F_1 males compared to controls

Dose Group	Mean Day Post-Partum For Complete Development
Control	43
LD	44
MD	45*
HD	45*

* - p<0.05

- Two significant weight changes were seen prior to pairing of the F_1 generation males and females. Male F_1 rats of the MD group had a significantly smaller body weight gain from Week 12-16 than the controls.

Body weight gains (g) Male Rats

Week 12-16	Control	LD	MD	HD
	40.1	39.3	30.2*	35.6

*- P<0.05

- Additionally, the LD female F_1 rats showed a significantly larger change in body weights from control for the time period of Start to Week 4.

Body weight gain (g) Female Rats

Start – Week 4	Control	LD	MD	HD
	118.8	128.2*	121.0	120.7

*- p<0.05

- Neither of these body weight change differences showed any dose response relationship and were unlikely due to drug treatment
- No significant effects seen on food intake in the F_1 generation

F_1 behavioral evaluation:

Locomotor Activity

- During Week 4, the HD male F_1 rats were more slightly active than the controls. During Week 4, the HD female F_1 rats were significantly more active (total ambulation score and total number of beams broken) than controls.

Locomotor Activity Scores – Week 4

Dose group	Total ambulation score	Total beams broken
Control M	100	345
HD M	111	369
Control F	115	396
HD F	155*	509*

* - p <0.05

Locomotor activity retested during Week 8 with offspring from all four dose groups (data below). No clear dose response relationship was seen, though the LD and MD animals did have significantly higher numbers on several scores. Unlike in Week 4, the HD female F_1 rats did not have higher ambulatory or total beam scores than the controls.

Locomotor Activity Scores – Week 8

Dose group	Total ambulation score	Total beams broken
Control M	105	325
LD M	143	428*
MD M	126	368
HD M	105	317
Control F	114	385
LD F	127	416
MD F	159**	481*
HD F	120	396

* - $p < 0.05$ ** - $p < 0.01$

Swimming Maze Test

- In this learning task there were two significant differences between the HD males and controls, with the HD males showing a decreased percentage of error free escapes in one of the five rounds of testing during Week 5. Additionally the HD males had a shorter time in the memory category. When retested during Week 6 in the design, with the exit ramp in the same position as it was for 5 trials during Week 5, the HD male rats found the escape ramp quicker than the controls. The results of this study show no toxicological impact of the prenatal and lactational exposure to CC-5013.

F_1 reproduction:

- The reproduction parameters for the bred F_1 generation males and females are presented below, taken from the sponsor's tables
- Some male rats in all dose groups failed to sire pregnancies. At necropsy, there were no macroscopic differences in the reproductive organs of these rats.

Pregnancy Parameters Of The F_1 Generation From An F_0 Generation Treated With CC-5013 From GD 6 – PND 21

	Control	LD 100 mg/kg/day	MD 300 mg/kg/day	HD 500 mg/kg/day
Males				
Number bred	20	20	20	20
Number died/euthanized	0	0	0	0
Number inducing pregnancy	18	18	15	16
Females				
Number bred	20	20	20	20
Died during pairing	0	0	1	0
Number alive after pairing	20	20	19	20
Number not pregnant	2	2	2	2
Number pregnant (%)	18 (90.0)	18 (90.0)	17 (89.5)	18 (90.0)
Number died/euthanized	0	0	0	0
Number with total embryo loss	0	0	0	0
Number with live embryos at GD 13	18	18	17	18

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- The uterine/implantation parameters for the bred F_1 females are recreated from the sponsor's table below. There was a significant drug dose effect on the number of embryos per female, with a decrease seen with increasing doses of CC-5013. There were also more dams with early intrauterine deaths in the CC-5013 treated groups, though not in a dose-response fashion.

Uterine/Implantation Parameters For The F_1 Generation Females From An F_0 Generation Treated With CC-5013 From GD 6 – PND 21

	Control	LD 100 mg/kg/day	MD 300 mg/kg/day	HD 500 mg/kg/day
Number females with live embryos GD 13	18	18	17	18
Mean number corpora lutea per female	17.8	19.4	18.2	17.6
Mean number implantations per female	16.8	17.6	16.8	15.4
Pre-implantation loss				
Mean %	5.1	7.2	7.7	11.2
Number of dams affected	8	7	11	12
Early intrauterine deaths				
Mean %	0.3	1.6	1.0	0.8
Number of dams affected	3	12**	9*	10*
Late intrauterine deaths				
Mean %	0.2	0.1	0.1	0.1
Number of dams affected	4	1	1	1
Post-implantation loss				
Mean %	3.4	9.2	6.3	5.8
Number of dams affected	7	13*	10	10
Mean # of embryos per female&	16.3	16.0	15.8	14.6

& - Significant dose response relationship $p<0.05$ – Trend to less embryos with increased dose of CC-5013.
* $p<0.05$ ** $p<0.01$

- Body weight changes of the pregnant F_1 female rats showed a significant dose response effect between Days 0 and 3 and the HD females had a significantly lower body weight change during the period of GD 6-10. The dams were euthanized on GD 13. The sponsor's table is recreated below.

Body Weight Changes During Gestation In The F_1 Generation Females From An F_0 Generation Treated With CC-5013 From GD 6 – PND 21

Days of Gestation	Control	LD 100 mg/kg/day	MD 300 mg/kg/day	HD 500 mg/kg/day
GD 0-3&	17.3	16.0	16.5	13.2
GD 3-6	9.9	11.6	8.4	13.1
GD 6-10	20.6	17.4	18.2	14.8**
GD 10-13	11.0	14.5	13.7	14.7

& - Significant dose response test – $p<0.05$ – Trend to decreased body weight gains with increased CC-5013 doses.
** - $p<0.05$

- No significant effects on food intake during gestation in the F_1 generation

F_2 findings:

No necropsy data was presented for the F_2 generation. Embryo data is presented under the F_1 reproduction heading.

Conclusions:

- No F_0 toxicity seen.
- Delayed sexual maturation in MD and HD males.
- Decreased weight gains during gestation for F_1 females, GD 0-3 a significant dose response relationship and during GD 6-10 significant decrease in HD group.
- Should an indication be sought for this drug where this study were required, this study may need to be redone with adequate dosing levels, as little to no parental toxicity was seen in the F_0 generation and it is possible that higher doses may have shown additional effects.

Reproductive toxicology conclusions

Fertility and Early Embryonic Development

The effects of CC-5013 on fertility and early embryonic development were studied in the rat. The doses used were based on the 13-week toxicology study in the rat and the dose-range finding study for the embryo-fetal development study in the rat. The HD of 500 mg/kg/day was expected to produce slight parental toxicity.

Parental toxicity was not seen in this study. There was no lethality nor any clinical signs noted that were drug-related. Prior to pairing the treated males and females, no differences were seen in body weight gains or food intake. Once dosing ceased on GD 6, the CC-5013-treated females exhibited a significant dose response relationship toward increased food intake and body weight gain when compared to control, although none of the dose groups were individually significantly different from control.

Although two MD and two HD females were positive for mating upon examination of the vaginal smear, they were not pregnant. While this was not seen in the control or LD groups, it is unlikely to be a treatment effect. Historical data from the laboratory that conducted the study showed a range of 0-4 rats that were not pregnant despite positive signs of mating.

Implantation data showed a significant dose response relationship in the mean number of implantations per female, with decreased implantations as the dose of CC-5013 increased. This parameter is used to calculate the pre-implantation loss, so therefore led to a significant dose response relationship in increased pre-implantation loss with increased doses of CC-5013. The sponsor notes that compared to historical data from the laboratory conducting this study, the control and LD rats had higher than normal mean number of implantations per female. In the six rat fertility studies conducted by the laboratory prior to this study, the mean number of implantation sites for control rats

ranged from 14.2 – 15.8, with an average of 15.1 for the six studies. The control group in this study had a mean implantation number of 15.9. The sponsor points to this as the reason for the statistically significant dose response test with decreased mean number of implantations with increased CC-5013 doses. Given the lack of other effects, and the laboratory's historical data, it is unlikely that this is a drug effect.

Seminology data showed significant decreases in straight line velocity, curvilinear velocity an average path velocity. These parameters, however, did not show a dose response relationship. The sponsor again claims that the significance is due to the higher than expected, based on historical data, velocity numbers in the sperm of the control rats. Data from the conducting laboratory's previous nine studies show that sperm velocity data from control rats were considerably lower than what was seen in this study. The lack of a dose response relationship in these sperm velocity parameters in the CC-5013 dosed animals, and the lack of any effects on the male rats' ability to impregnate the female rats, indicates that these findings are unlikely to be of any toxicological relevance.

It is noted that in none of the toxicology studies conducted, in any species, have histopathological changes been seen in the reproductive organs of the male or female animals.

This study is not generally needed for therapeutics intended for patients with advanced disease. Should further indications be sought, it is possible that this study would need to be conducted again utilizing higher doses to ensure that the lack of adverse fertility findings following CC-5013 treatment was not due to insufficient dosing. While it is important to identify a NOAEL for reproductive toxicology, it is also necessary to ensure adequate dosing so that an adverse effect is not missed because the doses tested were not sufficient.

Based on this study, doses up to 500 mg/kg during the pre-mating period and up to GD 6, produced no adverse effects on the parental rats or on the fertility/fecundity parameters. This dose is approximately 600 times the human dose of 10 mg, based on body surface area. It is not known how the AUC of the drug in the rats compares to that in the clinical setting, as these data do not exist for this patient population. Given that, comparison based on body surface area is the best comparison possible.

Embryo-Fetal Development

Embryo-fetal development effects of CC-5013 were investigated in two species, the rat and the New Zealand White rabbit. Both studies were preceded by dose range finding studies to set the appropriate dose levels for the pivotal trials. ICH S5A guidelines state that medicinal products should be studied in a rodent and non-rodent model. If negative in the rodent model, this should be confirmed in the second species. While the guideline recommends that the best rodent species is the rat and the best non-rodent

species is the rabbit, it also allows for the possibility that for certain compounds, one or both of these species may not be appropriate.

Rat

The rat was dosed with either 100, 300 or 500 mg/kg during organogenesis. The same doses were used in the range-finding study as well as the pivotal rat study. The sponsor claims that no maternal or embryo-fetal toxicity was seen in the range-finding study and that the HD from that study is an appropriate HD for the rat pivotal embryo-fetal development study. It would have been preferable to increase the HD level to achieve sufficient toxicity.

The range-finding study showed a decrease in the number of corpora lutea and implantations in the CC-5013 treated groups. Although not analyzed statistically, these parameters decreased with increasing CC-5013 doses. Although the sponsor claims this can not be a drug effect as treatment began after implantation, the potential for drug effect could not be ruled out. However, in the pivotal trial, with higher animal numbers and statistical analyses, this effect was not seen.

In the pivotal study, minimal impact of drug treatment was seen on body weight changes and food intake. Only during the first day of treatment was an effect seen on food intake. No dose groups differed significantly from control, but a test for a dose response relationship was significant, with higher doses of CC-5013 leading to lower food intake. Again, this was only seen on the first day of dosing and no impact of CC-5013 was seen on feeding after that point. Body weight gains were only significantly affected by the drug treatment in the first few days of dosing. During GD 6-7 and GD 8-9, the HD dams gained significantly less weight than the control rats. No other significant body weight effects were seen

The pivotal rat study also showed significant decreases in placental weight. This did not have a dose-response relationship and appeared to have no other signs of a toxicological relevance. The fetal weights were not impacted by this decrease in the placental weights. And though these decreased weights were significantly different from control, they appeared to be rather minor in size of the difference.

No effect of CC-5013 administration during organogenesis was seen on skeletal or external/visceral defects.

Historical data tells us that the limb-bud effects that are the hallmark of thalidomide teratogenicity are not seen in the rat model. Because of CC-5013's similarity in structure, the rat is not the animal model that would best examine the full range of potential CC-5013 developmental effects.

Rabbit

The New Zealand White rabbit was used as the non-rodent species to examine the embryo-fetal toxicity of CC-5013. In a dose range finding study, doses of 50, 100 or 150 mg/kg/day were administered to the rabbits and at all doses mortality and abortions were seen. Because of the maternal and embryo lethality seen in this study, there were only three litters from CC-5013 treated females evaluable, 2 at the 50 mg/kg/day dose and 1 at the 100 mg/kg/day. As this was a dose range finding study, minimal data was collected from any does that died, were euthanized, or lost their litters. In the few animals that were used in the evaluation, CC-5013 treatment at these doses decreased body weights and caused lowered food intake. The lethality and abortions seen nearly all occurred after the drug administration had concluded. It was also during this time that the decreases in food consumption were noted.

The sponsor chose 25 mg/kg/day as the HD in the pivotal rabbit study. Additional doses were 5 and 15 mg/kg/day of CC-5013 and a group given 250 mg/kg/day of thalidomide, a dose known to be cause the associated limb bud defects in the New Zealand White rabbit. While the CC-5013 treatment did cause several animals to abort or to have total embryo/fetal loss, ultimately the control group had 86% evaluable litters and the HD CC-5013 group had 82% evaluable litters. This parameter indicates that a dose higher than 25 mg/kg/day could have been used for this study and sufficient litters obtained for evaluation.

The pivotal study, as in the dose range finding study, showed that what maternal toxicity was seen with CC-5013, seemed to be more evident after the drug administration period had concluded. Food intake, though decreased from the time of dosing onward, was more dramatic in the later part of gestation. The CC-5013 dosing did lead to a trend toward increased early intrauterine deaths, a parameter indicative of toxicity earlier in gestation.

The sponsor did not conduct an adequate test in the New Zealand White rabbit, a thalidomide sensitive species. Given the sensitivity of food intake as an indicator of toxicity in the rabbit, animals that were exhibiting eating problems prior to dosing should not have been placed on study. While toxicity was certainly seen in this study, the top dose studied should have been higher than 25 mg/kg. All dose groups had comparable numbers of litters for evaluation at the end of the study and maternal toxicity was not of sufficient magnitude to preclude testing at higher dose levels.

Prenatal and Postnatal Development

The sponsor conducted the study in rats, under the standard recommended design based on the ICH S5A document. The doses were chosen based on the rat 13-week toxicology study where 300 mg/kg was the NOEL. Additionally, the range-finding embryo-fetal study in the rat (Report 1398/236-D6154) showed doses of 300 and 500

mg/kg produced only slight reductions in mean body weight gain. The HD (500 mg/kg) was expected to produce slight adult toxicity and the LD (100 mg/kg) was expected to be the NOAEL.

Maternal toxicity in the F_0 generation was not seen. Litter parameters showed minimal significant differences. There was a trend to a smaller percent of the litter being male pups as CC-5013 doses increased. This is a parameter of little toxicological relevance. Additionally, the LD rats had a significantly lower post-implantation survival index compared to control. However no significance was seen in the two higher doses and the lack of a dose response effect indicates that this result is unlikely to be a drug effect.

Physical and reflex development in the F_1 generation showed very minimal effects of prenatal exposure to CC-5013. The LD pups had a delay in pinna detachment, with less LD pups reaching this developmental milestone on PND 2 than the control pups. Again, there was no effect seen at the other doses and with no dose response effect, it is unlikely to be drug related. The MD and HD male F_1 pups had a delay in sexual maturation, as measured by balano-preputial separation.

Locomotor activity of the control and HD F_1 pups during Week 4 appeared to show a treatment effect, with the HD female pups having significantly increased total ambulation scores and total numbers of beams broken. This effect did not seem to be evidenced over time, as the test was conducted again at Week 8 with pups from all dose groups rather than just the control and HD. This study showed significant increases in MD females total ambulation scores and LD males and MD females total beams broken. There is no dose response effect, and the HD females do not show increases during this week. While the Week 4 effect may have been drug-related, without additional dose groups, this assumption is one that can not truly be confirmed. The Week 8 data do not show a long-term drug effect on increased locomotor activity.

Learning and memory were assessed in a swimming maze test. Although the male HD F_1 pups had a significantly lower number of error free escapes in one of the five trials conducted during the first round of tests, they also had a significantly shorter time finding the correct exit ramp when tested the for memory of the ramp's position the next week. This shows that in this test of learning and memory, there was no impact of prenatal CC-5013 exposure at the doses tested.

Males and females of the F_1 generation were mated and the effects of prenatal CC-5013 exposure on these parameters examined. The pregnant F_1 rats had a significant dose relationship on the body weight gains during the period of GD 0-3, with smaller weight gains with increasing doses of CC-5013. Additionally, during the GD 6-10 period, the HD pregnant F_1 rats had a significantly lower body weight gain than the controls. The only significant effect on the pregnancy parameters was seen on the number of dams with early intrauterine deaths. This was significantly higher in all the CC-5013 dose groups when compared to control. It was however, not dose-dependent. This, coupled with the lack of any other significant effects, makes it unlikely

that the prenatal CC-5013 exposure had any significant impact on fertility and pregnancy.

This study should also have utilized a higher dose for the HD group, allowing assurance that any negative effects seen were not due to insufficient maternal exposure. However this study is not required for this indication.

2.6.6.7 Local tolerance

Not done

2.6.6.8 Special toxicology studies

None

2.6.6.9 Discussion and Conclusions

Lenalidomide [3-(4'-amino-1,3-dihydro-1-oxo-2H-isoindol-2-yl)-2,6-piperidinedione] is a thalidomide analogue. It is a racemic mixture of S(-) and R(+) with a net optical rotation of zero. Lenalidomide inhibited the secretion of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6 and IL-12) and increased the secretion of anti-inflammatory cytokines (IL-10) from peripheral blood mononuclear cells. Lenalidomide inhibited, *in vitro*, the proliferation of Namalwa (B cell, Burkitt's lymphoma, deletion of chromosome 5) and Farage (non-Hodgkin's lymphoma B cell line) cells. In the repeat dose toxicity studies, multiple organ hemorrhage, renal toxicity, gastrointestinal tract inflammation and thymus and bone marrow atrophy were observed. Lenalidomide did not show mutagenic or clastogenic potential.

Reproductive toxicity was assessed in rats and rabbits. Fertility and early embryonic development and pre- and post-natal development were studied in the rat model. These studies are not normally submitted for indications such as MDS. Minimal toxicity was seen and there was little impact of lenalidomide treatment on the reproductive parameters measured. The dosing in these studies, however, may not have been sufficiently high enough to preclude the potential of positive findings. The required embryo-fetal development studies were conducted in rat and the rabbit. These studies are not acceptable for thorough examination of the potential reproductive developmental effects of lenalidomide. Study details and conclusions are presented in Section 2.6.6.6.

2.6.7 TOXICOLOGY TABULATED SUMMARY

General Toxicology:

Summary of Single and Multiple Dose Toxicology Studies

Species (Study #)	Duration/ Route	N/sex/ dose	STD_{10} ($mg/m^2/day$)	HNSTD ($mg/m^2/day$)	Significant finding
[redacted].CD-1(ICR)BR mice (1398/099)	Single IV dose	5	>120		One animal had enlarged heart weight, second animal had renal pelvic dilatation
[redacted].CD-1(ICR)BR mice (1398/098)	Single, oral by gavage	5	>6,000		None
Hsd.Brl:WH rats (1398/097)	Single, IV dose	5	>240		None
Hsd.Brl:WH rats (1398/096)	Single, oral by gavage	5	>12000		Unilateral renal pelvic dilatation in 2/5M
[redacted].(IGS)CDBR rats (1398/105) GLP	Daily x 7 Oral gavage	5		12,000	Kidney wt↑, urea↑ and creatinine↑(1/5 animals) at 3,000 and 12,000 $mg/m^2/day$
Cdl:CD(SD) IGSBR rats (1398/107) GLP	Daily x 28 Oral by gavage	10		6,000	Mortality: 1 F moribund in 1800 mg/m2 (considered non-drug related), ↑urea, creatinine and kidney weight, and moderate to severe tubular nephropathy /nephritis in the kidneys at 6000 $mg/m^2/day$
[redacted]CD® (SD)IGSBR rats,(1398/244) GLP	Daily x 26 week	20		1800	Pelvic mineralization and pyelitis
Monkey (M. fascicularis), GLP	Daily x 28 Oral gavage	3		24	No mortality, no adverse effects.
Cynomolgus monkeys (1398/191), GLP	Daily x 13 week	3		24	No toxicity at the highest dose tested.
Cynomolgus monkeys (1398/243), GLP	Daily x 52 week	6		24	Atrophy of the thymus. Gastrointestinal inflammation and atrophy of bone marrow and thymus at 48 and 72 mg/m2/day animals). Multiple organ hemorrhage, effects on bone marrow myelopoietic cells

Genetic Toxicology:

Study	Concentration or Dose	Results		
		Positive control	No metabolic (-S9)	Plus metabolic (+S9)*
Bacterial reverse mutation assay	5000 µg/plate	Yes	Negative	Negative
In vitro TK mouse lymphoma cells	162-2593 µg/ml	Yes	Negative	Negative
In vitro human peripheral blood lymphocytes	1271-2593 µg/ml	Yes	Negative	Negative
In vivo rat bone marrow micronucleus assay	500, 1000 and 2000 mg/kg	Cyclophosphamide	No increase in PCE/NCE ratio	

* No P450 metabolism observed in studies to address lenalidomide metabolism.

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Reproductive Toxicology:

Study	Route	Duration	Dose	Result
Rat (1398/245)	PO	Males for 2 weeks prior to pairing until euthanized Females for two weeks prior to pairing and then up until GD 6	100, 300 and 500 mg/kg/day	No parental toxicity. No significant treatment-related effects on fertility/fecundity or sperm parameters.
Rat (1398/236)	PO	Females dosed GD6-17 inclusive	100, 300 and 500 mg/kg/day	Range finding study. No maternal or embryo-fetal toxicity
Rat (1398/237)	PO	Females dosed GD6-17 inclusive	100, 300 and 500 mg/kg/day	Very minimal maternal toxicity – slight transient decreases in food intake and body weight gain. Decreases in placental weight with CC-5013. No adverse effects on embryo/fetus. No malformations or defects seen.
Rat (1398/246)	PO	Females dosed GD6-PND 21 inclusive	100, 300 and 500 mg/kg/day	No significant F_0 toxicity. Decreased body weight gain during early period of gestation in F_1 rats. Delayed sexual maturation in F_1 males of the MD and HD groups.
Rabbit (0329DC35.001)	PO	Females dosed GD8-10 inclusive	100 mg/kg/day	Screening study. No maternal and embryo-fetal toxicity
Rabbit (1398/225)	PO	Females dosed GD7-19 inclusive	50, 100 and 150 mg/kg/day	Range-finding study. All CC-5013 doses had mortality and abortions. Only 3 CC-5013 treated rabbits had evaluable litters.
Rabbit (1398/226)	PO	Females dosed GD7-19 inclusive	5, 15 and 25 mg/kg/day	Comparable number of evaluable litters across groups. Decreased weight gain and food intake with MD and HD CC-5013. Thalidomide group had limb malformations, CC-5013 did not. Trend toward increased overall malformations with increased CC-5013 doses. Insufficient dosing and confounding inappetence variable.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: Lenalidomide is approvable from pharmacology/toxicology perspective.

Unresolved toxicology issues (if any): The reproductive toxicology of lenalidomide needs to be fully addressed, as in the recommended Phase 4 commitments.

Recommendations: This NDA is approvable from a pharmacology/toxicology perspective.

Suggested labeling: Separate review will be conducted.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____Concurrence Yes __ No__

Appendix/Attachments: Appendix A and B

APPENDIX A

TABLE OF CONTENTS

2.6.2 PHARMACOLOGY

2.6.2.2 Primary pharmacodynamics

1 Differential cytokine modulation and T cell activation by two distinct classes of thalidomide analogues that are potent inhibitor of TNF-α.
2 Thalidomide and its analogues overcome drug resistance of human multiple myeloma cells to conventional therapy.
3 Amino-substituted thalidomide analogs: potent inhibitors of TNF-α production.

2.6.2.4 Safety pharmacology

1 CC-5013: Cardiovascular and respiratory effects in the anaesthetised dog following intravenous infusion (1398/124-D1140).

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

1 CC-5013: A study to determine the oral bioavailability in the rat, dog, and monkey (1398/124-D1140).
2 CC-1088, CC-4047, CC-5013 and CC-7025: Comparative absorption by the Caco-2 cell line (1398/109-D1140).

2.6.6 TOXICOLOGY

2.6.6.2 Single-dose toxicity

1 CC-5013: Single dose intravenous toxicity study in the mouse [approximation of the minimum lethal dose level] (1398/098-D6144).
2 CC-5013: Single dose oral toxicity study in the mouse [approximation of the minimum lethal dose level] (1398/098-D6144).
3 CC-5013: Single dose intravenous toxicity study in the rat [approximation of the minimum lethal dose level] (1398/097-D6144).
4 CC-5013: Single dose oral toxicity study in the rat [approximation of the minimum lethal dose level] (1398/096-D6144).

2.6.6.3 Repeat-dose toxicity

1 CC-5013: 7 day oral (gavage) range-finding toxicity study in the rat (1398/105-D6154).
2 CC-5013: 28 day oral (gavage administration) toxicity study in the rat (1398/107-D6154).
3 CC-4047 & CC-5013: 28 day oral (gavage administration) toxicity study in the monkey (1398/126-D6154).
4 CC-5013: 28 day (gavage administration) toxicity study in the monkey (1398/108-D6154).

6.6.6.4 Genetic toxicology

1 CC-5013: Reverse mutation in four histidine-requiring strains of *Salmonella typhimurium* and two tryptophan-requiring strains of *Escherichia coli* (1398/100-D5140).
2 CC-5013: Induction of chromosome aberrations in cultured human peripheral blood lymphocytes (1398/118-D5140).
3 CC-5013: Mutation at the thymidine kinase (*tk*) locus of mouse lymphoma L5178Y cells (MLA) using the technique (1398/95-D5140).

2.6.6.6 Reproductive and developmental toxicology

1 Developmental and reproductive toxicity screening study for effects on embryo-fetal development in rabbits (0329DC35.001).

2.6.2 PHARMACOLOGY

2.6.2.2 Primary pharmacodynamics

1 Differential cytokine modulation and T cell activation by two distinct classes of thalidomide analogues that are potent inhibitor of TNF-α. J. Immunol. 1999, 163:380-6. Vol. 1.3, p 477.

Conducted by Celgene Corporation, Warren, NJ and Laboratory of Cellular Physiology and Immunology, The Rockefeller University, New York. Six structural analogues of thalidomide have been evaluated for their effects on the production of monocyte cytokines and their immunomodulatory effects on T cells. CC-5013, a thalidomide analogue to be investigated in the present IND, was not evaluated in this published paper.

2 Thalidomide and its analogues overcome drug resistance of human multiple myeloma cells to conventional therapy. Vol. 1.3, p 484.

Conducted at the Department of Adult Oncology, Dana-Farber Cancer Institute, and Department of Medicine, Harvard Medical School, and Celgene Corporation. Results indicated that thalidomide and its analogues act directly on tumor cells via inducing apoptosis or G1 growth arrest. These agents also enhance the anti-multiple myeloma (MM) activity of dexamethasone.

(1) 3HTdR uptake of MM 1S cells and HS Sultan was inhibited in a dose dependent manner by thalidomide and its analogues (ImiD 1, ImiD 2 and ImiD 3). Selected cytokine inhibitory drugs (SelCID 1 and 3) at high doses (11 μM) also inhibited the proliferation of MM.1S cells.

(2) Thalidomide and ImiD only weakly inhibited 3HTdR uptake of Dox6, Dox40 (cells resistant to doxorubicin), MR20 (cells resistant to mitoxantrone) or LR5 (cells resistant to melphalan) in cultures.

(3) ImiDs reduced 3HTdR uptake of MM.1S cells and dexamethasone increased this inhibition of proliferation.

(4) IL-6 (50 ng/mL) increased DNA synthesis of MM.1S cells in cultures even in the presence of IMiDs.

(5) Thalidomide and IMiDs increased G1 growth in MM.1S cells and induced G1 growth arrest in both Hs Sultan and AS patient MM cells.

(6) IMiDs downregulated p21 expression in MM.1S cells and upregulated p21 in Hs Sultan cells and AS patient MM cells.

(7) IL-6 increased mitogen activated protein kinase tyrosine phosphorylation. This increased phosphorylation was partially blocked by PD98059 (MEK1 inhibitor).

3 Amino-substituted thalidomide analogs: potent inhibitors of TNF-α production. Bioorg. Med. Chem. Lett. 1999, 9:1625-30. Vol. 1.3, p 518.

Conducted by Celgene Corporation and Rockefeller University, and accepted for publication on 30 April 1999. The 4-amino substituted analogues of thalidomide (CC-5013 & CC-4047) inhibited tumor necrosis factor α (TNF-α) release in lipopolysaccharide (LPS) stimulated human PBMC.

TNF-α Inhibition in LPS Stimulated Human PBMC and Whole Blood by Thalidomide Analogues.

Compound #	Compound Name	TNF-α Inhibit (%). At 100 μM	TNF-α IC50 (nM)	Whole blood TNF-α IC50 (nM)
5a	CC-4047	95	13	25
(S)-5a	CC-4047	99	3.9	14
(R)-5a	CC-4047	85	93	73
8a	CC-5013	74	100	480

2.6.2.4 Safety pharmacology

1 CC-5013: Cardiovascular and respiratory effects in the anaesthetised dog following intravenous infusion (1398/124-D1140). Vol. 1.3, p 524.

Conducted by [redacted] in compliance with GLP and QA regulations. The study was initiated on 3 February 1999. Intravenous administration of CC-5013 at 2, 10 and 20 mg/kg did not produce any significant effect on cardiovascular and respiratory systems of the anesthetized dog when compared with the vehicle control (20% PEG 400) group.

The relevant safety pharmacology results are summarized below.

System	Species/ Number of animals	Route	Dose (mg/kg)	Time (min) of observation	Parameters measured	Criteria	Findings
CVS	Beagle dogs 2/sex/group	IV	0 2 10 20	0, 2, 10, 15, 30, 45, 60, 90, 120	Blood pressure	≥10%	N.S.*
					Heart rate	≥20%	N.S*.
					ECG	≥11%	N.S*
					Respiration**	≥20	N.S*

* = not significant

** - peak inspiratory and expiratory flow, tidal volume, minute volume and rate of respiration

Plasma concentrations increased in a dose-proportional manner as shown below.

Mean plasma concentration of CC-5013 after IV administration to dog

Dose (mg/kg)	Cmax (ng/mL) after 2 min	Cmax (ng/mL) after 30 min
2	2,719	1,027
10	13,110	6,084
20	24,843	13,656

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

1 CC-5013: A study to determine the oral bioavailability in the rat, dog, and monkey (Draft and final report: 1398/124-D1140). Vol. 1.3, p 697.

Conducted by ___ It is a draft report and compliance with GLP and QA regulations are not signed. The study was initiated on 5 May 1998. Single oral and intravenous administrations of CC-5013 to male rats, monkeys and dogs were well tolerated. Bioavailability was highest in dogs (88%) followed by rats (68%) and monkeys (50%).

Studies in rats

species and strain: Sprague Dawley rats
#/time point: Oral: 3 M
intravenous: 4 M
age: 12 weeks
weight: 165-225 g
dosage groups: oral: 100 mg/kg
intravenous: 10 mg/kg
route, form and volume: oral: suspension in 1% (w/v) aqueous carboxymethyl cellulose, 5 mL/kg.
intravenous: fine suspension in PEG 400:Intralipid 20 (1:4 v/v), 1.25 mL/kg
blood sampling: oral: pre-dose, 5, 10, 20, 30 min; 1, 2, 4, 6, 8, 12, 24, and 48 h after dosing
intravenous: pre-dose, 2, 5, 10, 20, 30, min; 1, 2, 4, 8, 12, 24, 48 h post-dose.

Results

Parameter	Oral	intravenous
Cmax (ng/mL)	26,023	18,137
Tmax (h)	0.5	0.03
AUC 0-48 h (ng.h/mL)	82,829	12,217
T1/2 (h)	2.3	3.0
Cl tot (mL/min/kg)	-	13.6
Vd (L/kg)	-	3.6
F (%)	68	-

Studies in dogs

species and strain: beagle dogs
#/group: 4M
age: 7-9 months
weight: 13.1 - 14.4 kg
dosage groups: oral: 80 mg/kg
Intravenous: 10 mg/kg

route, form, and volume: oral: suspension in 1% (w/v) aqueous carboxymethyl cellulose, 5 mL/kg.
intravenous: fine suspension in PEG 400:Intralipid 20 (1:4 v/v), 1.2 mL/kg
blood sampling: oral: pre-dose, 5, 10, 20, 30 min; 1, 2, 4, 6, 8, 12, 24, and 48 h after dosing.
intravenous: pre-dose, 2, 5, 10, 20, 30, min; 1, 2, 4, 8, 12, 24, 48 h post-dose.

Results

Parameter	Oral	Intravenous
Cmax (ng/mL)	45,111±13,525	28,270±9,160
Tmax (h)	1.3±0.5	0.03±0.0
AUC 0-48 h (ng.h/mL)	226,254±94,163	32,138±2,333
T1/2 (h)	6.4±4.2	2.1±0.6
Cl tot (mL/min/kg)	-	5.2±0.4
Vd (L/kg)	-	1.0±0.3
F (%)	88	

Studies in monkeys

species and strain: cynomolgus monkeys (Macaca fascicularis)
#/group: 4 M
age: 17-40 months
weight: 2.5- 3.55 kg
dosage groups: oral: 100 mg/kg
intravenous: 10 mg/kg
route, form, and volume: oral: suspension in 1% (w/v) aqueous carboxymethyl cellulose, 5 mL/kg.
intravenous: fine suspension in PEG 400:Intralipid 20 (1:4 v/v), 1.25 mL/kg
blood sampling: oral: pre-dose, 5, 10, 20, 30 min; 1, 2, 4, 6, 8, 12, 24, and 48 h after dosing
intravenous: pre-dose, 2, 5, 10, 20, 30, min; 1, 2, 4, 8, 12, 24, 48 h post-dose.

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Results

Parameter	Oral	Intravenous
Cmax (ng/mL)	21,580±4,316	28,733±5,437
Tmax (h)	1.5±0.6	0.03±0.0
AUC 0-48 h (ng.h/mL)	105,347±16,681	21,036±2,923
T1/2 (h)	13.4±9.2	1.3±0.1
Cl tot (mL/min/kg)	-	8.0±1.2
Vd (L/kg)	-	0.9±0.1
F (%)	50	-

2 CC-1088, CC-4047, CC-5013 and CC-7025: Comparative absorption by the Caco-2 cell line (1398/109-D1140). Vol. 1.4, p 843.

Conducted by — in compliance with GLP regulations. The study was initiated on 13 May 1998. CC-5013 was poorly absorbed across Caco-2 monolayer in a paracellular manner.

Solutions of test articles, CC-1088, CC-4047, CC-5013 and CC-7025 were applied to either the apical (0.5 mL) or basolateral (1.5 mL) compartments of the monolayer of Caco-2 cells. The transport experiments were carried out at 370C under aseptic conditions. The transepithelial electrical resistance (TEER) of each monolayer was recorded before and after test article administration.

Results

Apparent permeability of Caco-2 cell monolayers

Test article	Papp x 106 (cm/s) Apical to basolateral	Basolateral to apical	Estimated log P
CC-1088	8.0	18	0.45
CC-4047	34	39	-1.16
CC-5013	<1.7	3.08	-1.99
CC-7025	39	41	1.96

The log P was estimated using an atom/fragment contribution method (J Pharm Sci 1995; 84:83-92)

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2.6.6 TOXICOLOGY

2.6.6.2 Single-dose toxicity

1 CC-5013: Single dose intravenous toxicity study in the mouse [approximation of the minimum lethal dose level] (1398/098-D6144). Vol. 1.4, p 853.

Conducted by — in compliance with GLP and QA regulations. The study was initiated on March 30, 1998. There were no deaths and macroscopic changes in mice treated with CC-5013 at 40 mg/kg by intravenous administration.

species and strain: — CD-1(ICR)BR mice
#/sex/group: 5
age: 6 - 7 weeks
weight: 32 - 36 g (males), 23 - 26 g (females).
Drug and lot #: CC-5013, batch # 4068-34-A, lot 2 & batch # 40778-10-D, lot 3,
vehicle: 20% v/v PEG 400 in purified water
dosage groups: 40 mg/kg
route, form, volume, and infusion rate: i.v, 20 mL/kg, infusion into tail vein.
duration: single administration, followed by an observation period of 15 days.
Observations

Clinical signs: 0.5 h and 4 times within the first 4 hours, twice daily on days 2, 3, and 4, once daily from days 5 to 15.
Body weights: days -1, 1, 4, 8, and 15.
Gross pathology: day 15

Results:

Clinical signs: mortality: none
2/5 males were lethargic from 15 to 30 minutes after dosing.
Body weights: no effect
Gross pathology: enlarged heart in one male and renal pelvic dilatation in a second male.

2 CC-5013: Single dose oral toxicity study in the mouse [approximation of the minimum lethal dose level] (1398/098-D6144). Vol. 1.4, p 871.

Conducted by — The study report was submitted with signed and dated GLP and QA compliance statements. The study was initiated on March 30, 1998. There were no deaths, clinical signs or macroscopic changes following oral administration of 2 g/kg CC-5013 to mice.

species and strain: — CD-1(ICR)BR mice
#/sex/group: 5
age: six to seven weeks
weight: 28 to 31 g (males) and 24 to 27 g (females).
drug and lot #: CC-5013, batch # 4068-34-A, lot 2 and batch # 40778-10-D, lot 3
vehicle: 0.5% w/v aqueous solution of carboxymethyl cellulose, batch # 126H0367, by —
dosage: 2000 mg/kg
route, form, volume: oral by gavage, suspension in carboxymethyl cellulose, 20 ml/kg
duration: single administration, followed by an observation period of 15 days.
Observations
Clinical signs: 0.5 h and 4 times within the first 4 hours, twice daily on days 2, 3, and 4, once daily from days 5 to 15.
Body weights: days -1, 1, 4, 8, and 15.
Gross pathology: day 15

Results:

Clinical signs: no deaths and no clinical signs.
Body weights: no effect
Gross pathology: no changes

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3 CC-5013: Single dose intravenous toxicity study in the rat [approximation of the minimum lethal dose level] (1398/097-D6144). Vol. 1.4, p 888.

Conducted by — in compliance with GLP regulations. Quality Assurance was also included. The study was initiated on 30 March 1998. Single intravenous administration of CC-5013 at 40 mg/kg did not cause any mortality, clinical signs or macroscopic changes in rats.

species and strain: — Brl:WH rats
#/sex/group: 5
age: 10-11 weeks
weight: 309-338 g (males) and 188-211 g (females)
drug and lot #: CC-5013, batch # 4068-34-A (lot 2) and batch # 40778-10-D (lot 3)
vehicle: 20% v/v PEG 400 in water, batch # 37H0849 supplied by —
Dosage: 40 mg/kg
Route and volume: intravenous, 20 ml/kg
duration: single administration, followed by an observation period of 15 days.
Observations
Clinical signs: 0.5 h and 4 times within the first 4 hours, twice daily on days 2, 3, and 4, once daily from days 5 to 15.
Body weights: days -1, 1, 4, 8, and 15.
Gross pathology: day 15

Results:

Clinical signs: mortality: none
2 males were lethargic between 15-30 minutes after dosing.
Body weights: No effect on body weight gain
Gross pathology: One male - dark focus on the right anterior lobe of the lungs.
One female - slight uterine bilateral distension.
No other macroscopic changes

4 CC-5013: Single dose oral toxicity study in the rat [approximation of the minimum lethal dose level] (1398/096-D6144). Vol. 1.4, p 906.

Conducted by — in compliance with GLP and QA regulations. The study was initiated on 30 March 1998. There were no deaths in rats following oral administration of 2000 mg/kg of CC-5013.

species and strain: — Brl:WH rats
#/sex/group: 5
age: 10-11 weeks
weight: 288-320 g (males) and 184-218 g (females)
drug and lot #: CC-5013, batch # 4068-34-A (lot 2) and batch # 40778-10-D (lot 3).
vehicle: 0.5% w/v aqueous solution of carboxymethyl cellulose, batch # 126H0367, supplied by —

dosage: 2000 mg/kg
route, form, volume: oral by gavage, suspension in carboxymethyl cellulose, 20 ml/kg
duration: single administration, followed by an observation period of 15 days.
Observations
Clinical signs: 0.5 h and 4 times within the first 4 hours, twice daily on days 2, 3, and 4, once daily from days 5 to 15.
Body weights: days -1, 1, 4, 8, and 15.
Gross pathology: day 15

Results:

Clinical signs: no mortality, pale discolored feces on day 1.
Body weights: no effect on body weight gain
Gross pathology: unilateral (right) slight pelvic dilatation in 2 males and few pale and few dark foci on the lungs of one of these rats.

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2.6.6.3 Repeat-dose toxicity

1 CC-5013: 7 day oral (gavage) range-finding toxicity study in the rat (1398/105-D6154). Vol. 1.4, p 924.

Conducted by — .n compliance with GLP and QA regulations. The study was initiated on Dec. 22, 1998. Daily oral administration of CC-5013 to rats increased urea and creatinine levels and kidney weight in 1/5 low dose (500 mg/kg/day) and in 1/5 high dose (2000 mg/kg/day) male animals. There was no mortality or other adverse effects.

species and strain: — (IGS)CDBR rats
#/sex/group: 5
age: 6 weeks
weight: 174-193 g (males), 141-164 b (females)
drug, lot #, and % purity: CC-5013, batch # 40778-10-D, purity —
vehicle: 1% aqueous carboxymethyl cellulose,

dosage:

Group	Description	Dose (mg/kg/day)
1	Control	0
2	Low	500
3	Intermediate	1000
4	High	2000

route, form and volume: oral by gavage, suspension in carboxymethyl cellulose, 10 mL/kg
duration: once daily for 7 days.
Observations

Clinical signs: 0, 30, 60, 120, 240 minutes after dosing daily
Body weights: days 1, 3, 7 and 8.
Food consumption: daily
Hematology: day 8
Clinical chemistry day 8
Organ weights day 8
Gross pathology: day 8
Histopathology: Not performed
Toxicokinetics: 1, 2, 4, 8, and 24 hours after dosing on days 1 and 7.

Results:

Clinical signs: Mortality: 2 control females under anesthesia on day 8.
No abnormal clinical signs.
Body weights: No effect
Food consumption: Unaffected
Hematology:

Changes (%) from the concurrent control

Parameter	LD		MD		HD	
	♂	♀	♂	♀	♂	♀
Hg	7↓	-	10↓	-	9↓	-
RBC	9↓	-	11↓	-	13↓	-
PCV	7↓	-	9↓	-	8↓	-

- Less than 5% change

Clinical chemistry:

Group	Sex	# of animals	Urea	Creatinine
2	M	1/5	2 fold ↑	1.3 fold↑
4	M	1/5	2 fold ↑	3 fold ↑

No other effects on other male and female animals

Organ Weights: Kidney weight ↑ of the above mentioned two animals
Gross pathology: none
Toxicokinetics: No conclusions can be drawn due to contamination (controls) and swapped labeling between collection and analysis of samples.

2 CC-5013: 28 day oral (gavage administration) toxicity study in the rat (1398/107-D6154). Vol. 1.4, p 1209.

Conducted by — in compliance with GLP regulations. Quality Assurance was also included. The study was initiated on Dec. 22, 1998. The NOAEL for this 28-day oral toxicity study in the rat was 300 mg/kg/day (1800 mg/m2/day). Reduction in body weight gain, hemoglobin, and packed cell volume, increased prothrombin time and proteinuria, and moderate to severe tubular nephropathy/nephritis were observed in high dose rats (1000 mg/kg/day). Toxicokinetics showed that AUC increased less than proportional with dose in male and female animals.

species and strain: Sprague Dawley rats of — CD(SD)IGSBR strain
#/sex/group: 10 for main and 12 for satellite
age/weight: ♂: ~28 days / 183.5 - 253.6 g
♀: ~28 days / 145.8 - 185.8 g
satellite groups used for toxicokinetics: 3 groups, 12 animals/sex/group
drug, lot #, and % purity: CC-5013 (lot No. 6 &7, batch # 60854-02), — purity
control article/vehicle: 1% aqueous carboxymethyl cellulose (w/v)

dosage:

Group #	Description	Dose level (mg/kg/day)
1	Control	0
2	Low	100
3	Intermediate	300
4	High	1000

formulation: suspension in 1% carboxymethylcellulose

route of administration: oral by gavage, 10 mL/kg

duration: once daily for 28 days
Observations

Clinical signs: 0.5, 1, 2 hours post dose daily
Body weights: first day of dosing and then weekly
Food consumption: weekly
Ophthalmoscopy: all animals before treatment, and on control and high dose animals in Week 4.
Hematology: week 4
Urinalysis: weeks 1 & 4
Organs weighed: adrenals, brain, heart, kidney, liver, ovaries, pituitary, prostate, spleen, testes, thyroid,
Gross pathology: at study termination on Day 29
Histopathology: at scheduled study termination on Day 29.
Toxicokinetics: Three animals/sex from satellite groups were bled pre-dose, 15, 30, 60, 120, 240, 480, and 1440 minutes after dosing on days 1 and 28. The plasma was stored deep frozen.

Results:

Clinical signs: mortality: 1/10 MD ♀ killed on Day 29 due to poor conditions and considered non-drug related. Red staining on trayliners under two male and one female high dose animals during week 3 and under four high dose males during week 4 of dosing. This may be related to kidney changes.
Body weights:
Group mean body weight gains for high dose group from start to week 4.

Sex	Week 1	Week 2	Week 3	Week 4
Male	155.0±14.8	155.7±14.8	144.0±15.9	97.9±30.6*
Female	66.2±7.5	57.6±31.2	76.2±7.4	62.3±12.2

*p<0.001, ANOVA, regression and Dunnett's.

HD: ♂ body weight gain ↓ 37 %.

The body weight gain of all females and males (except group 4) did not change significantly when compared with controls.
Food consumption: high dose males (26%↓) and females (10%↓) during week 4.
Ophthalmoscopy: No effect
Electrocardiography: Not performed
Hematology: All these changes except neutrophil (large variations) were within 9% of the control values and are considered of limited biological significance for cancer patients.

Statistically significant group mean hematology findings during week 4 are given below.

Parameter	Sex	Group 1	Group 4
Hb (g/dL)	M	16.3±0.3	15.1±1.1**
PCV (%)	M	46.6±0.9	42.5±3.3**
MCV (fl)	M	58.9±2.2	55.1±2.4***
MCH (pg)	M	20.6±0.5	19.5±0.8**
Neutrophil	M	1.3±0.3	4.6±1.9***
PT (S)	M	19.5±1.2	20.9±2.0 DR*
	F	19.6±0.8	21.2±1.9*

* p<0.05 ** p<0.01 *** p<0.001 DR = significant dose response test

Clinical chemistry: Group mean urea and creatinine levels were increased in high dose males (8/10) and females (4/10).

Changes (%) in blood chemistry parameters compared to the control (Week 4)

Parameter	MD		HD	
	M	F	M	F
AST	-	-	17 ↓**	13 ↓ *
ALT	-	-	32 ↓ ***	13 ↓
IN PHOS	-	-	14 ↑ *	14 ↑*
UREA	-	-	262 ↑ ***	97 ↑*
T BILI	-	-	32 ↑*	22 ↑
CREAT	6↓ *	-	125 ↑*	47 ↑*
TOT CHOL	-	-	59 ↑***	9 ↓
TRIGS	-	-	50 ↑*	53 ↑*

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

Urinalysis: HD, week 4 -↑ proteinuria, blood and CC-5013 in the urine.

Organ Weights: HD - ↑kidney weight (62% in ♂**, 42% in ♀*)
*$p<0.05$ **$p<0.001$

Gross pathology: Kidney appearance in high dose animals

Parameter	Males	Females
Number examined	10	10
Dark area	1	
Large	8	3
Pale area	3	1
Mottled	5	
Pelvic dilatation	1	1
Pale focus	2	2
Irregular surface	2	2
Pale	1	3
Firm		1

Histopathology: Histopathology of Kidneys

Parameter	Male				Female			
	G1	G2	G3	G4	G1	G2	G3	G4
Number examined	10	10	10	10	10	10	10	10
Corticomedullary mineralization				1	6	1	5	8
Hydronephrosis	1	2		4			3	1
Tubular nephropathy/nephritis				9				4

Toxicokinetics: The AUC did not increase linear with dose.

Dose (mg/kg/day)	Sex	Day	Cmax (ng/mL)	Tmax (h)	AUC (ng.h/mL)
100	M	1	22,089	1	83,869
		28	21,049	1	74,922
	F	1	22,952	1	137,694
		28	18,508	1	65,836
300	M	1	52,322	1	147,092
		28	20,802	1	127,275
	F	1	51,192	4	348,894
		28	24,341	1	141,884
1000	M	1	67,515	1	265,332
		28	52,851	2	783,647
	F	1	59,101	4	379,256
		28	56,179	4	352,443

3 CC-4047 & CC-5013: 28 day oral (gavage administration) toxicity study in the monkey (1398/126-D6154). Vol. 1.5, p 1457.

Conducted by — in compliance with GLP regulations. Quality Assurance was also included. The study was initiated on Oct. 2, 1998. The repeat dose NOAEL was 0.2 mg/kg/day (2.4 mg/m2/day) for CC-4047 and 2.0 mg/kg/day (24 mg/m2/day) for CC-5013 for primates via oral gavage once daily for 28 days. 2 mg/kg/day was the only dose tested for CC-5013. 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 0-24 h and Cmax values for CC-5013 did not change significantly between days 1 and 28.

species and strain: cynomolgus monkeys, Macaca fascicularis
#/sex/group: 3
age: 15 to 25 months
weight: 1.6 to 2.9 kg
satellite groups used for toxicokinetics or recovery: None
drug, lot # and % purity: CC-5013 (— lot # 3, batch # 40778-10-4, — purity)
CC-4047 (— lot # 8, batch # 40753-09, purity not stated)

dosage groups

Group #	Description	Dose level (mg/kg/day)
1	Control	0
2	CC-4047 low	0.2
3	CC-4047 high	2.0
4	CC-5013 high	2.0

formulation/vehicle: suspension in 1% carboxymethylcellulose
route, volume: oral (gavage), 4 mL/kg
Observations

Clinical signs: daily
Body weights: weekly
Food consumption: weekly
Ophthalmoscopy: pretreatment and week 4
EKG: pretreatment and week 4
Hematology: blood samples were withdrawn from the femoral vein/artery before treatment and on Days 3, 7, 10, 14, 17, 21, 24 and 28.
Urinalysis: pretreatment and in week 4.
Organs weighed: adrenals, brain, heart, kidney, liver, ovaries, pituitary, prostate, spleen, testes, thyroid, uterus
Gross pathology: day 29
Histopathology: day 29
Toxicokinetics: predose, 30 min, 1, 2, 4, 8, and 24 hours after dosing on Days 1, 14 and 28.

Results:

Clinical signs: Moribund sacrifice: 1M 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].
Loose feces: 1 M & 1 F 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.

Number of animals with finding

Group	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:

Toxicokinetic Parameters of CC-4047 at 0.2 mg/kg/day

Parameter	Male		Female	
	Day 1	Day 28	Day 1	Day 28
Cmax (ng/mL)	79	113	125	83
Tmax (h)	1.7	16.0	1.7	8.0
AUC 0-24 h (ng.h/mL)	195	2,258	734	1,493

Toxicokinetic Parameters of CC-4047 at 2.0 mg/kg/day

Parameter	Male		Female	
	Day 1	Day 28	Day 1	Day 28
Cmax (ng/mL)	356	735	385	651
Tmax (h)	2.	4.5	0.8	1.3
AUC 0-24 h (ng.h/mL)	4,882	8,116	5,134	6,133

Toxicokinetic Parameters of CC-5013 at 2.0 mg/kg/day

Parameter	Male		Female	
	Day 1	Day 28	Day 1	Day 28
Cmax (ng/mL)	1,590	1,5040	1,692	1,552
Tmax (h)	0.5	0.8	0.5	0.5
AUC 0-24 h (ng.h/mL)	3,138	2,865	2,778	2,765

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4 CC-5013: 28 day (gavage administration) toxicity study in the monkey (1398/108-D6154). Vol. 1.6, p 1811.

Conducted by ~~[redacted]~~ in compliance with GLP regulations. Quality Assurance was also included. The study was initiated on Dec. 10, 1998. The administration of CC-5013 at 20 mg/kg/day for 28 days by oral gavage to monkeys produced lymphoreticular and hemopoietic systems toxicity (↓M/E ratio, altered marrow hemopoiesis, hyalinised germinal centers in spleen) and nephrotoxicity (↑BUN and ↑creatinine). Toxicokinetic data did not show accumulation of the drug in the primate over the period of the study.

species and strain: cynomolgus monkeys (Macaca fascicularis)
age: 15 to 25 months
weight: 1.60 to 2.50 kg

satellite groups used for toxicokinetics or recovery: none
drug, lot #, and % purity: CC-5013 — lot # 5, batch # 60832-01,
formulation/vehicle: suspension in 1% carboxymethyl cellulose (w/v)

dosage groups:

Group	Description	Dose (mg/kg/day)	Animals/group Male	Female
1	Control	0	3	3
2	Test	20	3	3

route, volume: orally by gavage, 4 mL/kg
Observations

Clinical signs: daily
Body weights: weekly
Food consumption: weekly
Ophthalmoscopy: pretreatment and in week 4
EKG: pretreatment and in week 4.
Hematology: on days 3, 7, 10, 14, 17, 21 and 28.
Clinical chemistry day 29
Urinalysis: pretreatment and in week 4
Organ weights: day 29
Organs weighed: adrenals, brain, heart, kidney, liver, ovaries, pituitary, prostate, spleen, testes, thyroid and uterus.
Gross pathology: day 29
Histopathology: day 29
Toxicokinetics: pre-dose, 0.5, 1, 2, 4, 8, and 24 hours after dosing on days 1 and 28.

Results:

Clinical signs: moribund: 1♂, group 2, sacrificed on day 18
vomiting: occasional [1♂ control, and 2♂ and 1♀ test group]
Body weights: no effect
Food consumption: unaffected
Ophthalmoscopy: no treatment related abnormalities
Electrocardiography: heart rate unaffected
Hematology: large variations, no significant differences.
Bone marrow (myelogram) examination:

Group/sex	Total erythropoietic cells	Total myelopoietic cells	Myeloid/erythroid ratio
1/M	48.8±1.8	47.4±2.3	1.0±0.1
2/M	48.3±4.7	48.9±5.2	1.1±0.2
1/F	45.7±2.6	51.5±2.5	1.1±0.2
2/F	63.7±2.2	30.3±3.3	0.5±0.1

The myeloid/erythroid ratio for treated males was comparable with the concurrent male controls.

Clinical chemistry: moribund (2 M) - ↑urea (up to 14 fold), creatinine (up to 8 fold)
other animals - no difference

Urinalysis: unaffected
Organ Weights: Combined thyroid/parathyroid weight ↓44% (absolute) and 45% (relative to b.w.)
Gross pathology: unaffected

Histopathology:

Organ	Finding	Group 1 M	2 M	1 F	2 F (# of animals/3 total animals)
Femur +marrow	Altered hemopoiesis				2
Sternum +marrow	Altered hemopoiesis				2
Liver	Focal necrosis		1		1
Spleen	Hyalinised germinal centers		2		3
Adrenal	Mineralization		1		
Kidney	Inflammatory cell foci		1	1	3
	Pyelitis		1		
	Cyst				1
Stomach	Lymphocytic gastritis		1		2
Thymus	Atrophy		2		3
Brain	Mineralization				1

Toxicokinetics: Toxicokinetic Parameters of CC-5013 at 20 mg/kg/day

Parameter	Male Day 1	Male Day 28	Female Day 1	Female Day 28
Cmax (ng/mL)	5,329	6,604	3,572	6,573
Tmax (h)	0.83	0.75	0.83	0.67
AUC 0-24 h (ng.h/mL)	22,234	19,017	12,970	16,269

6.6.6.4 Genetic toxicology

1 CC-5013: Reverse mutation in four histidine-requiring strains of Salmonella typhimurium and two tryptophan-requiring strains of Escherichia coli (1398/100-D5140). Vol. 1.6, p 1982.

Conducted by — in compliance with GLP and QA regulations. The study was initiated on 1 April 1998. CC-5013 did not induce mutation in Ames test at concentrations up to 5,000 µg/plate with or without metabolic activation (S-9).

Method: Ames test
Strains/Species/Cell line: Salmonella typhimurium (TA98, TA100, TA1535, TA1537) and Escherichia coli (WP2 pKM101, WP2 uvrA pKM101)
Test Agent Stability: Stable
Metabolic Activation System: Aroclor 1254 induced rat liver post-mitochondrial fraction (S-9)
Controls, Vehicle: Dimethyl sulphoxide
Positive Controls and 2-aminoanthracene: 2-Nitrofluorene, sodium azide, 9-aminoacridine, 4-nitroquinoline,
Exposure Conditions: 370C
Incubation times: 3 days
Doses used: Range finding - 8, 40, 200, 1000, 5000 µg/plate
Definite exp. - 312.5, 625, 1250, 2500, 5000 µg/plate
Analysis: 100±10%
No. plates/replicates analyzed: 3 without and with S-9
Counting method: Electronically using — Colony counter or manually

Results:
Summary of mean revertant colonies

Compound	Dose (µg/plate)	S-9	TA98	TA100	TA1535	TA1537	WP2 Pkm101	Wp2 UVRa Pkm101
DMSO	100 µl	-	23±2	112±5	13±3	8±2	18±2	165±12
CC-5013	5000	-	24±4	135±6	13±3	6±4	23±6	136±15
Positive controls	2 – 10	-	1151±41	910±80	472±14	803±63	587±74	1346±214
DMSO	50 µl	+	28±8	133±10	18±4	8±3	21±8	149±15
CC-5013	5000	+	29±5	140±13	15±4	9±2	27±6	195±18
Positive controls	5	+	1762±171					572±17

Conclusions: No sign of toxicity and no mutagenic potential in the presence or absence of metabolic activation system.

APPEARS THIS WAY
ON ORIGINAL

2 CC-5013: Induction of chromosome aberrations in cultured human peripheral blood lymphocytes (1398/118-D5140). Vol. 1.7, p 2055.

Conducted by [redacted] in compliance with GLP and QA regulations. The study was initiated on 30 April 1998. Two independent experiments using human lymphocyte cultures from a male and a female donor were performed. CC-5013 did not induce chromosome aberrations in cultured human peripheral blood lymphocytes.

Experiment 1 (Donor sex: Male)

Method:

S-9	Treatment + recovery (hours)	Concentration (µg/mL) CC-5013	Positive control	Vehicle control
-	20 + 0	1271, 1815, 2593	NQO*, 5ug/mL	DMSO***
+	3 + 17	1271, 1815, 2593	CPA**, 12.5 µg/mL	DMSO***

*NQO – 4-nitroquinoline 1-oxide
** CPA – cyclophosphamide
*** DMSO – dimethyl sulphoxide

Strains/Species/Cell line: Human lymphocyte
Test Agent Stability: CC-5013, batch number 40778-10-D, stable.
Metabolic Activation System: S-9 prepared from male SD rats
No. of slides analyzed: Duplicate
Counting method: Microscopic

Results:
Cells with structural aberrations

Treatment (µg/mL)	S-9	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance	Mitotic index
Solvent	-	200	1	1		2.0
CC-5013 (2593)	-	200	2	1	NS	2.1
*NQO (5)	-	141	48	46	P ≤0.001	
Solvent	+	200	1	1		7.0
CC-5013 (2593)	+	200	1	1	NS	5.3
**CPA, 12.5	+	200	31	25	P ≤0.001	

*NQO – 4-nitroquinoline 1-oxide **CPA – cyclophosphamide

Conclusions: The study was accepted as valid (final concentration was 10 mM). No chromosomal aberration was induced by CC-5013.

Experiment 2 (Donor sex: female)
Method:

S-9	Treatment + recovery (hours)	Concentration (µg/mL) CC-5013	Positive control	Vehicle control
-	20 + 0	1660, 2074, 2593	NQO*, 2.5µg/mL	DMSO***
+	3 + 17	1660, 2074, 2593	CPA**, 12.5 µg/mL	DMSO***
-	44 + 0	2593		DMSO***
+	3 + 41	2593		DMSO***
-	3 + 17	2593		DMSO***

*NQO – 4-nitroquinoline 1-oxide ** CPA – cyclophosphamide *** DMSO – dimethyl sulphoxide

Cell line: Human peripheral blood lymphocytes
Test Agent Stability: Stable
Metabolic Activation System: S-9
No. slides: Duplicate
Counting method: Microscopy

Results:

Cells with structural aberrations

Treatment (µg/mL)	S-9	Treatment + recovery (hours)	Cells scored	Cells with aberration including gaps	Cells with aberration excluding gaps	Significance	Mitotic index
Solvent	-	20 + 0	200	4	4		7.5
CC-5013, (2593)	-	20 + 0	200	6	4	NS	4.3
NQO, (2.5)	-	20 + 0	200	37	32	P ≤0.001	-
Solvent	+	3 + 17	200	0	0		7.3
CC-5013 (2593)	+	3 + 17	200	2	0	NS	6.5
CPA, (12.5)	+	3 + 17	200	59	53	P ≤0.001	-
Solvent	-	44 + 0	200	2	1		6.1
CC-5013 (2593)	-	44 + 0	200	5	4	NS	3.4
Solvent	+	3 + 41	200	0	0		4.7
CC-5013 (2593)	+	3 + 41	200	0	0	NS	4.9

Conclusions: The study was accepted as valid. No chromosomal aberration was induced by CC-5013 in the presence or absence of S-9.

3 CC-5013: Mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells (MLA) using the — technique (1398/95-D5140). Vol. 1.7, p 2122.

Conducted by — in compliance with GLP and QA regulations. The study was initiated on 6 April 1998. Two independent experiments were conducted. CC-5013 did not increase mutant frequency at the thymidine kinase locus of mouse lymphoma cells in the presence or absence of S-9.

Method:
Cell line: L5178Y TK +/- mouse lymphoma
Test Agent Stability: Stable and used within 2 hours after initial formulation.
Metabolic Activation System: S-9
Negative Controls, Positive Controls: DMSO; 4-nitroquinoline 1-oxide, benzo(a)pyrene
Exposure Conditions: 3 hours rocking at 370C.
Incubation and sampling times: 8 days
Doses:
Range finding: 112.5, 225, 450, 900, 1800, and 3546 µg/mL
Definite: 162.5, 325, 650, 1300, 2000, 2250, and 2593 µg/mL
Analysis: — by HPLC.
No. of plates: 2 for survival and 4 for TFT resistance
Cytotoxic endpoints: 63% relative survival in the absence of S-9 and 94% relative survival in the presence of S-9. ICH S2A criteria were met based on limited solubility and 10-mM final concentration.

Results:

Treatment (µg/mL)	S-9	%RS***	MF x 10-6 ****
Control (0)	-	100	64.6
Control (0)	+	100	77.9
CC-5013 (162.5)	-	95.4	85.6
CC-5013 (162.5)	+	101.9	NA
CC-5013 (2593)	-	77.5	90.4
CC-5013 (2593)	+	87.5	95.1
NQO* (0.1)	-	70.4	390.9
BP**(3)	+	48.1	861.3

* 4-nitroquinoline 1-oxide **benzo(a)pyrene
*** percentage relative survival **** mutant frequency

Second experiment gave similar results.

Conclusions: The study was accepted as valid. CC-5013 did not increase mutant frequency in mouse lymphoma cells in the presence or absence of S-9.

2.6.6.6 Reproductive and developmental toxicology

1 Developmental and reproductive toxicity screening study for effects on embryo-fetal development in rabbits (0329DC35.001). Vol. 1.7, p 2188

Conducted by [redacted] in compliance with GLP and QA regulations. The study was initiated on 15 July 1997. In this screening study, CC-5013, CC-4013, CC-4047, CC-6022, thalidomide, or acetylsalicylic acid [ASA] pretreatment followed by thalidomide were administered orally by intubation to pregnant rabbits from gestation days 8 through 10 inclusive. Thalidomide treatment (250 mg/kg/day) increased the number of resorptions, lowered fetal body weight, and caused abnormal limb development (abnormal curvature and rotation of limbs and missing digits) in rabbits. Thalidomide analogues (CC-5013, CC-4013, CC-4047, CC-6022) at a dose level of 100 mg/kg/day did not effect embryo-fetal development or cause any maternal toxicity. ASA pretreatment did not decrease the teratogenic potential of thalidomide.

species and strain: Oryctolagus cuniculus/New Zealand White rabbit/ — SPF
#/group: 4
age: 7.75 - 8.0 months
weight: 3.5 - 4.9 kg
satellite groups used for toxicokinetics or recovery: none
drug and lot #:

Compound	Lot #	Supplier
CC-4013	40566-05-A	Celgene Corp
CC-4047	40563-12-A	"
CC-5013	40553-46-C	"
CC-6022	40562-20-C	"
Thalidomide	574-574-96-005	"
Acetylsalicylic acid	46H1053	—
Carboxymethyl cellulose	33H0576	"

% purity: not provided
formulation/vehicle: suspension in carboxymethyl cellulose
dosage:

Group #	Test material	Dose level (mg/kg/day)
1-vehicle control	1% carboxymethyl cellulose	0
2-positive control	Thalidomide	250
3- control	ASA/thalidomide*	100/250
4-test	CC-4013	100
5-test	CC-4047	100
6-test	CC-5013	100
7-test	CC-6022	100

*Animals were dosed with 100 mg/kg acetylsalicylic acid approximately 2 hours prior to 250 mg/kg thalidomide administration.

Route and volume: oral by intubation, 3 ml/kg

Observations

Clinical signs: Twice daily during dosing period and daily from gestation day 11 and onwards.
Body weights: Gestation days 0, 6, 8, 9, 10, 15, 22, and 29
Gross pathology: Gestation day 29

Results:

Mortality: None
Premature deliveries: None
Clinical signs: None in any group
Body weights: No significant difference
Maternal gross pathology: No treatment related findings.
Cesarean Sections:
Maternal cesarean data: No significant differences in mean total corpora lutea, mean implantation, mean viable or nonviable fetuses per doe/group.

Thalidomide treated animals showed significantly lower fetal body weight and increased resorptions.

Fetal Gross observation:Control and test treated animals - no external gross findings

Positive control animals - abnormal curvature or rotation of the forelimbs or hindlimbs, missing and/or abnormal digits and thick and shortened tail were observed.

Conclusions: The oral administration of 100 mg/kg/day of CC-5013, CC-4013, CC-4047, and CC-6022 during gestation days 8 through 10 did not cause any adverse effects on the embryo-fetal development in the rabbit. This study was uninformative since doses causing maternal toxicity were not used, nor were doses as high as thalidomide used. Thalidomide treatment at 250 mg/kg/day during gestation days 8 to 10 resulted in abnormal limb development. The abnormalities included abnormal curvature and rotation of limbs and missing digits. Richard et al. (J Pharm. Exp Thera. 1996; 277:1649) had shown that intraperitoneal administration of acetylsalicylic acid (ASA) reduced the incidence of abnormal limb development in thalidomide-treated does. ASA is an irreversible prostaglandin H synthetase inhibitor. Oral administration of ASA before thalidomide treatment did not reduce abnormal limb development compared to thalidomide alone treated animals in the present study.

APPENDIX B

TABLE OF CONTENTS

2.6.2 PHARMACOLOGY

2.6.2.2 Primary pharmacodynamics

1. Inhibition of TNF-α in Peripheral Blood Mononuclear Cells and Human Whole Blood

CC-5013 was assessed in vitro for ability to inhibit TNF-α production following liposaccharide (LPS)-stimulation of human peripheral blood mononuclear cells (PBMC) or human whole blood. CC-5013 was dissolved in DMSO and added to tissue culture plates containing PBMCs. Cells were incubated for 20 hours with CC-5013 and supernatants were collected for determination of TNF-α levels. The whole human blood assay was run in a similar fashion to the PBMC assay except heparinized whole human blood was plated directly onto microtiter plates. The assay was then continued like that of the PBMC assay. The IC50 of CC-5013 for inhibiting the production of TNF-α following LPS-stimulation of PBMC was ~100 nM; the IC50 for inhibition of TNF-α production in human whole blood was ~480 nM. Thalidomide, in contrast, displayed an IC50 of ~194 nM for inhibiting TNF-α production following LPS-stimulation of PBMC.

2. Modulation of Pro-Inflammatory and Anti-Inflammatory Cytokines

CC-5013 was assessed in vitro for ability to inhibit interleukin production following liposaccharide (LPS)-stimulation of human peripheral blood mononuclear cells (PBMC). CC-5013 was tested at its TNF-α IC50, 3-fold TNF-α IC50 and 10-fold TNF-α IC50. C-5013 exhibited dose-dependent inhibition (all concentrations) of LPS-stimulated production of the pro-inflammatory cytokines IL-1β and IL-6 by PBMC. It also increased (all concentrations) the production of the anti-inflammatory cytokine IL-10 by LPS-stimulated PBMC.

2.6.2.4 Safety pharmacology

1. Effects of Oral CC-5013 on General Activity and Behavior (Irwin Test) in the Rat

This safety pharmacology study (#1398/437) conducted by ——— ——— in accordance with GLP regulations, examined the effects of orally administered CC-5013 on the general activity and behavior of the rat. Groups of six male Wistar rats received a single administration of CC-5013 (500, 1000 or 2000 mg/kg) or vehicle (1% carboxymethylcellulose solution) by oral gavage. Observations in the Irwin test battery were performed at 30, 60, 90, 180 and 300 min after dosing. The animals were kept for 7 days following the day of dosing for general observation.

The oral administration of CC-5013 at dose levels of 500, 1000 and 2000 mg/kg produced no effects on behavior or general activity in male rats when compared to the vehicle-treated animals in this study.

Toxicokinetic data obtained from a previous 7-day toxicity study (— # 1398/105) in which CC-5013 was administered orally to male Sprague-Dawley rats at dose levels of 500, 1000 and 2000 mg/kg for 7 days showed mean Cmax values of 56,047, 73,773 and 68,045 ng/ml, respectively. In a 28 day oral toxicity study in male and female Sprague-Dawley rats — # 1398/107), initial doses of 100, 300 and 1000 mg/kg yielded Cmax values of 22,089, 52,322 and 67,515 ng/ml of CC-5013, respectively, and AUC values of 83,869, 147,092 and 265,332 ng•h/ml, indicating that systemic exposure was likely achieved in Wistar rats at the dose levels used for the Irwin test.

2. Effects of CC-5013 on Cloned HERG Channels Expressed in Mammalian Cells

This safety pharmacology study (#031205.DFN), conducted by — , and in accordance with GLP regulations, examined the in vitro effects of CC-5013 on ionic currents in voltage-clamped human embryonic kidney (HEK-293) cells that express the human gene HERG. CC-5013 was dissolved in physiological saline with 0.3% DMSO and added to the cell culture. Four concentrations (22, 66, 204 and 787μM) of CC-5013 were tested (3 cells/conc.). The vehicle was used as the negative control and terfenadine and E4031 were used as positive controls.

CC-5013 inhibited HERG current amplitude by 7.7% after the highest concentration (787μM) tested (Table 1). The vehicle control reduced HERG current amplitude by 0.7%; the positive control agent, terfenadine, at a concentration of 60nM inhibited HERG current amplitude by 79%. The IC50 for the inhibitory effect of CC-5013 on HERG current could not be determined but was estimated to be higher than 787uM.

Table 1. Inhibition of HERG Current.

Treatment	Concentration	N=	Mean % Inhibition of HERG Current Amplitude
Vehicle	-	3	-0.7 (increase in current)
CC-5013	22 μM	3	0.7
	66 μM	3	2.6
	204 μM	3	2.5
	787 μM	3	7.7
Terfenadine	60 nM	3	79.0

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

1. In Vitro Binding of CC-5013 to Rat, Rabbit, Monkey and Human Plasma Proteins

This study (#1398/295) conducted by —, in accordance with GLP regulations, examined the extent of in vitro binding of CC-5013 to plasma proteins. Radiolabelled (C14) CC-5013 was added to plasma obtained from rats, rabbits, monkeys and humans at concentrations of 30, 100, 1000, 3000 and 10,000 ng/ml and incubated at 37oC for 15 min prior to microfiltration. The filtrate was analyzed for radioactivity by liquid scintillation spectroscopy. Plasma protein binding was calculated from the equation:

$$\% \text{ free fraction} = \frac{\text{radioactive conc. of filtrate}}{\text{radioactive conc. of unfiltered sample}} \times 100$$

$$\% \text{ binding} = 100 - \% \text{ free fraction}$$

All species showed a low degree of binding of 14C-CC-5013 to plasma proteins over a wide range of concentrations (Table 2). The corresponding free fraction was similar for rat (81.1%), rabbit (81.2%), monkey (77.3%) and human (70.8%).

Table 2. Plasma Protein Binding*

Species	Mean % Binding to Plasma Protein
Rat	18.9
Rabbit	18.8
Monkey	22.7
Human	29.2

* Mean values obtained with CC-5013 concentrations of 30 to 10000 ng/ml

2. Metabolism of CC-5013 by Human Liver Microsomes In Vitro

This study (#1398/335) conducted by — in accordance with GLP regulations, investigated the metabolism of CC-5013 to determine the involvement and affinity of specific CYP450 isozymes using human liver microsomes.

Radiolabelled CC-5013 (10 uM) was added to suspensions of pooled human liver microsomes and incubated for up to 60 min. The metabolic activity was stopped by the addition of acetronitrile, the mixture was centrifuged and aliquots of the supernatant were analyzed by HPLC for detection of metabolic products.

No in vitro metabolism of CC-5013 was observed in incubations with human liver microsomes after 10 or 60 min. The radiochromatogram revealed only a single peak, consistent with CC-5013, and no other metabolic products. Thus, it appears that CC-5013 is resistant to CYP450 phase I metabolism.

3. Metabolism of CC-5013 in Isolated Human Hepatocytes

This study (#1398/348) conducted by — , in accordance with GLP regulations, investigated the metabolism of CC-5013 in isolated human hepatocytes to determine the extent of Phase II (conjugative reactions) metabolism.

Solutions of 14C-CC-5013 in the incubation medium (Leibovitz L-15 medium) were prepared and added to suspensions of human hepatocytes to achieve final CC-5013 concentrations of 1, 5 or 25 μM. The samples were incubated at 37oC for up to 6 hours. Control incubations were performed in the absence of hepatocytes. After incubation, methanol was added to terminate metabolic activity and the samples (in duplicate) were centrifuged and aliquots of the supernatant were analyzed by HPLC for CC-5013 and metabolic products. A solution of 14C-7-ethoxycoumarin (50 μM) was used as the positive control.

The HPLC analysis showed no metabolism of CC-5013 at any of the concentrations tested. Minor peaks observed on the radiochromatogram were considered to be hydrolysis products as they were formed in the negative control (CC-5013 without hepatocytes) The recovery of 14C-CC-5013 from each incubation with human hepatocytes in suspension was > 95% (Table 3). The HPLC analysis showed Phase II metabolic products formed with the positive control, 14C-7-ethoxycoumarin (Table 4).

Table 3

Recovery of (^{14}C)-CC-5013 from each incubation with fresh human hepatocytes in suspension culture

Nominal concentration of (^{14}C)-CC-5013 (μM)	Incubation period (h)	Replicate	% recovery	
			Donor 1	Donor 2
1	0	1	104	97.4
		2	104	95.7
	1	1	103	99.1
		2	104	91.6
	2	1	101	102
		2	112	98.6
	4	1	109	95.2
		2	106	109
	6	1	110	111
		2	105	112
5	0	1	102	100
		2	107	103
	1	1	104	103
		2	107	101
	2	1	104	111
		2	107	106
	4	1	105	111
		2	109	111
	6	1	108	105
		2	106	105
25	0	1	105	101
		2	102	99.3
	1	1	106	104
		2	101	105
	2	1	105	105
		2	107	104
	4	1	103	106
		2	107	106
	6	1	105	106
		2	109	111

Table 4

Metabolism of [^{14}C]-7-ethoxycoumarin by fresh human hepatocytes (donor 1) in suspension culture

Incubation period (h)	Replicate	% Formation/metabolism			
		Glucuronide	7-OH coumarin	Sulphate	7-EC
0	1	-	1.3	-	98.3
	2	-	1.4	-	97.6
	Mean	-	**1.3**	-	**97.9**
0.25	1	1.7	0.4	1.4	92.2
	2	1.3	0.5	0.9	95.0
	Mean	**1.5**	**0.5**	**1.2**	**93.4**
1	1	4.3	-	1.9	83.8
	2	3.8	-	2.3	82.9
	Mean	**4.1**	-	**2.1**	**83.4**

- not detected

Metabolism of [^{14}C]-7-ethoxycoumarin by fresh human hepatocytes (donor 2) in suspension culture

Incubation period (h)	Replicate	% Formation/metabolism			
		Glucuronide	7-OH coumarin	Sulphate	7-EC
0	1	-	1.3	-	97.7
	2	-	1.3	-	95.4
	Mean	-	**1.3**	-	**96.6**
0.25	1	3.8	0.8	1.0	86.3
	2	2.9	0.7	0.5	88.5
	Mean	**3.3**	**0.7**	**0.7**	**87.4**
1	1	7.8	2.5	3.5	52.4
	2	8.0	1.3	3.4	54.3
	Mean	**7.9**	**1.9**	**3.4**	**53.3**
2	1	10.1	2.9	4.2	37.5
	2	10.2	2.0	3.8	38.7
	Mean	**10.2**	**2.4**	**4.0**	**38.1**
3	1	13.1	-	5.0	29.2
	2	12.0	2.3	5.1	25.5
	Mean	**12.6**	**2.3**	**5.0**	**27.4**

- not detected

2.6.6 TOXICOLOGY

2.6.6.3 Repeat-dose toxicity

1. 13-Week Oral Toxicity Study in the Rat

Study Facility: —
Study No: 1398/206
Study Dates: 10/16/00 – 1/17/01

GLP Compliance: Compliance with GLP regulations attested.
QA Report: Yes

Animals: Male and female — CD (SD)IGSBR rats (M, 135-214 gm; F, 137-183 gm) were housed in groups of five of same sex per cage and maintained on — Rodent Diet #1 food and tap water ad libitum.

Drug Administration: CC-5013 (Batch # 61240-04) was suspended in 1% aqueous carboxymethyl cellulose and administered orally by gavage. Analysis of the test formulation showed the actual concentrations used were — of the intended amounts.

Dose Levels: 0 (vehicle), 75, 150 and 300 mg/kg/day (10/sex/group for main study; 3/sex/group for toxicokinetic satellite study).

Observations/Measurements: The animals were observed daily for mortality and clinical signs of toxicity. Body weights were recorded prior to dosing and at weekly intervals during the dosing period. Food consumption was determined weekly. Ophthalmoscopic examinations were performed on control and high dose animals predose and during Week 12 of the dosing period. Blood samples were obtained from the lateral tail vein for all main study animals during Week 13 of dosing for hematology and clinical chemistry analyses. Urine was collected for analysis during Week 12. Blood samples were obtained from the satellite groups on Day 1 and during Week 13 of dosing for measurement of plasma levels of CC-5013. At the end of the dosing period, the animals were killed and examined macroscopically. The following organs were removed and weighed: adrenals, brain, heart, kidneys, liver, mesenteric and mandibular lymph nodes, ovaries, pituitary, prostate, spleen, testes and epididymides, thymus, thyroid and parathyroids. Sections from the following major tissues and organs from control and high dose main study animals and tissues with gross lesions from all main study groups were examined microscopically: adrenals, aorta, brain, cecum, colon, duodenum, epididymis, esophagus, eye, Harderian gland, head, heart, ileum, jejunum, kidneys, lachrymal gland, larynx, liver, mandibular and mediastinal lymph nodes, lungs, mammary gland, nasal turbinates, optic nerves, ovaries, pancreas, parathyroid, pituitary gland, prostate, rectum, salivary gland, sciatic nerve, seminal vesicles, skin, spinal cord, spleen, sternum with bone marrow, stomach, testes, thymus, thyroid, tongue, trachea, urinary bladder, uterus, vagina and Zimbal gland.

Results

Mortality and Clinical Signs
No animals died during the study and no clinical signs of toxicity were observed.

Body Weight
Mean body weights for treated animals throughout the study were comparable to control. Body weight gain was slightly lower than control for mid and high dose males (~5% lower) and for mid and high dose females (6% and 8% lower, respectively).

Food Consumption
Food consumption for treated animals was comparable to control.

Ophthalmoscopy
There were no ophthalmoscopic findings for treated and control animals.

Hematology, Clinical Chemistry and Urinalysis
Hematology parameters for treated animals were comparable to control.

Clinical chemistry parameters for treated animals were comparable to control.

In Week 12 of the study, the presence of unidentified crystals was observed in urine samples from the majority of mid and high dose males and females.

Organ Weights
Organ weights were reported as unadjusted (absolute wt) or adjusted to overall mean necropsy body weight. The sponsor carried out statistical analysis on the adjusted organ weights. Lower than control adjusted liver weight was noted for mid dose males and females and for high dose females; lower than control adjusted thymus weight was noted for high dose females (Table 1).

Table 1. Organ Weights

Organ	Sex	Dose Group (mg/kg/day)			
		0 (Control)	75	150	300
Liver weight, gm					
Adjusted wt. (Unadjusted wt)	M	11.03 (11.01)	10.49 (10.89)	10.16* (9.95)	10.59 (10.42)
Adjusted wt. (Unadjusted wt)	F	6.85 (6.94)	6.58 (6.64)	6.46* (6.35)	6.42* (6.38)
Thymus weight, gm					
Adjusted wt. (Unadjusted wt)	F	0.295 (0.298)	0.247 (0.250)	0.255 (0.251)	0.243* (0.242)

* Significantly different from control (0) value ($p<0.05$).

Gross and Microscopic Pathology
No treatment-related gross findings were observed.

Histopathologic examination revealed unilateral renal tubular nephropathy in one high dose female. The sponsor considered this finding to be a background finding rather than treatment related. No other microscopic findings were observed.

Toxicokinetics

CC-5013 plasma concentrations increased with increasing doses for both males and females. Mean CC-5013 plasma concentrations and AUCs on Day 1 were comparable to plasma concentrations and AUCs seen in Week 13 for both males and females (Table 2).

Table 2. Toxicokinetic Results

Dose (mg/kg/day)	Dose ratio #	Sex	C_{max} (ng/mL)				T_{max} (hour)	
			Day 1	Day 1 ratio #	Week 13	Week 13 ratio #	Day 1	Week 13
75	1.0	M	13027.4	1.0	15907.9	1.0	1.0	1.0
150	2.0		20206.2	1.6	22179.4	1.4	1.0	1.0
300	4.0		27431.8	2.1	19494.7	1.2	2.0	2.0
75	1.0	F	10885.6	1.0	13017.8	1.0	1.0	0.5
150	2.0		21115.7	1.9	23561.9	1.8	2.0	1.0
300	4.0		30746.7	2.8	33377.2	2.6	1.0	1.0

Dose (mg/kg/day)	Dose ratio #	Sex	$AUC_{(0-24h)}$ (ng.h/mL)			
			Day 1	Day 1 ratio #	Week 13	Week 13 ratio #
75	1.0	M	45025.7	1.0	60347.8	1.0
150	2.0		75056.5	1.7	76803.0	1.3
300	4.0		132202.6	2.9	87030.6	1.4
75	1.0	F	38833.4	1.0	59824.9	1.0
150	2.0		100622.0	2.6	108191.6	1.8
300	4.0		157666.6	4.1	136486.0	2.3

normalised to 75 mg/kg/day dose level

2. 13-Week Oral Toxicity Study in Monkeys (Vol 3, pg. 695)

Study Facility: —
Study No.: 1398/191
Study Dates: Study initiation, 12/23/99; study termination 6/12/00
GLP Compliance: Compliance with GLP regulations attested.
QA Report: QA report is unsigned; this is a draft of the final report.

Animals: Cynomolgus monkeys (12 M, 12 F; 1.75 to 2.55 kg, 15 to 29 mo. old at time of dosing). The animals of the same group and sex were housed together and fed a fixed daily amount of fresh fruit, biscuits and forage mix. Water was provided ad libitum.

Drug Administration: CC-5013 (Batch # 60990-03) was dissolved in 1% aqueous carboxy-methylcellulose and administered orally by gavage; control animals received the drug vehicle.

Dose Levels: 0, 0.2, 1.0 and 2.0 mg/kg/day (3/sex/group).

Observations/Measurements: Animals were observed at least twice daily for mortality and clinical signs of toxicity. Body weights were recorded once weekly and before necropsy. As food was provided communally into each pen, individual food consumption values could not be recorded. Ophthalmoscopic examinations were performed pre-treatment and in week 12 of treatment. ECG recordings were obtained pre-treatment and in week 13 of treatment. Venous blood samples were obtained via a femoral vein, pretreatment and in week 13, for hematology and clinical chemistry analyses. Urine samples were collected overnight from all animals, pretreatment and in week 12 of treatment, for urinalysis. On day 1 and during week 13 of treatment, blood samples were obtained from animals in each dose group, predose and at 0.5, 1, 2, 4, 8, 12 and 24 hours after dosing, for toxicokinetic analysis. Animals were anesthetized, killed by exsanguination and examined for macroscopic pathology. Major organs were removed and weighed; sections of tissues and organs from all animals were fixed onto slides, stained and examined microscopically (Table 1).

Table 1. Histopathology Inventory for IND # —

Study No.	1398/191
Species	Monkey
Adrenals	X
Aorta	X
Axillary lymph node	
Brain	X
Cecum	X
Cervix	
Colon	X
Duodenum	X
Epididymis	
Esophagus	X
Eye	X
Fallopian Tubes	
Gall Bladder	X

Study No.	1398/191
Species	Monkey
Gross Lesions	X
Harderian Gland	
Head	
Heart	X
Ileum	X
Injection Site	Not applicable
Jejunum	X
Kidneys	X
Lachrymal Gland	X
Larynx	
Liver	X
L nodes, cervical	
L nodes, mandibular	X
L nodes, mediastinal	
Lungs	X
Mandibular Gland	
Mammary Gland	X
Nasal Turbinates	
Optic Nerves	X
Ovaries	X
Pancreas	X
Parotid gland	
Parathyroid	X
Pituitary Gland	X
Prostate	X
Rectum	X
Salivary Gland	X
Sciatic Nerve	X
Seminal Vesicles	X
Skeletal Muscle	X
Skin	X
Spinal Cord	X
Spleen	X
Sternum with bone marrow	X
Stomach	X
Testes	X
Thymus	X
Thyroid	X
Tongue	X
Tonsil	X
Trachea	X
Urinary Bladder	X
Uterus	X
Vagina	X
Zymbal Gland	

Results

Mortality and Clinical Signs
One male in the low dose (0.2 mg/kg/day) group was killed and necropsied in week 9 due to poor health. No other treatment-related clinical signs of toxicity were observed.

Body Weight
There were no adverse effects of treatment on body weight gain except for the male in the low dose group that showed a weight loss prior to early termination from the study.

Ophthalmology
No treatment-related ophthalmic lesions were observed.

Heart Rate and Rhythm
No treatment-related effects on heart rate or rhythm were noted.

Hematology, Clinical Chemistry and Urinalysis
No treatment-related effects on hematology, clinical chemistry and urinalysis parameters were noted.

Gross and Microscopic Pathology
No gross or microscopic effects of CC-5013 treatment were observed. Gross and microscopic findings in both control and treated animals were consistent with the usual pattern of findings in animals of this strain and age.

Toxicokinetics
On day 1, the maximum plasma concentration of CC-5013 was reached between 0.5 and 2 hours after dosing. Cmax and AUC0-24 values increased with dose for both males and females; the increase of Cmax and AUC0-24 values was approximately proportional to the increase in dose. In week 13, the increase of the Cmax and AUC0-24 for the females was approximately proportional to the increase in dose with the exception of the increase in Cmax value between doses 0.2 and 2 mg/kg/day (6.6-fold) which was slightly lower than the increase in dose (10-fold). The increase in Cmax and AUC0-24 values for the males was approximately proportional to the increase in dose. Cmax and AUC0-24 values generally were lower in week 13 as compared to Day 1 for both males and females (Table 1).

Table 1. Toxicokinetic Results

Dose (mg/kg/day)	Dose ratio #	Sex (n=3)	C_{max} (ng/mL)				T_{max} (hour)	
			Day 1	Day 1 ratio #	Week 13	Week 13 ratio #	Day 1	Week 13
0.2	1.0	M	118.4	1.0	83.8	1.0	1.0	1.0
1	5.0		743.8	6.3	359.2	4.3	0.7	1.3
2	10.0		1257.1	10.6	986.6	11.8	0.7	1.2
0.2	1.0	F	116.9	1.0	125.4	1.0	0.8	0.7
1	5.0		645.9	5.5	509.9	4.1	0.5	0.7
2	10.0		1451.2	12.4	830.0	6.6	0.8	1.3

Dose (mg/kg/day)	Dose ratio #	Sex (n=3)	$AUC_{(0-24h)}$ (ng.h/mL)			
			Day 1	Day 1 ratio #	Week 13	Week 13 ratio
0.2	1.0	M	250.6	1.0	NC	-
1	5.0		1438.9	5.7	1023.9	1.0 *
2	10.0		3212.1	12.8	2742.7	2.7 *
0.2	1.0	F	234.7+	1.0	241.6	1.0 #
1	5.0		1510.5	6.4	1225.0	5.1 #
2	10.0		3216.0	13.7	2275.1	9.4 #

normalised to 0.2 mg/kg/day dose level
* normalised to 1.0 mg/kg/day dose level
+ mean $AUC_{(0-24h)}$ calculated using 2 animals only
NC = Not calculated, insufficient data available

3. Fifty-Two Week Oral (Gavage) Toxicity Study in the Monkey

Study Facility: —
Study No: 1398/243
Study Dates: Initiation of dosing, 9/12/01; final necropsy, 9/12/02

GLP Compliance: Compliance with GLP regulations attested.
QA Report: Yes

Animals: Male and female cynomolgus monkeys (males, 1.9 kg - 2.4 kg; females 1.8 kg – 3.1 kg; 83 to 134 weeks old at the time of dosing). The animals of the same group and sex were housed together in pens (2 m high with at floor area of 3.25 m2) and fed a fixed daily amount of fresh fruit, biscuits and forage mix. Water was provided ad libitum.

Drug Administration: CC-5013 (Batch # 61457-06) was dissolved in 1% aqueous carboxymethylcellulose and administered orally by gavage; control animals received the drug vehicle.

Dose Levels: 0, 1, 2, 4 and 6 mg/kg/day (6/sex for control and 6 mg/kg groups; 4/sex for 1, 2 and 4 mg/kg dose groups).
Dose selection was based on 4-week and 13-week toxicity studies in which the 2 mg/kg/day dose was identified as a NOEL and 20 mg/kg was associated with toxicity (nephrotoxicity and adverse effects on lymphoreticular organs and hemopoiesis in bone marrow. The 1 mg/kg dose has been shown in clinical trials to have a therapeutic effect.

Observations/Measurements: The animals were observed twice daily for mortality and clinical signs of toxicity. ECGs were recorded and ophthalmic exams were performed predose and in study weeks 25 and 50. Body weights were measured weekly. Food consumption was visually monitored throughout the study. Venous blood samples were collected at various intervals after dosing on Day 1 and in week 52 for toxicokinetics. Venous blood samples were also collected predose and in weeks 13, 26 and 52 for hematology and clinical chemistry. Urine was collected predose and in weeks 12, 25 and 50 for urinalysis. At the end of the treatment period, 4 monkeys/sex/group were anesthetized with pentobarbital, killed by exsanguination and necropsied; the remaining 2 monkeys/sex from control and high dose groups were maintained treatment free for 7 weeks. Blood samples were obtained from the recovery groups for hematology and clinical chemistry analyses; these animals were necropsied. Major organs from all animals were excised and weighed and sections of the following tissues and organs were fixed onto slides and examined microscopically.

(• - Tissues preserved) († - Organs weighed) (š - Tissues examined)

Ref. no	Tissue / organ	(•)	(†)	(š)
0	Blood sample			
0	Bone marrow smear (sternum)	•ac		
1 + 2	Eyes + optic nerves	•b		š
3	Skin	•		š
3	Mammary	•		š
4	Muscle	•		š
5	Sciatic nerves (x2)	•		š
6	Femur with bone marrow and articular surface	•		š
7	Sternum with bone marrow	•		š
8	Rib			
9	Liver (x2)	•	†	š
9	Mesenteric lymph nodes	•		š
10	Spleen	•	†	š
10	Pancreas	•		š
10	Adrenals	•	†	š
11 + 12	Kidneys	•	†	š
13	Stomach	•		š
14	Gall bladder	•		š
14	Duodenum	•		š
14	Jejunum	•		š
15	Ileum	•		š
15	Caecum	•		š
15	Colon	•		š
16	Rectum	•		š
17 + 18	Uterus	•	†	š
18	Urinary bladder	•		š
18	Prostate	•	†	š
18	Seminal vesicles	•		š

Ref. no	Tissue / organ	(•)	(†)	(š)
19 + 20	Testes + epididymides	•	†	š
19 + 20	Ovaries	•	†	š
21	Thymus	•		š
21	Salivary glands	•		š
21	Mandibular lymph nodes	•		š
22	Thyroids + parathyroids	•	†	š
23 + 23.1 + 23.2	Heart (x3)	•	†	š
24	Lungs (x2)	•		š
25	Oesophagus	•		š
25	Aorta (LS + TS)	•		š
26	Tongue	•		š
27	Pituitary	•	†	š
28-32	Brain (x5)	•	†	š
32	Spinal cord cervical	•		š
33	Spinal cord thoracic	•		š
33	Spinal cord lumbar	•		š
34	Trachea	•		š
35	Vagina	•		š
36	Lacrimal glands	•		
99	Additional tissue			
99.1	Injection sites			
99.2	Animal identification	•		
99.3				
99.4				
99.5				
100	Gross lesions	•		š

Fixative: 10% Neutral Buffered Formalin except where stated as:
a - Methanol
b - Davidson's
c - see clinical pathology section of protocol

Left and right organs will be weighed together.
Lungs to include mainstem bronchi.
Bone tissue designated for histopathological examination will be decalcified using Kristensons fluid.
LS - longitudinal section
TS - transverse section

• Denotes tissues preserved from all animals š = Denotes tissues examined microscopically
†Denotes organs removed and weighed

Results

Mortality and Clinical Signs of Toxicity

Nine monkeys (one male and two females at 4 mg/kg/day and four males and 1 female at 6 mg/kg/day and one replacement male at 4 mg/kg/day) were killed prior to week 20 due to signs of toxicity (Table 5). One control female was removed from the study in week 46 due to a fracture of the left forearm. The remainder of the animals administered 4 or 6 mg/kg/day (including replacement animals, 1 male and 1

female in the 4 mg/kg/day group and 1 male in the 6 mg/kg/day group) were terminated after study week 20, or in the case of two monkeys/sex in the 6 mg/kg/day group were terminated on completion of the 7 week treatment-free period.

Table 5. Animals Terminated Early Due to Toxicity

Dose Group	Sex	Animal No.	Time of Early Sacrifice	Clinical Signs/Condition
0 (Control)	F	28	Wk 46	Fracture of left forearm
4 mg/kg/day	F	41	Wk 15	Eye bruises, swollen head
		42a	Wk 6	Thin, sunken eyes, moribund
6 mg/kg/day	F	47	Wk 18	Sluggish, sores on mouth and body, pale gums, labored respiration
4 mg/kg/day	M	17b	Wk 6	Thin, sunken eyes, moribund
		50	Wk 18	Labored respiration
6 mg/kg/day	M	19	Wk 19	Hind leg skin lesions, bruising on left hind leg, pale mucous membranes.
		20	Wk 11	Sluggish, semi-conscious, mouth sores pale gums swollen hind paw, moribund.
		21	Wk 10	Thin, sluggish, dehydrated, sunken eyes, moribund.
		24c	Wk 2	Hunched back, thin, sluggish, sunken eyes, liquid feces, moribund.

a Replaced with monkey #51 on Day 43
b Replaced with monkey #50 on Day 43
c Replaced with monkey #49 on Day 29

Clinical signs observed in animals treated with 1 and 2 mg/kg/day were comparable to control.

Ophthalmoscopic Exams
There were no treatment-related ophthalmoscopic findings.

ECG Recordings
Unremarkable

Body Weights
There was no adverse effect on body weight for males and females treated with 1 and 2 mg/kg/day (Figures 1 & 2). Marked weight loss (between 8% and 27% of pretreatment weight) was observed for several of the decedent animals (1 male and 1 female in the 4 mg/kg/day group and 2 males in the 6 mg/kg/day group).

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Figure 1

Group mean body weights - males

Test article	Control	CC-5013			
Group	1	2	3	4	5
Level (mg/kg/day)	0	1	2	4	6

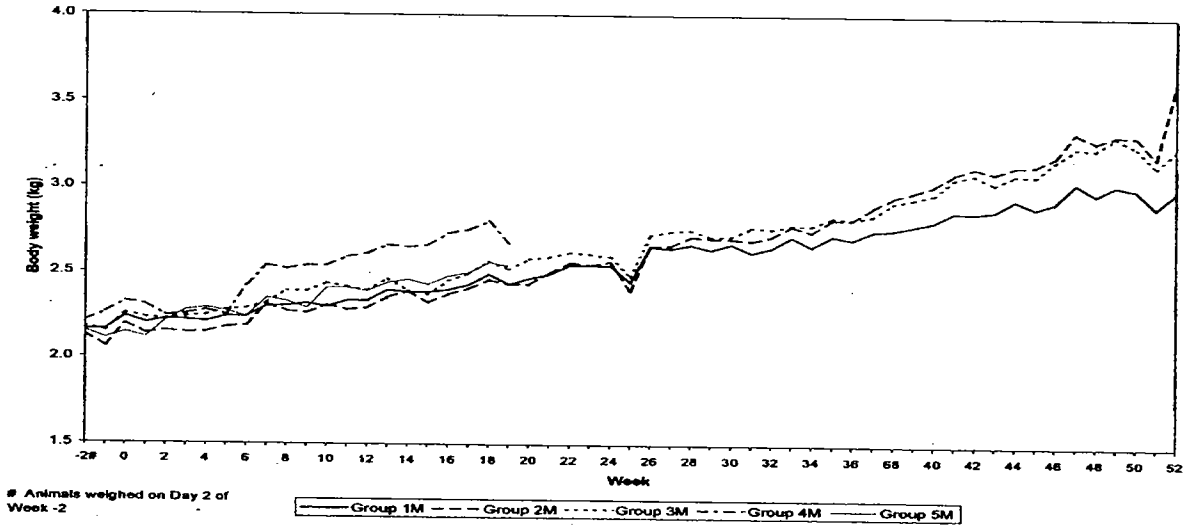

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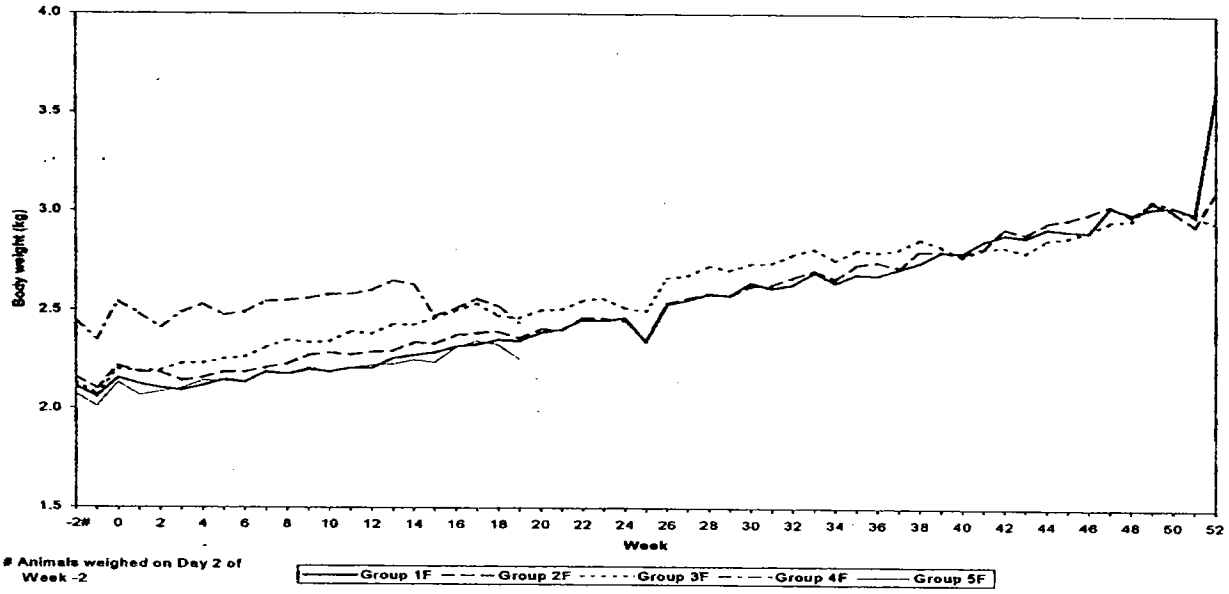
Figure 2

Group mean body weights - females

Test article	Control	CC-5013			
Group	1	2	3	4	5
Level (mg/kg/day)	0	1	2	4	6



Hematology

For males and females administered 1 mg/kg/day of CC-5013, hematology parameters were unaffected through 52 weeks of treatment. There was a slight, and inconsistent, suppression of white blood cell counts in males given 2 mg/kg/day when compared to control (Table 6).

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Table 6

Group mean haematology
Occasion: Week 52

Test article	Control		CC-5013		
Group	1	2	3	4	5
Level (mg/kg/day)	0	1	2	4	6

Group Sex	Total WBC 1000/cmm	N 1000/cmm	L 1000/cmm	M 1000/cmm	E 1000/cmm	B 1000/cmm	LUC 1000/cmm
1M Mean	13.6	6.9	5.9	0.2	0.3	0.1	0.1
SD	3.7	2.4	2.0	0.1	0.1	0.1	0.1
2M Mean	11.6	5.7	5.1	0.3	0.3	0.1	0.2
SD	3.7	3.5	2.4	0.1	0.1	0.1	0.1
3M Mean	6.7	2.8	3.3	0.3	0.2	0.1	0.1
SD	0.9	0.7	0.1	0.1	0.1	0.1	0.1
1F Mean	12.9	4.6	7.3	0.5	0.3	0.1	0.2
SD	2.6	1.5	1.2	0.2	0.1	0.1	0.1
2F Mean	8.5	3.0	4.5	0.4	0.5	0.1	0.2
SD	1.4	0.9	1.5	0.1	0.1	0.1	0.1
3F Mean	10.6	5.8	4.0	0.3	0.5	0.0	0.2
SD	2.9	3.6	1.2	0.1	0.3	0.1	0.1

Several of the decedent male and female monkeys in the 4 and 6 mg/kg/day groups had lower than control platelet counts (up to 23% lower), reduced hemoglobin concentration, lower red blood cell counts, packed cell volume and white blood cell counts (up to 40% lower). These decrements appeared to result from hemorrhage observed at necropsy. In those animals previously administered 6 mg/kg/day and maintained on a treatment-free period for 7 weeks, the platelet and white blood cell counts were similar to concurrent control values.

The majority of 4 and 6 mg/kg/day males and females that were terminated after 19 weeks of treatment had a lower than control myeloid/erythroid ratio (0.2 to 0.7 vs. ≥1.0 for control) due to a reduction in the proportion of myelopoietic cells. At the completion of the treatment free period, bone marrow cellularity of males and females at 6 mg/kg/day was comparable to control.

Clinical Chemistry
Higher than control urea (2- to 6-fold higher) and creatinine (1.3- to 2.3-fold higher) levels were observed in decedent males and females in the 4 and 6 mg/kg/day groups. No effects on clinical chemistry parameters were noted for males or females in the 1 and 2 mg/kg/day groups throughout the treatment period.

Urinalysis
Urinalysis parameters for treated animals surviving to terminal sacrifice were comparable to control.

Organ Weights
Organ weights for treated males and females surviving to terminal sacrifice were comparable to control.

Macroscopic Observations

Early Sacrifice: Macroscopic examination of decedents in the 4 and 6 mg/kg/day male and female groups showed widespread red and/or dark discolorations throughout the body, but most notably in the skin/subcutis, alimentary tract and lymphoid organs (Tables 7a & 7b).

Terminal Sacrifice: Most tissues from males and females in the 1 and 2 mg/kg/day groups were macroscopically unremarkable and comparable to control. However, small thymus was noted in 2 females from the 1 mg/kg/day group and 1 female from the 2 mg/kg/day group.

Microscopic Observations

Early Sacrifice: Microscopic findings from decedents in the 4 and 6 mg/kg/day male and female groups consisted of hemorrhage in multiple organs, gastrointestinal tract inflammation and lymphoid and bone marrow atrophy. Hemorrhage was a prominent feature in 5 monkeys (3M and 2F) and correlated with the red and/or dark discolorations seen macroscopically, and with the low platelet count seen in hematology analysis (Tables 7a & 7b). Inflammation of the gastrointestinal tract was also a notable finding in 4 animals that exhibited clinical signs of toxicity. Atrophy of bone marrow and thymus was noted in all decedent animals treated with 4 or 6 mg/kg/day of CC-5013.

Terminal Sacrifice: Atrophy of the thymus, similar to that noted in the decedents, was observed in most males and females treated with 1 or 2 mg/kg/day of CC-5013. This correlated with small thymus noted macroscopically. There were no other microscopic findings related to CC-5013 treatment.

Table 7a
Treatment Associated Findings- Males

Group	Animal #	Terminal Body Wt., g (Effect)	Hemorrhage	Small Thymus	GI Inflammation	Thymic Atrophy	Low Platelets*
Control	1	2862	-	-	-	-	
	2	3249	-	-	-	-	
	3	3155	-	-	-	-	
	4	2885	-	-	-	-	
(Recovery)	5	2702	-	-	-	-	
(Recovery)	6	2522	-	-	-	-	
1 mg/kg	7	2960	-	-	-	-	
	8	3228	-	-	-	minimal	
	9	3171	-	-	-	-	
	10	4163	-	-	-	moderate	
2 mg/kg	11	3303	-	-	-	minimal	
	12	3483	-	-	-	minimal	
	13	2667	-	-	-	minimal	
	14	3868	-	-	-	slight	
4 mg/kg	15	2694	-	-	-	slight	
	16	2700	-	-	-	slight	
	17†	1644 (wt.loss)	present	yes	slight	severe	
	18	2896	-	-	-	minimal	
	50 (R) †	2645	-	-	-	moderate	yes
6 mg/kg	19†	2460	present	-	slight	slight	yes
	20†	2318	present	-	slight	severe	
	21†	1822 (wt.loss)	present	yes	slight	moderate	
	22	2624	-	-	-	-	
	23	2878	-	-	-	-	
	24†	1794 (wt.loss)	present	yes	slight	severe	
	49 (R)	3885	present	-	-	slight	

(R) = Replacement animal † = Sacrificed early due to toxicity Dash line (-) indicates absence of effect

* Less than 100 thousand/ccm; (Normal range =300-600 thousand/ccm)

Table 7b
Treatment Associated Findings- Females

Group	Animal #.	Terminal Body Wt., g (Effect)	Hemorrhage	Small Thymus	GI Inflammation	Thymic Atrophy	Low Platelets*
Control	25	2966	-	-	-	-	
	26	2589	-	-	-	-	
	27	3581	-	-	-	-	
	28†	2942	-	-	-	-	
(Recovery)	29	2647	-	-	-	-	
(Recovery)	30	2397	-	-	-	-	
1 mg/kg	31	3231	present	-	-	slight	
	32	2965	-	yes	-	moderate	
	33	3282	-	-	-	-	
	34	2823	-	yes	-	moderate	
2 mg/kg	35	3486	-	-	-	slight	
	36	3106	-	-	-	moderate	
	37	2770	-	yes	-	minimal	
	38	3054	-	-	-	minimal	
4 mg/kg	39	2491	-	-	-	moderate	yes
	40	2470	present	-	slight	slight	
	41†	2978	present	-	slight	moderate	yes
	42†	2461 (wt. loss)	present	-	slight	severe	
	51 (R)	2824	-	-	-	slight	
6 mg/kg	43	2340	present	-	-	minimal	yes
	44	2188	present	-	-	slight	yes
	45	2242	-	-	-	moderate	
	46	2422	-	-	-	-	yes
(Recovery)	47†	2228	present	-	slight	severe	yes
(Recovery)	48	2627	-	-		-	

(R) = Replacement animal † = Sacrificed early due to toxicity Dash line (-) indicates absence of effect

* Less than 100 thousand/ccm; (Normal range =300-600 thousand/ccm)

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Toxicokinetics

CC-5013 was readily absorbed following oral administration and Cmax and AUC increased (near proportionally) with increasing dose with no appreciable differences between males and females (Tables 7 and 8). Mean Cmax and AUC values were similar on Day 1 and in Week 52 for the 1 and 2 mg/kg/day groups (The 4 and 6 mg/kg/day groups were terminated at Week 20; the Cmax and AUC values for these groups were lower during Week 19 compared to values seen on Day 1). Tmax was reached between 0.5 and 2 hours across all treated groups.

Table 6. Toxicokinetics-Cmax

CC-5013 - Mean C_{max}

Dose (mg/kg/day)	Dose Ratio#	Group	Sex	Mean C_{max} (ng/mL)					
				Day 1	Day 1 ratios#	Week 19	Week 19 ratios#	Week 52	Week 52 ratios#
1	1	2	M	744.5	1.0	488.1	1.0	625.3	1.0
2	2	3		1746.6	2.3	772.8	1.6	1389.6	2.2
4	4	4		3446.1	4.6	1813.4	3.7	X1	X1
6	6	5		5777.2	7.8	2697.9	5.5	X1	X1
1	1	2	F	1120.8	1.0	458.9	1.0	615.4	1.0
2	2	3		1600.7	1.4	884.1	1.9	1817.5	3.0
4	4	4		2981.1	2.7	1677.9	3.7	X1	X1
6	6	5		5611.0	5.0	2630.3	5.7	X1	X1

# normalised to 1 mg/kg/day dose
X1 = Dose group withdrawn from study due to adverse toxicity

Table 7. Toxicokinetics- AUC

CC-5013 - Mean $AUC_{(0\text{-}24h)}$

Dose (mg/kg/day)	Dose Ratio#	Group	Sex	Mean $AUC_{(0\text{-}24h)}$ (ng.h/mL)					
				Day 1	Day 1 ratios#	Week 19	Week 19 ratios#	Week 52	Week 52 ratios#
1	1	2	M	2146.8	1.0	1092.6	1.0	2125.7	1.0
2	2	3		4108.6	1.9	1695.6	1.6	4412.5	2.1
4	4	4		8655.2	4.0	4763.0	4.4	X1	X1
6	6	5		14975.8	7.0	6852.5	6.3	X1	X1
1	1	2	F	2316.4	1.0	1102.9	1.0	2249.2	1.0
2	2	3		3814.1	1.6	1948.4	1.8	4919.6	2.2
4	4	4		8708.2	3.8	3950.7	3.6	X1	X1
6	6	5		15189.8	6.6	7525.2	6.8	X1	X1

# normalised to 1 mg/kg/day dose
X1 = Dose group withdrawn from study due to adverse toxicity

6.6.6.4 Genetic toxicology

1. Rat Bone Marrow Micronucleus Assay

Study Facility: —
Study No: 1398/187
Study Dates: 4/19/00 to 7/07/00

GLP Compliance: Compliance with GLP regulations attested.
QA Report: Yes

Animals: Male — SD-BR (CD) rats (208-247gm) were housed in groups of 3 and maintained on — — diet and tap water ad libitum.

Drug Administration: CC-5013 (Batch # 60832-01, 4068-34-A and 60990-03; — pure) was suspended in 1% carboxymethylcellulose and administered orally by gavage.

Dose Levels: 0 (vehicle), 500, 1000 and 2000 mg/kg (6 rats/dose group). The high dose is the recommended maximum dose according to ICH (S2A) Guideline on Genotoxicity Testing (1995).

Procedure: Rats were dosed once daily for two consecutive days with vehicle or CC-5013. The positive control, cyclophosphamide (40 mg/kg), was given as a single dose. The animals were observed for clinical signs of toxicity during the study days. Twenty-four hours after the second dose of vehicle or CC-5013, and 24 hours after the single dose of cyclophosphamide, the rats were killed by asphyxiation and a single femur from each rat was removed. Bone marrow was removed from each femur, suspended in bovine serum and smeared onto slides. Slides were air-dried, fixed and examined microscopically. The relative proportion of polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) were determined from at least 1000 cells per slide to calculate a PCE/NCE ratio (a lower than control ratio is evidence of bone marrow toxicity). At least 2000 PCEs per slide were examined for the presence of micronuclei and the number of micronucleated PCEs/slide was recorded. The mean frequency of micronucleated PCE in each CC-5013 treated group was compared to that of vehicle control. The test agent was considered positive if a statistically significant increase above control frequency of micronucleated PCE was observed at any dose and the frequency of micronucleated PCE at that dose exceeded the historical vehicle control range.

Results

Clinical Signs
No animals receiving vehicle, CC-5013 or cyclophosphamide exhibited clinical signs of toxicity.

Bone Marrow Analysis

Rats treated with CC-5013 exhibited group mean PCE/NCE ratios that were within the historical control range and were comparable to the value for the concurrent vehicle control group. The mean frequencies of micronucleated PCEs for all CC-5013 groups did not differ significantly from that of the concurrent vehicle control. The positive control agent, cyclophosphamide, caused a significant ($p < 0.001$) increase above control frequency of micronucleated PCE. These results show CC-5013 to be negative in this genotoxicity assay.

Treatment group (mg/kg/day)	Kill time (hours)	Mean ratio PCE/NCE	Group mean frequency of micronucleated PCE ± SD (per 1000 cells)
Vehicle control	24	1.21	0.33 ± 0.52
500	24	1.34	0.42 ± 0.49
1000	24	1.47	0.17 ± 0.26
2000	24	1.48	0.25 ± 0.42
CPA, 40+	24	0.78	8.17 ± 4.08

+ Administered as a single dose
SD Standard deviation

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	21-880
SERIAL NUMBER:	001
DATE RECEIVED BY CENTER:	04/07/2005
DRUG NAME:	Revlimid® (Lenalidomide)
INDICATION:	Myelodysplastic syndrome (MDS)
SPONSOR:	Celgene Corporation 86 Morris Avenue, Summit, NJ 07901
DOCUMENTS REVIEWED:	Electronic submission
REVIEW DIVISION:	Division of Oncology Drug Products (HFD-150)
PHARM/TOX REVIEWER:	M. Anwar Goheer, Ph.D. Kimberly Benson, Ph.D.
PHARM/TOX SUPERVISOR:	John K. Leighton, Ph.D., D.A.B.T.
ACTING DIVISION DIRECTOR:	Robert Justice, M.D., M.S.
PROJECT MANAGER:	Carl Huntley, R.Ph., MBA

Date of review submission to Division File System (DFS): December 9, 2005

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: The non-clinical studies submitted to this NDA provide sufficient information to support the use of lenalidomide (Revlimid®) in patients with transfusion-dependent anemia due to low- or intermediate-1-risk myelodysplastic syndromes (MDS) associated with a deletion 5q cytogenetic abnormality with or without additional cytogenetic abnormalities.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings: Lenalidomide (3-(4' aminoisoindoline-1-one)-1-piperidine-2, 6-dione; CC- 5013; IMiD-3 and Revlimid ®) is a thalidomide analogue. It is a racemic mixture of S (-) and R (+) forms. The in vitro and in vivo characterization of pharmacological properties of lenalidomide had demonstrated that the drug inhibits the secretion of pro-inflammatory cytokines (TNF-α, IL-1ß, IL-6 and IL-12) and increases the secretion of anti-inflammatory cytokine (IL-10) from peripheral blood mononuclear cells (PBMC), induces T-cell proliferation (IL-2, IFN-γ), inhibits cell proliferation (MM, Burkitt's lymphoma) and inhibits angiogenesis (Knight-R, Semin Oncol 2005; 32: 24-30 & Dredge et al., Microvasc Res. 2005; 69: 56-63). Lenalidomide inhibits the expression of cyclooxygenase- 2 (COX-2) but did not affect COX-1 in vitro. This may translate into adverse effects that need to be fully explored in clinical trials. In addition to these immune effects, there is evidence that thalidomide and its analogues may act directly on tumor cells, via inducing apoptosis or G1 growth arrest.

The oral administration of lenalidomide at dose levels of 3, 6 and 12 g/ m^2 produced no effects on behavior or general activity in male rats. Intravenous administration of the drug at doses up to 400 mg/m^2 did not produce any significant effect on cardiovascular and respiratory systems of the anesthetized dog. In vitro, lenalidomide inhibited the cloned human potassium channel (hERG) current by 8% only at the highest concentration tested (787 µM).

Lenalidomide did not inhibit or induce any of the major cytochrome P450 isozymes *in vitro* and *in vivo* indicating limited potential for P450-related drug-drug interactions. Distribution of radioactivity in the fetal tissues of pregnant rat was low after oral administration but fetal brain showed more activity than maternal brain. The highest concentrations were found in the kidney (cortex and medulla), liver, spleen and the mucosa of the GI tract of rats.

During traditional toxicity assessment, lenalidomide was administered to rodents (mice, rats) and non rodents (monkeys) for 1, 7, and 28 days and 13, 26, and 52 weeks. Single dose administration of lenalidomide up to 6 g/m^2 in mice and 12 g/ m^2 in rats did not cause any adverse effects. Daily oral administration of lenalidomide at 6 g/m^2 to rats for 28 days was associated with moderate to severe tubular nephropathy/nephritis, which was attributed to precipitation of the lenalidomide in the kidney. Once daily oral administration of lenalidomide to rats at doses of 450, 900 or 1800 mg/m^2/day for 26 weeks was mainly associated with reduced body weight gain (12%) for high dose males and reversible pelvic mineralization in the kidney of all treated animals.

Oral administration of lenalidomide to cynomolgus monkeys at dose levels of 12, 24, 48, or 72 mg/m^2/day for 52 weeks was associated with hemorrhage in multiple organs, gastrointestinal tract inflammation and lymphoid and bone marrow atrophy. Dosing at 48 and 72 mg/m^2/day was discontinued after 20 weeks of treatment due to toxicity and mortalities. A reversal of the macroscopic and microscopic findings seen in decedent and the terminal sacrifice was noted in 7 week treatment-free recovery animals. It is clear that this species is much more sensitive to lenalidomide than rodents.

Lenalidomide did not induce mutation in the Ames test, chromosome aberrations in cultured human peripheral blood lymphocytes, or mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells. Lenalidomide did not increase morphological transformation in Syrian Hamster Embryo assay or induce micronuclei in the polychromatic erythrocytes of the bone marrow of male rats.

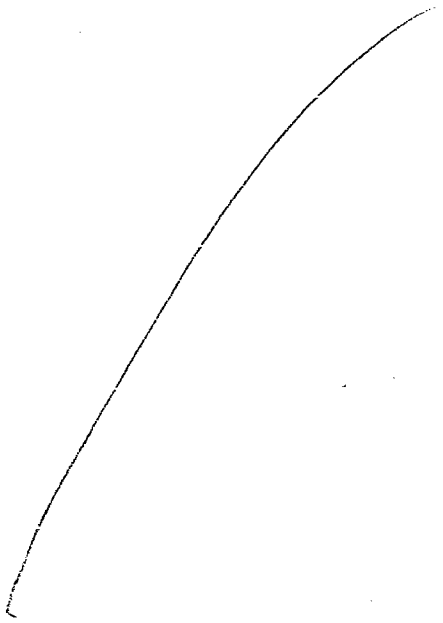
Reproductive and developmental toxicity: Reproductive studies were conducted with lenalidomide, examining the effects on fertility and early embryo development, embryo-fetal development, and pre- and post-natal development. Only the embryo-fetal development studies are required for drugs with oncologic indications. These studies have not been adequately conducted at this time. The first study, conducted in a rat, showed very slight maternal toxicity and no fetal malformations. The rat, however, is not an adequate species for the full assessment of lenalidomide's developmental effects, given the structural similarity to thalidomide. Historical data indicates that the rat is not sensitive to the full range of thalidomide's teratogenic effects.

An additional developmental study was conducted in the rabbit, with a concurrent thalidomide dose group. This study had a confounding variable with some rabbits not eating prior to the study and all these rabbits had a negative outcome in the study. Additionally, the highest dose tested did not meet the standard criteria for sufficient drug exposure.

B. Pharmacologic activity: Both lenalidomide and thalidomide have been shown to increase the secretion of anti-inflammatory cytokine IL-10 from LPS-stimulated PBMC, stimulates T-cells proliferation and production of IL-2 and IFN-γ ..Both inhibit the secretion of pro-inflammatory cytokines TNF-α, IL-1ß, and IL-6. In addition to these immune effects, there is evidence that thalidomide and its analogues may act directly on tumor cells, via inducing apoptosis or G1 growth arrest. Exact mechanisms of action however remain unknown.

C. Nonclinical safety issues relevant to clinical use: Inflammation of the gastrointestinal tract and atrophy of the bone marrow, thymus, and lymphoid tissues were observed during repeat dose toxicity studies (up to 12 months) in cynomolgus monkeys. Embryo-fetal developmental toxicity has not been adequately addressed. The structural similarity of lenalidomide to thalidomide, a known human teratogen, suggests developmental risk. Lenalidomide also inhibits expression of COX-2 in vitro but not COX-1. This finding should be fully explored in clinical trials.

B. Recommendations on labeling:



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___ § 552(b)(5) Deliberative Process

✓ § 552(b)(5) Draft Labeling

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