

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

206843Orig1s000

PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: NDA 206-843
Supporting document/s: SN 034
Applicant's letter date: February 13, 2015
CDER stamp date: February 13, 2015
Product: Daclatasvir
Indication: Anti-Hepatitis C Virus
Applicant: Bristol-Myers Squibb Company (BMS)
Review Division: Division of Antiviral Products
Reviewer: L. Peyton Myers, PhD
Supervisor/Team Leader: Hanan Ghantous, PhD, DABT
Division Director: Debra Birnkrant, MD
Project Manager: Sohail Mosaddegh, Pharm.D.

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 206-843 are owned by BMS or are data for which BMS has obtained a written right of reference. Any information or data necessary for approval of NDA 206-843 that BMS does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 206-843.

TABLE OF CONTENTS

1 EXECUTIVE SUMMARY	3
1.1 INTRODUCTION	3
1.2 BRIEF DISCUSSION OF NONCLINICAL FINDINGS	3
1.3 RECOMMENDATIONS	5
2 DRUG INFORMATION	7
2.1 DRUG	7
2.2 RELEVANT INDS, NDAs, BLAs AND DMFs	7
2.7 REGULATORY BACKGROUND	7

Table of Figures

Figure 1 - Structure of DCV	7
-----------------------------------	---

1 Executive Summary

1.1 Introduction

Daclatasvir (DCV, 60-mg), is a Hepatitis C Virus (HCV) nonstructural protein 5A (NS5A) inhibitor proposed for the treatment of HCV. DCV inhibits HCV NS5A protein with high affinity. DCV is proposed to be used with sofosbuvir (Sovaldi®).

1.2 Brief Discussion of Nonclinical Findings

Nonclinical studies were reviewed in the first review cycle for NDA 206-843. Please refer to NDA 203-843 for the review of the nonclinical data (archived on August 28, 2014). Briefly, the data are summarized below.

Nonclinical studies were conducted in the mouse, rat, dog, rabbit (embryofetal) and cynomolgus monkey. Studies included safety pharmacology, secondary pharmacology, PK/ADME, single-dose and repeat-dose toxicity, carcinogenicity studies (2 year study in rats and 6 month study in transgenic mice), genotoxicity, reproductive toxicity as well as local tolerance studies, immunotoxicity, and phototoxicity.

Safety pharmacology was evaluated for cardiotoxicity, CNS toxicity, and respiratory effects as part of the single- and repeat-dose (single-agent and combination) GLP toxicity studies conducted with DCV. *In vitro* assays evaluated effects on receptor and ion channel ligand binding and relative inhibition of enzyme activity. No significant safety pharmacology signals were detected. Minor CV (cardiovascular) effects were noted *in vivo* in rabbits with an exposure margin of 91x compared to the C_{max} at the recommended human exposure.

DCV was highly protein bound (95.1% to 99.5% in mouse, rat, rabbit, dog, and monkey serum; 95.6% in human serum). After IV single-dose administration, DCV was rapidly eliminated in mice (~1 hr) as well as rats, dogs, and monkeys, ($t_{1/2}$ of ~3.5-4.5 hrs). Distribution studies indicated that (consistent with the indication), DCV concentrates predominantly in the liver. The steady-state volume of distribution for DCV was greater than the reported total body water volumes, indicative of extravascular distribution. The blood-to-plasma DCV concentration ratio values, which were similar in humans and animals, suggested that DCV is distributed preferentially into plasma in most species. CYP3A4 was identified as the major enzyme responsible for the metabolism of DCV and in the formation of BMS-805215 (the primary circulating human metabolite). Although multiple metabolites were detected in animals, there were no unique human metabolites.

The main findings observed with DCV in the nonclinical studies included liver findings (increased weight and enzyme activity) as well as an adrenal effect (hypertrophy and vacuolation). In rats dosed for 1 month, DCV induced only minimal and reversible hepatic changes that included slight increases in serum ALT levels and a minimal

increase in liver weights without any liver histologic changes. No liver effects were notable in the 6 month study in rats. In monkeys dosed 4 months, liver enzymes (AST, ALT) increased with dose with histological correlates (mononuclear-cell infiltration in centrilobular areas of the liver, minimal/slight bile-duct hyperplasia and Kupffer-cell hyperplasia/ hypertrophy and minimal/moderate rarefaction of cytoplasm in centrilobular hepatocytes). In both rats and monkeys, adrenal gland findings included increases in adrenal gland size/weight, morphologic hypertrophy, and/or hyperplasia of cortical cells in the zona fasciculata and/or zona reticularis, increases in urine corticosterone levels (rats), and changes in cytoplasmic vacuolation. In rats, there was also a finding of increased urine output concurrent with increased water consumption in rats (with no associated adverse effects). The adrenal activity was monitored in early clinical trials (cortisol), but no effects were noted. Liver abnormalities were noted in clinical trials (AST/ALT elevations) but were not dose limiting.

Combination toxicology studies were performed with DCV and asunaprevir (ASV), as well as with pegylated-Interferon Alfa-2B + ribavirin (pIFN/RBV). (b) (4)

DCV was neither genotoxic nor carcinogenic in nonclinical studies (2-year rat study and 6-month transgenic mouse study). In a fertility study, male fertility parameters were affected in rats. Mean pre-implantation loss was noted in litters sired by treated males at increased compared to controls. Furthermore, spermatogenic effects were noted. Exposure margins at the NOAEL (no observed adverse effect level) for male fertility parameters were 4.6-fold (rat). Female fertility parameters were not affected with exposure margins at the recommended daily human exposure of 24-fold. DCV was also not a selective developmental toxicant when administered to pregnant rats or rabbits during organogenesis. All reproductive toxicity findings were secondary to maternal toxicity with exposure margins at the recommended daily human exposure of 6-fold (rat) and 22-fold (rabbit). DCV was also excreted in milk at ~2x the concentration of maternal plasma. No new toxicities relative to those observed in adult rats were seen in juvenile rats administered DCV. The AUC value at the NOAEL for juvenile toxicity was 4-fold the recommended daily human exposure.

Local tolerance studies showed no irritation effects with DCV. However, DCV is a potential dermal sensitizer. DCV was shown to be potentially phototoxic in an *in vitro* study evaluating the absorption of light by DCV. However, a follow up phototoxicity study (single oral dose) in Long Evans rats was negative. Furthermore, no ocular or other photo-related toxicity was noted in any of the repeat dose toxicity.

The submitted studies represent a complete nonclinical toxicology package for DCV.

1.3 Recommendations

1.3.1 Approvability

There are no nonclinical pharmacology and toxicology issues which would preclude the approval of DCV 60 mg tablets

1.3.2 Additional Non Clinical Recommendations

None.

1.3.3 Labeling

Suggested labeling:

8.1 Pregnancy

Risk Summary

No data with DAKLINZA in pregnant women are available to inform a drug-associated risk. In animal reproduction studies in rats and rabbits, no evidence of fetal harm was observed with oral administration of daclatasvir during organogenesis at doses that produced exposures up to 6 and 22 times, respectively, the recommended human dose (RHD) of 60 mg. However, embryofetal toxicity was observed in rats and rabbits at maternally toxic doses that produced exposures of 33 and 98 times the human exposure, respectively, at the RHD of 60 mg [*see Data*]. Consider the benefits and risks of DAKLINZA when prescribing DAKLINZA to a pregnant woman.

In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively.

Data

Animal Data

Daclatasvir was administered orally to pregnant rats at doses of 0, 50, 200, or 1000 mg/kg/day on gestation days 6 to 15. Maternal toxicity (mortality, adverse clinical signs, body-weight losses, and reduced food consumption) was noted at doses of 200 and 1000 mg/kg/day. In the offspring, malformations of the fetal brain, skull, eyes, ears, nose, lip, palate, or limbs were observed at doses of 200 and 1000 mg/kg. The dose of 1000 mg/kg was associated with profound embryoletality and lower fetal body weight. No malformations were noted at 50 mg/kg/day. Systemic exposure (AUC) at 50 mg/kg/day in pregnant females was 6-fold higher than exposures at the RHD.

In rabbits, daclatasvir was initially administered at doses of 0, 40, 200, or 750 mg/kg/day during the gestation days 7 to 19. Daclatasvir dosing was modified due to vehicle toxicity during the study to doses of 20, 99, and 370 mg/kg/day, respectively. Maternal toxicity was noted at doses of 200/99 and 750/370 mg/kg/day with adverse clinical signs, severe reductions in body weight, and food consumption. Mortality and euthanasia occurred in multiple dams at 750/370 mg/kg/day. At 200/99 mg/kg/day, fetal effects included increased

embryofetal lethality, reduced fetal body weights, and increased incidences of fetal malformations of the ribs as well as head and skull. No malformations were noted at in rabbits 40/20 mg/kg/day. Systemic exposures (AUC) at 40/20 mg/kg/day were 22-fold higher than exposures at the RHD.

In a pre- and postnatal developmental study, daclatasvir was administered orally at 0, 25, 50, or 100 mg/kg/day from gestation day 6 to lactation day 20. At 100 mg/kg/day maternal toxicity included mortality and dystocia; developmental toxicity included slight reductions in offspring viability in the perinatal and neonatal periods and reductions in birth weight that persisted into adulthood. There was neither maternal nor developmental toxicity at doses up to 50 mg/kg/day. Systemic exposures (AUC) at this dose were 3.6-fold higher than the RHD. Daclatasvir was present in rat milk with concentrations 1.7- to 2-fold maternal plasma levels.

8.2 Lactation

Risk Summary

No information regarding the presence of daclatasvir in human milk, the effects on the breastfed infant, or the effects on milk production is available. Daclatasvir is present in the milk of lactating rats [*see Use in Specific Populations (8.1)*]. The development and health benefits of breastfeeding should be considered along with the mother's clinical need for DAKLINZA and any potential adverse effects on the breastfed infant from DAKLINZA or from the underlying maternal condition.

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis and Mutagenesis

A 2-year carcinogenicity study in Sprague Dawley rats and a 6-month study in transgenic (Tg rasH2) mice were conducted with daclatasvir. In the 2-year study in rats, no drug-related increase in tumor incidence was observed at doses up to 50 mg/kg/day (both sexes). Daclatasvir exposures at these doses were approximately 6-fold (males and females) the human systemic exposure at the therapeutic daily dose. In transgenic mice no drug-related increase in tumor incidence was observed at doses of 300 mg/kg/day (both sexes).

Daclatasvir was not genotoxic in a battery of *in vitro* or *in vivo* assays, including bacterial mutagenicity (Ames) assays, mammalian mutation assays in Chinese hamster ovary cells, or in an *in vivo* oral micronucleus study in rats.

Impairment of Fertility

Daclatasvir had no effects on fertility in female rats at any dose tested. Daclatasvir exposures at these doses in females were approximately 24-fold the human systemic exposure at the therapeutic daily dose. In male rats, effects on reproductive endpoints at 200 mg/kg/day included reduced prostate/seminal vesicle weights, minimally increased dysmorphic sperm, as well as increased mean pre-implantation loss in litters sired by treated males. Daclatasvir exposures at the 200 mg/kg/day dose in males were approximately 26-fold the human systemic exposure at the therapeutic daily dose. Exposures at 50 mg/kg/day in males

produced no notable effects and was 4.7-fold the exposure in humans at the recommended daily dose.

2 Drug Information

2.1 Drug

CAS Registry Number
1214735-16-6

Generic Name
Daclatasvir, DCV

Code Name
BMS-790052

Chemical Name
[[[1,1'-Biphenyl]-4,4'-diylbis[1H-imidazole-4,2-diyl(2S)-2,1-pyrrolidinediyl[(1S)-1-(1-methylethyl)-2-oxo-2,1-ethanediy]]]biscarbamic acid, dimethyl ester, dihydrochloride

Molecular Formula/Molecular Weight
C₄₀H₅₀N₈O₆·2HCl /811.80 (free base 738.88)

Structure or Biochemical Description

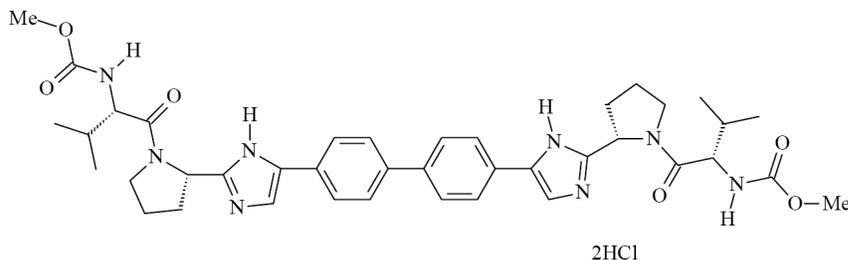


Figure 1 - Structure of DCV

Pharmacologic Class
Hepatitis C Virus NS5A inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 79599 – primary IND for DCV

NDA 206-843 – primary review archived on August 28, 2014

2.7 Regulatory Background

DCV is an NS5A inhibitor proposed for the treatment of HCV in combination with sofosbuvir. DCV was originally submitted on March 31, 2014 and the primary Pharmacology/Toxicology (P/T) review was archived on August 28, 2014. The NDA was resubmitted on February 13, 2015 in response to the Complete Response. The suggested label in the current review is modified from the original P/T review as it includes PLLR format changes that were implemented by the FDA in 2015.

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

/s/

LAINÉ P MYERS
06/29/2015

HANAN N GHANTOUS
06/29/2015

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: NDA 206-843
Supporting document/s: 000, 003
Applicant's letter date: March 31, 2014
CDER stamp date: March 31, 2014
Product: Daclatasvir
Indication: Anti-Hepatitis C Virus
Applicant: Bristol-Myers Squibb Company (BMS)
Review Division: Division of Antiviral Products
Reviewer: L. Peyton Myers, PhD
Supervisor/Team Leader: Hanan Ghantous, PhD, DABT
Division Director: Debra Birnkrant, MD
Project Manager: Sohail Mosaddegh, Pharm.D.

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 206-843 are owned by BMS or are data for which BMS has obtained a written right of reference. Any information or data necessary for approval of NDA 206-843 that BMS does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 206-843.

TABLE OF CONTENTS

1	EXECUTIVE SUMMARY	6
1.1	INTRODUCTION	6
1.2	BRIEF DISCUSSION OF NONCLINICAL FINDINGS	6
1.3	RECOMMENDATIONS	7
2	DRUG INFORMATION	10
2.1	DRUG	10
2.2	RELEVANT INDS, NDAs, BLAs AND DMFs	11
2.3	DRUG FORMULATION	11
2.4	COMMENTS ON NOVEL EXCIPIENTS	11
2.5	COMMENTS ON IMPURITIES/DEGRADANTS OF CONCERN	12
2.6	PROPOSED CLINICAL POPULATION AND DOSING REGIMEN	12
2.7	REGULATORY BACKGROUND	12
3	STUDIES SUBMITTED.....	13
3.1	STUDIES REVIEWED.....	13
3.2	STUDIES NOT REVIEWED	15
3.3	PREVIOUS REVIEWS REFERENCED.....	15
4	PHARMACOLOGY	15
4.1	PRIMARY PHARMACOLOGY	15
4.2	SECONDARY PHARMACOLOGY	15
4.3	SAFETY PHARMACOLOGY	16
5	PHARMACOKINETICS/ADME/TOXICOKINETICS	17
5.1	PK/ADME.....	17
5.2	TOXICOKINETICS	21
6	GENERAL TOXICOLOGY.....	21
6.1	SINGLE-DOSE TOXICITY	21
6.2	REPEAT-DOSE TOXICITY	25
7	GENETIC TOXICOLOGY	77
7.1	<i>IN VITRO</i> REVERSE MUTATION ASSAY IN BACTERIAL CELLS (AMES).....	77
7.2	<i>IN VITRO</i> ASSAYS IN MAMMALIAN CELLS.....	79
7.3	<i>IN VIVO</i> CLASTOGENICITY ASSAY IN RODENT (MICRONUCLEUS ASSAY).....	79
7.4	OTHER GENETIC TOXICITY STUDIES.....	80
8	CARCINOGENICITY	81
9	REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY	91
9.1	FERTILITY AND EARLY EMBRYONIC DEVELOPMENT.....	91
9.2	EMBRYONIC FETAL DEVELOPMENT	95
9.3	PRENATAL AND POSTNATAL DEVELOPMENT	107

10	SPECIAL TOXICOLOGY STUDIES	112
11	INTEGRATED SUMMARY AND SAFETY EVALUATION	123
12	APPENDIX/ATTACHMENTS	131

Table of Tables

Table 1- DCV Tablet Formulation (60 mg)	11
Table 2 - Quality Standards for Excipients Used in the Manufacture of DCV Tablets ...	12
Table 3. Proposed DCV drug substance impurity specifications	12
Table 4. Proposed DCV drug substance residual solvent specifications.....	12
Table 5 - In Vitro Evaluations of DCV as an Inhibitor of Drug Metabolizing Enzymes and Transporters.....	16
Table 6 - TK Summary, Study DN06064	22
Table 7 - TK for BMS-79005 in TPGS-free Vehicle, Study DS10042	23
Table 8 - TK of BMS-790052 in Dogs (single dose), Study DS06211	24
Table 9 - PK Summary of BMS-790052 and Metabolites, Study DM06053	25
Table 10 - Mean Serum Cmax AUC Values for BMS-790052 in Mice, Study DT06107	26
Table 11 - Mean Concentration of BMS790052 in Tissues of Mice Given BMS-790052, Study DT06107	26
Table 12 - TK Summary, CBYB6F1 Mice, Study DM10043	27
Table 13 – Study Design: 5-Day Range Finding Study in Mice.....	29
Table 14 – Study Design: 28-Day Toxicology Study in Mice	29
Table 15 – Hematology: 28-Day Toxicology Study in Mice	30
Table 16 – Organ Weight: 28-Day Toxicology Study in Mice	31
Table 17 – Histopathology: 28-Day Toxicology Study in Mice.....	32
Table 18 – Toxicokinetics: 28-Day Toxicology Study in Mice.....	33
Table 19 - TK Summary, Study DN06068	34
Table 20 – Study Outline: 1 Month Rat Study	35
Table 21 – Clinical Chemistry: 1 Month Rat Study	37
Table 22 – Urinalysis: 1 Month Rat Study	38
Table 23 – Gross Pathology: 1 Month Rat Study	38
Table 24 – Organ Weight Changes: 1 Month Rat Study	39
Table 25 – Histopathology: 1 Month Rat Study	39
Table 26 – Mean TK Parameters for BMS-790052: 1 Month Rat Study.....	40
Table 27 – Mean TK Parameters for BMS-805215 (Metabolite): 1 Month Rat Study ...	40
Table 28 – Study Outline: 6 Month Rat Study	42
Table 29 – Mean Water Consumption Increases: 6 Month Rat Study.....	43
Table 30 – APTT Prolongation in the Treatment Animals: 6 Month Rat Study.....	43
Table 31 – Clinical Chemistry Changes: 6 Month Rat Study	44
Table 32 – Urinalysis: 6 Month Rat Study	45
Table 33 – Organ Weight Changes: 6 Month Rat Study	45
Table 34 – Histopathology: 6 Month Rat Study	46
Table 35 – Toxicokinetic Summary for BMS-790052 in Plasma: 6 Month Rat Study	47

Table 36 – Liver, Plasma, and Liver/Plasma Ratios: 6 Month Rat Study	47
Table 37 – Liver Concentrations of Parent and Metabolite: 6 Month Rat Study	47
Table 38 - Toxicokinetic Parameters (Mean ± SD) of BMS-790052 in Plasma of Dogs Receiving Once-daily Oral Doses of 3, 15 or 100/50 mg/kg for up to 28 Days; Study DS07058	52
Table 39 - Toxicokinetic Parameters (Mean ± SD) of BMS-805215 in Plasma of Dogs Receiving Once-daily Oral Doses of 3, 15 or 100/50 mg/kg for up to 28 Days; Study DS07058	52
Table 40 - Mean Plasma Toxicokinetic Parameters for BMS-790052, BMS-805215, and BMS-795853; Study DS07214	53
Table 41 - Incidence and Frequency of Soft/Liquid Feces During the Dosing Period ...	55
Table 42 - BMS-790052-related Changes in AL T and AST (Relative to Pretest) and their Incidence	56
Table 43 - BMS-790052 related Organ Weight changes	57
Table 44 - Incidence of Histopathological Findings	59
Table 45 - Mean Toxicokinetic Parameters for BMS-790052 a,b,c	60
Table 46 - Mean BMS-790052 Concentrations in Liver, Bile and Plasma at Necropsy ..	60
Table 47 - Incidence and Frequency of Soft/Liquid Feces During the Dosing Period ...	62
Table 48 - Incidence and Frequency of Soft/Liquid Feces During the Recovery Period ..	63
Table 49 - Incidence of BMS-790052-Related Gross Findings	64
Table 50 - BMS-790052-Related Organ-Weight Changes	65
Table 51 - Incidence of BMS-790052-Related Microscopic Findings in End-of-Dose Animals	66
Table 52 - Incidence of BMS-790052-Related Microscopic Findings in	66
Table 53 - Mean Toxicokinetic Parameters for BMS-790052 a	67
Table 54 - TK Values for BMS-790052 and BMS-650032 in Rats Co-Administered BMS-790052 and BMS-650032 for 1 Month	70
Table 55 - Mean Liver and Plasma Concentrations, and Liver-to-Plasma Concentration Ratios, at Necropsy for Rats Co-Administered BMS-790052 and BMS-650032 for 1 Month	71
Table 56 - TK Summary of pIFN and RBV in Monkeys, Study 08147	72
Table 57 - TK Summary of BMS-790052, pIFN, and RBV in Monkeys, Study 08077 ...	74
Table 58 - TK Summary of BMS-790052 and BMS-650032, Study 8143.....	75
Table 59 - TK Summary of BMS-790052 (DCV) and BMS-650032 (ASV), Study 9008 ..	77
Table 60- Toxicokinetic Parameters of BMS-790052 and BMS-805215 in Male Rats, Study DS08011	80
Table 61 - Experimental Design, Study DN11082	82
Table 62 - Survival, Study DN11082	83
Table 63 - Cause of Death, Study DN11082	84
Table 64 - Clinical Observations, Study DN11082	85
Table 65 - Incidence of BMS-790052-Related Gross Findings, Study DN11082	85
Table 66 - Non-Neoplastic Findings. Study DN11082	86
Table 67 - TK Summary, Study DN11082	86
Table 68 - Experimental Design, Study DN11083	88
Table 69 - Survival Summary, Study DN11083	89
Table 70 - Incidence of Raise Area (Papilloma) of the Right Pinna, Study DN11083 ...	90

Table 71 - Incidence of BMS-790052-related Microscopic Non-Neoplastic Findings, Study DN11083	90
Table 72 - TK Summary, Study DN11083	91
Table 73 - Experimental Design, Study DN08034	92
Table 74 - TK Summary, Study DN08034	93
Table 75 - Incidence of Drug-Related Gross Findings, Study DN08034	94
Table 76 - TK (Main Study), Study 07051	96
Table 77 - TK (Extension Study), Study 07051	96
Table 78 – Mortality (EFD study in rabbits)	99
Table 79 - TK (EFD study in rabbits)	100
Table 80 - Experimental Design, Study DN08011	104
Table 81 - TK Values for BMS-790052, Study DN08011	105
Table 82 - Toxicokinetic Summary in Dams (LD 10)	110
Table 83 - BMS-790052 in Maternal Plasma and Milk and Pup Plasma at 2 Hours Postdose on LD 10	110
Table 84 - TK in Juvenile Rats, Study DN11038	113
Table 85 - Study Design (Juvenile Toxicology Study)	114
Table 86 - TK Parameters in Dosing Week 10 in Juvenile Rats, Study DN12004	116
Table 87 - Bovine Opacity Results, BMS-790052	116
Table 88 - LLNA Results, BMS-790052	117
Table 89 - LLNA Results, Positive Control (HCA)	117
Table 90 - Mean Plasma Toxicokinetic Parameters on Day 1, Study DS07186	119
Table 91 - Mean Concentrations in Bile, Liver, and Plasma at Scheduled Necropsy (Days 4 & 10), Study DS07186	119
Table 92 - TK Summary, BMS-790052, Study DM11028	120
Table 93 - Mean Toxicokinetic Parameters for BMS-790052, Study DS07207	122
Table 94 – Exposure Margins for Oral Toxicity Studies	130

Table of Figures

Figure 1 - Structure of DCV	11
Figure 2 - Proposed pathways for the in vivo biotransformation of [¹⁴ C]BMS-790052 in mice, rats, dogs, monkeys, and humans	20
Figure 3 - Survival Curves (Male), Study DN11082	83
Figure 4 - Survival Curves (Female), Study DN11082	84
Figure 5 - Group mean body weights (males), Study DN08034	93

1 Executive Summary

1.1 Introduction

Daclatasvir (DCV, 60-mg), is a Hepatitis C Virus (HCV) nonstructural protein 5A (NS5A) inhibitor proposed for the treatment of HCV. DCV inhibits HCV NS5A protein with high affinity. DCV is proposed to be used with other anti-HCV drug products. DCV is a novel compound and a first in class.

1.2 Brief Discussion of Nonclinical Findings

Nonclinical studies were conducted in the mouse, rat, dog, rabbit (embryofetal) and cynomolgus monkey. Studies included safety pharmacology, secondary pharmacology, PK/ADME, single-dose and repeat-dose toxicity, carcinogenicity studies (2 year study in rats and 6 month study in transgenic mice), genotoxicity, reproductive toxicity as well as local tolerance studies, immunotoxicity, and phototoxicity.

Safety pharmacology was evaluated for cardiotoxicity, CNS toxicity, and respiratory effects as part of the single- and repeat-dose (single-agent and combination) GLP toxicity studies conducted with DCV. *In vitro* assays evaluated effects on receptor and ion channel ligand binding and relative inhibition of enzyme activity. No significant safety pharmacology signals were detected. Minor CV (cardiovascular) effects were noted *in vivo* in rabbits with an exposure margin of 91x compared to the C_{max} at the recommended human exposure.

DCV was highly protein bound (95.1% to 99.5% in mouse, rat, rabbit, dog, and monkey serum; 95.6% in human serum). After IV single-dose administration, DCV was rapidly eliminated in mice (~1 hr) as well as rats, dogs, and monkeys, ($t_{1/2}$ of ~3.5-4.5 hrs). Distribution studies indicated that (consistent with the indication), DCV concentrates predominantly in the liver. The steady-state volume of distribution for DCV was greater than the reported total body water volumes, indicative of extravascular distribution. The blood-to-plasma DCV concentration ratio values, which were similar in humans and animals, suggested that DCV is distributed preferentially into plasma in most species. CYP3A4 was identified as the major enzyme responsible for the metabolism of DCV and in the formation of BMS-805215 (the primary circulating human metabolite). Although multiple metabolites were detected in animals, there were no unique human metabolites.

The main findings observed with DCV in the nonclinical studies included liver findings (increased weight and enzyme activity) as well as an adrenal effect (hypertrophy and vacuolation). In rats dosed for 1 month, DCV induced only minimal and reversible hepatic changes that included slight increases in serum ALT levels and a minimal increase in liver weights without any liver histologic changes. No liver effects were notable in the 6 month study in rats. In monkeys dosed 4 months, liver enzymes (AST, ALT) increased with dose with histological correlates (mononuclear-cell infiltration in

centrilobular areas of the liver, minimal/slight bile-duct hyperplasia and Kupffer-cell hyperplasia/ hypertrophy and minimal/moderate rarefaction of cytoplasm in centrilobular hepatocytes). In both rats and monkeys, adrenal gland findings included increases in adrenal gland size/weight, morphologic hypertrophy, and/or hyperplasia of cortical cells in the zona fasciculata and/or zona reticularis, increases in urine corticosterone levels (rats), and changes in cytoplasmic vacuolation. In rats, there was also a finding of increased urine output concurrent with increased water consumption in rats (with no associated adverse effects). The adrenal activity was monitored in early clinical trials (cortisol), but no effects were noted. Liver abnormalities were noted in clinical trials (AST/ALT elevations) but were not dose limiting.

Combination toxicology studies were performed with DCV and asunaprevir (ASV), as well as with pegylated-Interferon Alfa-2B + ribavirin (pIFN/RBV). ^{(b) (4)}

DCV was neither genotoxic nor carcinogenic in nonclinical studies (2-year rat study and 6-month transgenic mouse study). In a fertility study, male fertility parameters were affected in rats. Mean pre-implantation loss was noted in litters sired by treated males at increased compared to controls. Furthermore, spermatogenic effects were noted. Exposure margins at the NOAEL (no observed adverse effect level) for male fertility parameters were 4.6-fold (rat). Female fertility parameters were not affected with exposure margins at the recommended daily human exposure of 24-fold. DCV was also not a selective developmental toxicant when administered to pregnant rats or rabbits during organogenesis. All reproductive toxicity findings were secondary to maternal toxicity with exposure margins at the recommended daily human exposure of 6-fold (rat) and 22-fold (rabbit). DCV was also excreted in milk at ~2x the concentration of maternal plasma. No new toxicities relative to those observed in adult rats were seen in juvenile rats administered DCV. The AUC value at the NOAEL for juvenile toxicity was 4-fold the recommended daily human exposure.

Local tolerance studies showed no irritation effects with DCV. However, DCV is a potential dermal sensitizer. DCV was shown to be potentially phototoxic in an *in vitro* study evaluating the absorption of light by DCV. However, a follow up phototoxicity study (single oral dose) in Long Evans rats was negative. Furthermore, no ocular or other photo-related toxicity was noted in any of the repeat dose toxicity.

The submitted studies represent a complete nonclinical toxicology package for DCV. It is recommended that DCV be approved as a treatment for HCV infection in combination with other antiretroviral agents in adults.

1.3 Recommendations

1.3.1 Approvability

There are no nonclinical pharmacology and toxicology issues which would preclude the approval of DCV 60 mg tablets

1.3.2 Additional Non Clinical Recommendations

None.

1.3.3 Labeling

Suggested Pharmacology/Toxicology labeling:

8 USE IN SPECIFIC POPULATIONS



(b) (6)

1 Page(s) of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page

Daclatasvir was not genotoxic in a battery of *in vitro* or *in vivo* assays, including bacterial mutagenicity (Ames) assays, mammalian mutation assays in Chinese hamster ovary cells, or in an *in vivo* oral micronucleus study in rats.

Fertility

Daclatasvir had no effects on fertility in female rats at any dose tested. Daclatasvir exposures at these doses in females were approximately 24-fold the human systemic exposure at the therapeutic daily dose. In male rats, effects on reproductive endpoints at 200 mg/kg/day included: reduced prostate/seminal vesicle weights, minimally increased dysmorphic sperm, as well as increased mean pre-implantation loss in litters sired by treated males. Daclatasvir exposures at the 200 mg/kg/day dose in males were approximately 26-fold the human systemic exposure at the therapeutic daily dose. Exposures at 50 mg/kg/day in males produced no notable effects and was 4. ^(b)₍₄₎ fold the exposure in humans at the recommended daily dose.

(b) (4)

2 Drug Information

2.1 Drug

CAS Registry Number

1214735-16-6

Generic Name

Daclatasvir, DCV

Code Name

BMS-790052

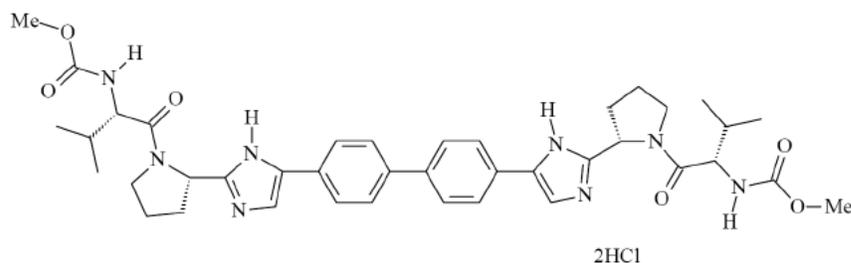
Chemical Name

[[[1,1'-Biphenyl]-4,4'-diylbis[1H-imidazole-4,2-diyl(2S)-2,1-pyrrolidinediyl[(1S)-1-(1-methylethyl)-2-oxo-2,1-ethanediyl]]]biscarbamic acid, dimethyl ester, dihydrochloride

Molecular Formula/Molecular Weight

C₄₀H₅₀N₈O₆.2HCl /811.80 (free base 738.88)

Structure or Biochemical Description

**Figure 1 - Structure of DCV**

Pharmacologic Class

Hepatitis C Virus NS5A inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 79599 – primary IND for DCV

2.3 Drug Formulation**Table 1- DCV Tablet Formulation (60 mg)**

Component	Quality Standard	Function	Quantity per Tablet	
			(%w/w)	(mg)
				(b) (4)
Daclatasvir Dihydrochloride (BMS-790052-05) ^a	In-house ^b	Active	22.0	66.00
Anhydrous Lactose ^c	NF/Ph.Eur./JP			(b) (4)
Microcrystalline Cellulose	NF/Ph.Eur./JP			
Croscarmellose Sodium	NF/Ph.Eur./JP			
Silicon Dioxide	NF/Ph.Eur. ^d /JPE			
Magnesium Stearate	NF/Ph.Eur./JP			
				(b) (4)
Opadry [®] Green ^e	In-house ^f			(b) (4)
				(b) (4)
Total Tablet Weight				315.00

2.4 Comments on Novel Excipients

No novel excipients.

Table 2 - Quality Standards for Excipients Used in the Manufacture of DCV Tablets

Excipient	Quality Standard
Anhydrous Lactose	NF/Ph.Eur./JP
Microcrystalline Cellulose	NF/Ph.Eur./JP
Croscarmellose Sodium	NF/Ph.Eur./JP
Silicon Dioxide	NF/Ph.Eur. ^a /JPE
Magnesium Stearate	NF/Ph.Eur./JP



(b) (4)

2.5 Comments on Impurities/Degradants of Concern

Overall, the proposed daclatasvir specifications summarized below are considered acceptable from a pharmacology/toxicology perspective.

Table 3. Proposed DCV drug substance impurity specifications

Impurity	Specification
BM	(b) (4)
individual unspecified impurities	(b) (4)

Table 4. Proposed DCV drug substance residual solvent specifications

Solvent	Specification
	(b) (4)

For complete discussion, see the impurity/degradant review by Dr. Mark Powley in Appendix A.

2.6 Proposed Clinical Population and Dosing Regimen

DCV is indicated at 60 mg QD (orally) for adults with HCV genotype (b) (4) infection and compensated liver disease (including cirrhosis).

2.7 Regulatory Background

DCV was evaluated in clinical trials under IND 79599.

3 Studies Submitted

3.1 Studies Reviewed

Absorption

- Single Dose PK – Study 930022297
- *In vitro* studies – Studies 930051246, 9300066616

Distribution

- *In vitro* studies – Studies 930022297, 930069481, 9300029499
- *In vivo* studies – Studies 930022297, DM07005, 930066888, 930066617, 930066612
- Study NCPK 26: Placental Transfer, Lacteal Excretion, and Tissue Distribution of Radioactivity in Pregnant Female SD Rats and Tissue Distribution of Radioactivity in Male and non-Pregnant Female SD Rats Following Oral Administration of ¹⁴C-BMS-790052

Metabolism

- Studies – 930022297, 930057616, 930041493, 930066897, 930022297, 930063706, 930022297, 930037531, 9300066871, 9300068344, 930047552, 930066867

Excretion

- Studies – 930039863, 930039861, 930065128, 930039859, 930037202, 93003858, 930041493, 930041493, 930022297

PK Drug Interactions

- Studies – 930022297, 930066927, 930051248, 930053239, 930066894, 930066932, 930066614, 930053227, 930022297

Safety Pharmacology

- Study DS07155 – BMS-790052 and BMS-805215; In Vitro Safety Pharmacology Screen of Receptor and Ion-Channel Binding and Enzyme Activity.
- Study DT 07080 – Effects on cardiac potassium (HERG/IKR) and cardiac sodium channels
- Study DT 06122 - Effects on cardiac electrophysiology in anesthetized rabbits
- Study DT 06153 - Single dose oral exploratory cardiovascular telemetry study in dogs
- Study DT07076 - BMS-790052; Effects on Cardiac Ion Channels and on Rabbit Purkinje Fiber Action Potentials.

Single Dose Toxicology

- Study DS10043 – Range finding study in CByB6F1 Hybrid Mice
- Study DS07063 – Range finding study in Mice
- Study DN06043 – Range finding study in Rats

- Study DS10042 – Range finding study in Rats
- Study DS07054 – Range finding study in Rats
- Study DS06211 – Range finding study in Dogs
- Study DM06053 – Range finding study in Monkeys

Repeat Dose Toxicology

- Study DT06107 – 4 day exploratory study in Mice
- Study DM10043 – 2 week Toxicology study in Mice
- Study DS080152 – 28-Day Exploratory Oral Toxicity Study in CBYB6F1 Hybrid Mice with a Preliminary 5-Day Range-Finding Toxicity Study
- Study DN06068 – 2 week Oral Exploratory Toxicology Study in Rats
- Study DS07055 – One-Month Oral Toxicity Study in Rats
- Study DS08002 – Six-Month Oral Toxicity Study in Rats
- Study DS07058 – 1 Month Oral Toxicity Study in Dogs
- Study DS07214 – 1 Month Oral Investigative Toxicity Study in Monkeys
- Study DS08039 – 4 Month Repeat Dose Toxicology Study in Monkeys
- Study DS08003 – 9 Month Repeat Dose Toxicology Study in Monkeys

Combination Toxicology Studies

- Study DS08126 – 1 month oral combination study in rats (BMS-790052 and BMS-650032)
- Study DS08147 – 1 week range finding study (pIFN/RBV) in monkeys
- Study DS08077 – 2 week study in monkeys with BMS-790052, pIFN, RBV
- Study DS08143 – 1 month study in monkeys with BMS-790052 and BMS-650032
- Study DM09008 – 3 month study in monkeys with BMS-790052 and BMS-650032

Genetic Toxicology and Carcinogenicity

- Study DS06143 – Exploratory Ames assay (non-GLP)
- Study DS06219 – Exploratory Ames Assay (non-GLP)
- Study DS07070 – GLP Ames Assay
- Study 964256 – GLP Ames Assay
- Study DS07064 – Chromosomal Aberration Assay
- Study DS06195 – Exploratory Micronucleus Assay (non-GLP)
- Study DS08011 – Rat Micronucleus Assay
- Study DN11083 – TgRAS Mouse carcinogenicity study
- Study DN11082 – 2 year Rat Carcinogenicity study

Reproductive Toxicology

- Study DN07054 – Range Finding repro study in Rats
- Study DN07051 – Range Finding repro study in Rabbits
- Study DN08034 – Fertility and early EFD study in Rats
- Study DN08012 – EFD study in Rabbits
- Study DN12003 – Pre- and Post-natal development in Rats

Special Toxicology Studies

- Study DN11038 – Juvenile Rats (3 week range finding, non-GLP)
- Study DN12004 – Juvenile Rats (10 week, GLP)
- Study 1212/0348 – Skin Sensitization in the mouse
- Study 1212/0349 – Skin Sensitization in the rabbit
- Study 07AE50.350079 – Opacity Assay with Bovine Corneas
- Study DS07186 – Nine Day Oral Investigative Toxicity Study in Dogs
- Study DM11028 – A Three-Month Oral Qualifying Toxicity Study in Rats
- Study DS07109 – *In vitro* Phototoxicity Study in 3T3 fibroblasts
- Study DS07207 – *In vivo* Phototoxicity Study in Rats

3.2 Studies Not Reviewed

All nonclinical safety studies relating to BMS-790052 were reviewed. See *Clinical Virology review(s) for nonclinical studies relating to Virology*.

3.3 Previous Reviews Referenced

All reviews from IND 79,599 are integrated into this review.

See Dr. Min Min's statistical review of the carcinogenicity studies for NDA 206-843.

4 Pharmacology

4.1 Primary Pharmacology

DCV is proposed as an NS5A inhibitor. The primary pharmacology assay(s) for NS5A inhibition activity (potency, cytotoxicity, and selection and characterization of drug resistance) were reviewed by the Clinical Virology Reviewers (Dr. Patrick Harrington and Dr. Lalji Mishra). Please see the Clinical Virology reviews for more information.

4.2 Secondary Pharmacology

In vitro, DCV was evaluated as an inhibitor of various CYPs and transporters. Results are summarized below. No major effects were noted in the ligand binding panel.

Table 5 - *In Vitro* Evaluations of DCV as an Inhibitor of Drug Metabolizing Enzymes and Transporters

Enzyme/Transporter	IC50 (μM) ^a	Comments
CYP3A	11 and 31.8 ⁸³	Data from human liver microsomes; DCV was a reversible and time-dependent inhibitor of CYP3A.
CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and CYP2D6	> 40 ⁸³	Data from human liver microsomes.
UGT1A1	12.7 ⁸⁴	
Digoxin transport	4.4 ¹	Caco-2 cell assay
P-gp	> 7 ⁸⁵	Inhibition at the highest tested concentration (7 μM) was 12.7%.
BCRP	10.9 ± 8.6 ⁸⁶	
MRP2	32 ± 7.7 ⁸⁷	
OATP1B1	2.3 ¹	
OATP1B3	5.7 ± 1.3 ⁸⁸	
OATP2B1	41.8 ± 4.0 ⁸⁹	
NTCP	ND ⁹⁰	Daclatasvir did not show any inhibition of NTCP at the tested concentrations of up to 16 μM.
OAT1	> 8 ⁹¹	Inhibition at the highest tested concentration (8 μM) was 27.9%
OAT3	> 8 ⁹¹	Inhibition at the highest tested concentration (8 μM) was 25.8%
OCT1	1.4 ⁹¹	
OCT2	7.3 ⁹¹	
BSEP	5.92 ⁹⁰	

Abbreviations: CYP: cytochrome P450; BCRP: breast cancer resistance protein; BSEP: bile salt export pump; MRP: multiple drug resistance protein; ND: not determined. NTCP: sodium-taurocholate cotransporting polypeptide. OAT: organic anion transporter; OATP: organic anion transporting polypeptide; OCT: organic cation transporter; P-gp: P-glycoprotein; UGT: uridine diphosphoglucuronosyltransferase;

^a For the purpose of in vitro to in vivo extrapolation, these IC50 values were converted to Ki using $K_i = [IC_{50}]/2$, which should be reasonable under the experimental conditions used for the assays.

4.3 Safety Pharmacology

CV (cardiovascular), CNS (central nervous system), and respiratory systems were evaluated as part of the single- and repeat-dose toxicology studies. In addition, *in vitro* and *in vivo* safety pharmacology studies were conducted with DCV, as well as the metabolites BMS-805215 (pyrrolidine-hydroxylated rearranged DCV -- the only circulating human metabolite) and BMS-795853 (descarboxymethyl DCV).

CARDIOVASCULAR

CV evaluations were included as part of the repeat dose toxicology studies.

In vitro, DCV was a mild inhibitor of hERG potassium channels (26.2% and 50.6% at 10 and 30 μ M, respectively, with an IC_{50} of 29.2 μ M). DCV was also a mild inhibitor of sodium channels (50.5% and 59.4% at 10 μ M), but not L-type calcium channels.

In an *in vivo* study in anesthetized rabbits, DCV was administered intravenously (IV) at 0 (vehicle), 1, 3, 10, and 30 mg/kg. There were no effects on ECG parameters at 1, 3, or 10 mg/kg (plasma concentrations \leq 72.9 μ g/mL), but at 30 mg/kg (156.9 μ g/mL), DCV moderately increased QRS duration ($29 \pm 1\%$) and mildly increased PR ($19 \pm 3\%$), AH ($16 \pm 4\%$) and HV ($10 \pm 1\%$) intervals. These effects indicate a slowed cardiac conduction within His-Purkinje system and ventricles, as well as in the atrioventricular (AV) node at 30 mg/kg of DCV. The effects of DCV on rabbit cardiac conduction only at the highest doses was consistent with the mild hERG and sodium channel findings observed *in vitro*.

No DCV-related CV (heart rate or ECG) changes were observed in dogs administered a single dose at \leq 150 mg/kg/day, or repeated daily doses at \leq 100 mg/kg/day for \leq 1 month. In monkeys, there were no DCV-related CV (heart rate or ECG) changes observed at \leq 300 mg/kg/day dosed for 4 months or \leq 150 mg/kg/day dosed for 9 months. At the highest doses, DCV exposures in dogs was \sim 9.6x (AUC) and in monkeys was \sim 2.7x (AUC) recommended daily human exposure.

PULMONARY

Pulmonary evaluations were included as part of the repeat dose toxicology studies. No significant effects were noted.

NEUROLOGIC

Neurologic evaluations were included as part of the repeat dose toxicology studies. No significant effects were noted.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Pharmacokinetic evaluations were included as part of the repeat dose toxicology studies.

Absorption and Bioavailability

Generally, absorption of DCV after oral administration in the animals studied was rapid. The time at which maximum concentration (C_{max}) in plasma was achieved (T_{max}) was 0.5 to 2.9 hours. The absolute oral bioavailability of DCV was 123% in mice, 50% in rats, 144% in dogs, and 38% in monkeys, indicating good oral absorption in mice, rats, and dogs, consistent with the good absolute oral bioavailability (67%) in humans. Data from *in vitro* and/or *in vivo* studies indicate that DCV is a substrate for P-glycoprotein (P-gp), but is not a substrate for human breast cancer resistance protein (BCRP). The absorption of DCV was pH-dependent in dogs. The pH-dependent absorption of DCV was confirmed in humans treated with famotidine or omeprazole; bioavailability decreased as gastric pH was increased.

Distribution

In vitro protein binding of DCV was similar in animals and humans (98.2%, 98.3%, 96.5%, 99.5%, 95.1%, and 95.6% in mouse, rat, dog, rabbit, monkey, and human serum, respectively).

The steady-state volume of distribution (V_{ss}) for DCV (3.6, 5.4, and 2.2 L/kg in rats, dogs, and monkeys, respectively) was greater than the reported total body water volumes, indicative of extravascular distribution. The tissue distribution of radioactivity was similar in pigmented Long-Evans rats receiving a single oral dose of ^{14}C -DCV and albino Sprague-Dawley rats receiving single (male and female) and multiple (male only) oral doses of ^{14}C -DCV. Drug-derived radioactivity was rapidly absorbed and widely distributed.

In Long Evans rats, ^{14}C -DCV was highest in the adrenal gland, bile, liver, cecum, small intestine, and stomach. Low concentrations of radioactivity were observed in tissues of the vascular/lymphatic, secretory, fatty, dermal, reproductive, skeleton/muscular, respiratory, and ocular organ systems (eye and uveal tract). In SD (albino) rats, tissue radioactivity concentrations were similar as in the Long Evans (pigmented) rats.

In pregnant Sprague Dawley rats given oral ^{14}C -DCV, there was rapid distribution of radioactivity into maternal and placental tissues. Radioactivity was detected in the fetal liver only at 4 hours, with a fetal liver-to-maternal plasma ratio of 0.19. In all other fetal tissues examined, radioactivity was either not detected or was BLQ. These results indicate that DCV and/or its metabolites cross the placenta in rats, but the distribution of radioactivity in fetal tissues was limited.

In nursing rats which were exposed to ^{14}C -DCV orally, radioactivity was detected in rat milk at 4 hours after dosing. The milk-to-maternal plasma concentration ratios based on C_{max} and AUC were 1.55 and 1.27, respectively.

Results from these drug distribution studies, conducted with ^{14}C -DCV, were consistent with the results from the repeat dose toxicology studies conducted with non-labeled DCV which indicated that DCV concentrates in the liver (mice, rats, dogs, and monkeys) and has limited penetration in brain (mice and rats). Furthermore, in studies using P-gp (an ABC transporter) knockout mice, the brain-to-plasma AUC ratio for DCV was about 3.4x and 5x higher in P-gp knock-out mice than those in wild-type mice after IV and oral administration, respectively. This suggests that the lack of brain distribution may be due to active P-gp transport of DCV in mice.

Metabolism

The *in vitro* rate of DCV metabolism in liver microsomes was similar in mouse, rat, dog, monkey, and human. In hepatocytes, however, the rate of DCV metabolism was lower in rat and human than in mouse, dog, and monkey. Eleven metabolites of DCV were formed in liver microsomes, liver S9 fractions, and hepatocytes of various species.

DCV was the predominant radioactive component in mouse, rat, rabbit, bile duct-cannulated (BDC) dog, and monkey plasma, representing 75% to 90%, 85% to 88%, 90% to 93%, 87.5% to 93.9%, and 74% to 86% of plasma radioactivity, respectively. In intact mice, rats, rabbits, and monkeys, 19%, 28%, 26%, and 27% of the dose, respectively, was recovered as metabolites in excreta. In bile duct-cannulated (BDC) rats, BDC dogs, and BDC monkeys, 36%, 17%, and 33% of the dose, respectively, was recovered as metabolites. Overall, in intact animals, the predominant metabolites identified in excreta were BMS-805215 (pharmacologically active) in monkeys (17.5% of the dose), BMS-795853 (pharmacologically active) in mice (6.3% of the dose), and BMS-952328 (desimidazo DCV-2-oxo-naphthyl ethanoic acid) in mice, rats, and rabbits (7.4%, 9.8%, and 1%, of the dose, respectively). In the bile duct-cannulated studies, the predominant metabolites were BMS-805215 in rats and monkeys (10.5% and 12.6% of the dose, respectively) and BMS-795853 in dog (6.0% of the dose). Other metabolites identified in bile, feces, or urine represented < 5% of the dose.

In humans given ^{14}C -DCV orally, 94.3% of the dose was recovered through 240 hours (10 days) post-dose, of which 58.9% and 30.1% of the dose was recovered as intact parent and metabolites, respectively. The formation of metabolites mainly involved

oxidation. *In vivo* metabolite profiles were qualitatively similar in animals and humans and there were no unique human metabolites.

Figure 2 - Proposed pathways for the *in vivo* biotransformation of ¹⁴C-BMS-790052 in mice, rats, dogs, monkeys, and humans

(b) (4)



Excretion

The elimination of DCV involved multiple pathways. Mostly, excretion was via the fecal route (70 – 91%, monkeys < mice < rats rabbits). In most species (mice < rats < rabbits < monkeys), only 0.4% to 1.3% of the dose appeared in urine as DCV. Dogs were slightly different than the other species, with 24.5%, 29.7%, and 8.75% of administered radioactivity recovered in bile, feces, and urine respectively.

In a fate study, 17-27% of the drug was found as metabolites in the species studied (dogs < mice < rats < rabbits < monkeys).

Humans had a similar profile as the rodents and monkeys: 6.61% in urine and 87.7% in feces.

PK Drug Interactions

Combination toxicology studies were performed to evaluate drug/drug interactions. No major effects were noted. Please see repeat dose toxicology section for the review of the combination toxicology data.

5.2 Toxicokinetics

Toxicokinetic evaluations were included as part of the repeat dose toxicology studies.

6 General Toxicology

6.1 Single-Dose Toxicity

Study title: BMS-790052, A Single-Dose Oral Exporatory TK and Tolerability Study in Male CByB6F1 Hybrid Mice

Study no.: DS10043
GLP compliance: No

Key Study Findings

The purpose of this study was to evaluate a TPGS (Vitamin E d- α -tocopheryl polyethylene glycol 1000 succinate)-containing and TPGS-free vehicles on BMS-790052 pharmacokinetics. Mice were dosed in a single oral gavage at 100, 300, 600, or 1000 mg/kg to groups of 20 male mice. No toxicity was noted at any dose. The exposure (AUC) of BMS-790052 was 0.2x to 0.7x lower in TPGS-free vehicle compared to TPGS-containing vehicle.

Study title: BMS-790052, SINGLE-DOSE ORAL TOXICITY STUDY IN MICE

Study no.: DS07063
GLP compliance: Yes
QA statement: Yes

Key Study Findings

BMS-790052 was administered by oral gavage to 4 groups of mice (5 mice/sex/group) at single doses of 0 (vehicle control), 100, 300, or 1000 mg/kg. All mice survived to the end of the study and there were no drug related findings or changes in clinical signs, body weight, food consumption, physical examinations, and gross pathology at any dose. The high dose (1000 mg/kg) was the NOEL for this study.

Study title: BMS-790052, SINGLE-DOSE ORAL TOXICOKINETIC AND TOLERABILITY STUDY IN RATS

Study no.: DN06064
GLP compliance: No

Key Study Findings

BMS-790052 was administered to rats (6/sex/group) at single doses of 30, 100, or 300 mg/kg.

Systemic exposures to BMS-790052, and a metabolite (BMS-795853), were dose related. Increases in AUC for both male and female rats after a single dose appeared to be more than proportional to the dose increment from 30 to 100 mg/kg, but less than proportional to the dose increment between the two higher doses (100 and 300 mg/kg). Exposure (AUC) to BMS-790052 in females was 7 to 32% higher than that in males over the dose range studied. The metabolite (BMS-795853) to parent (BMS-790052) plasma AUC ratios were 0.8 to 1.7% and approximately 0.8% in male and female rats, respectively, over the dose range studied.

Table 6 - TK Summary, Study DN06064

Parameter	Study Day	BMS-790052 Dose (mg/kg)					
		30		100		300	
		Male	Female	Male	Female	Male	Female
BMS-790052 C _{max} (nM)	1	4839 ± 736	5894 ± 1189	22084 ± 12799	26441± 4675	26147 ± 1688	33497 ± 2104
BMS-790052 AUC(0-last) (nM*h)	1	39212	41962	174108	230024	415216	519757
BMS-795853 C _{max} (nM)	1	62 ± 67	58 ± 15	120 ± 8	121 ± 21	197 ± 43	198 ± 44
BMS-795853 AUC(0-last) (nM*h)	1	681	349	1627	1727	3345	4308

All rats survived to the end of the study. No drug-related effects on body weight or clinical signs were noted in males or females at 30 or 100 mg/kg or in females at 300 mg/kg. NOAEL was the high dose of 300 mg/kg for this study.

Study title: BMS-790052, SINGLE-DOSE ORAL EXPLORATORY TOXICOKINETIC AND TOLERABILITY STUDY IN MALE RATS

Study no.: DS10042
GLP compliance: No

Key Study Findings

The study was conducted to determine the toxicokinetics and tolerability of BMS-790052 in a Vitamin E-d- α -tocopheryl polyethylene glycol 1000 succinate (TPGS)-free vehicle given as a single-oral dose to male rats to support dose and formulation selection for a definitive 2-year carcinogenicity study in rats. BMS-790052 was administered at doses of 12.5, 25, 50, or 100 mg/kg to groups of 6 male rats.

Table 7 - TK for BMS-790052 in TPGS-free Vehicle, Study DS10042

Parameter	BMS-790052 Doses (mg/kg)			
	12.5	25	50	100
C _{max} (µg/mL)	1.60	2.99	8.10	14.5
AUC(0-24h) (µg•h/mL)	9.58	34.4	77.8	123

When compared to a similar TPGS-containing vehicle, use of the TPGS-free vehicle resulted in minimal ($\leq 2.6\times$) to no differences in mean BMS-790052 AUC values across the dose range of 12.5 to 100 mg/kg. No toxicity was noted at any dose. NOAEL for this study was 100 mg/kg.

Study title: BMS-790052, SINGLE-DOSE ORAL TOXICITY STUDY IN RATS

Study no.: DS07054
 GLP compliance: Yes
 QA statement: Yes

Key Study Findings

BMS-790052 was administered by oral gavage to groups of 5 rats/sex at single doses of 0 (vehicle control), 100, 300, or 1000 mg/kg. All animals survived to the end of the study, and there were no drug-related gross- or microscopic-pathology findings. Transient decreases in food consumption occurred during days 1-3 in all groups. Transient body weight losses occurred at 300 and 1000mg/kg. At 1000 mg/kg a low incidence of transient hair coat soiling was observed. Due to the transient weight loss at 300 and 100 mg/kg, the NOAEL was 100 mg/kg.

Study title: BMS-790052, SINGLE-DOSE ORAL EXPLORATORY TOXICOKINETIC AND TOLERABILITY STUDY IN DOGS

Study no.: DS06211
 GLP compliance: No

Key Study Findings

BMS-790052 was administered as a single dose by oral gavage to 4 groups of dogs (2 dogs/sex/group) at doses of 0 (vehicle control), 15, 50, or 150 mg/kg. Systemic exposure (AUC) to BMS-790052 following administration of single oral doses was approximately equal to (male) or greater than (female) dose proportional between 15 and 50 mg/kg, but less than dose proportional from 50 to 150 mg/kg (both sexes).

Table 8 - TK of BMS-790052 in Dogs (single dose), Study DS06211

Dose (mg/kg)	C _{max} (nM)		AUC _{last} (μM•h)	
	Male	Female	Male	Female
15	2940	2804	15.9	16.1
50	7089	18116	63.4	208.3
150	7713	11573	95.9	150.1

At doses of 50 and 150 mg/kg in females, drug-related vomitus (with ingesta or white material) occurred after dosing on Day 1 (1/2 and 2/2 at 50 mg/kg and 150 mg/kg, respectively). The T_{max} of BMS-790052 did not appear to change in female dogs which had vomitus. Additional drug-related findings were limited to reduced food consumption on Day 1. There were no drug-related body weight changes, and no clinical-pathology, or physical examination findings. Although there was vomiting in the dogs at 50 and 150 mg/kg, there were no other effects. The NOAEL is considered to be 150 mg/kg.

Study title: BMS-790052, SINGLE-DOSE ORAL TOXICOKINETIC AND TOLERABILITY STUDY IN MONKEYS

Study no.: DM06053
GLP compliance: No

Key Study Findings

BMS-790052 was administered as a single oral dose to 3 groups of 1 monkey/sex at doses of 15, 50, or 150 mg/kg to determine the tolerability and toxicokinetics profile of BMS-790052. Systemic exposure in monkeys to BMS-790052 following single oral administration at 15, 50, and 150 mg/kg was dose-related. Increases in AUC(0-last) for males and females were greater than dose proportional from 15 to 50 mg/kg and approximately dose proportional from 50 to 150 mg/kg. BMS-790052 AUC values in females were approximately 2-fold the AUC values in males at all doses tested. All animals survived the study. There were no drug-related clinical observations, effects on body weight or feeding behavior, or changes in serum chemistry parameters. Due to the lack of any significant effects at any dose, the high dose (150 mg/kg) was considered to be the NOEL.

Table 9 - PK Summary of BMS-790052 and Metabolites, Study DM06053

Parameter	Dose (mg/kg)					
	15		50		150	
	M	F	M	F	M	F
<u>BMS-790052</u>						
C _{max} (μM)	1.2	1.8	3.9	6.0	8.1	8.9
AUC (0-last) (μM•h)	3.1	6.1	18.2	34.4	53.5	96.3
T _{max} (h)	2.0	2.0	2.0	2.0	2.0	2.0
<u>BMS-795853 (Met-4)</u>	M	F	M	F	M	F
C _{max} (μM)	-	-	-	0.1	-	0.3
AUC (0-last) (μM•h)	-	-	-	-	-	1.2
T _{max} (h)	-	-	-	4.0	-	4.0
<u>BMS-805215 (Met-2)</u>	M	F	M	F	M	F
C _{max} (μM)	0.3	0.5	1.7	4.3	7.4	7.9
AUC (0-last) (μM•h)	0.8	1.5	6.9	17.4	42.7	72.6
T _{max} (h)	2.0	2.0	2.0	2.0	2.0	4.0

N = 1 animal/sex/dose group

A dash (-) indicates that samples were below the lower limit of quantification (< 0.01 μM).

6.2 Repeat-Dose Toxicity

Study title: **4-DAY ORAL EXPLORATORY TOXICITY STUDY IN MICE**

Study no.: DT06107
 Study report location: EDR
 Conducting laboratory and location: BMS, Wallingford, CT
 Date of study initiation: Protocol date – 21 July 2006
 GLP compliance: No
 QA statement: No
 Drug, lot #, and % purity: BMS-790052, Batch 7M23810, 90.5% pure

Key Study Findings

This was an exploratory study in mice. BMS-790052 was given to male and female CD1 (ICR) mice once daily by oral gavage for 4 days at dosages of 0, 15, 50 or 100 mg/kg/day. Serum mean C_{max} AUC values on Day 4 increased nearly proportionally as dosage increased. At 8 and 24 hours post dose on Day 4, mean concentrations of BMS-790052 in liver were higher than those in serum (about 2.5×) whereas the concentrations in heart were about one-third those in serum.

Table 10 - Mean Serum Cmax AUC Values for BMS-790052 in Mice, Study DT06107

Dosage mg/kg/day	Mean Cmax		Composite AUC	
	µM (µg/mL)		µM×h (µg × h/mL)	
	Male	Female	Male	Female
15	5.38 (3.98)	4.04 (2.99)	34.1 (25.2)	25.9 (19.1)
50	18.3 (13.5)	13.1 (9.68)	106 (78.0)	78.2 (57.7)
100	27.5 (20.3)	26.5 (19.6)	165 (122)	171 (126)

Table 11 - Mean Concentration of BMS790052 in Tissues of Mice Given BMS-790052, Study DT06107

Dosage (mg/kg/day)	Serum µM		Liver µM		Heart µM	
	Male	Female	Male	Female	Male	Female
8 hours						
15	1.58	1.39	3.40	2.95	0.37	0.44
50	5.73	5.19	14.4	11.7	2.52	1.55
100	10.1	6.95	29.1	14.8	4.31	2.58
24 hours						
15	ND	ND	0.03	0.06	ND	ND
50	0.10	0.05	0.23	0.11	0.03	ND
100	0.67	0.85	1.63	1.98	0.17	0.21

Values represent the mean of 2 or 3 animals.

ND = Not determined as concentration was below the lower limit of quantification.

There were no compound-related clinical signs or effects on body weight and no compound-related changes in serum chemistry, hematology, and histopathology. BMS-790052 was well tolerated in mice given dosages ≤ 100 mg/kg/day for 4 days.

Study title: BMS-790052, TWO-WEEK ORAL TOXICITY STUDY IN CBYB6F1 HYBRID MICE

Study no.: DM10043
 Study report location: EDR
 Conducting laboratory and location: BMS, Mount Vernon, Indiana
 Date of study initiation: Protocol date - 23-Aug-2010
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: BMS-790052, Batch 7M23810, 90.5% pure

Key Study Findings

This study was conducted to determine the potential toxicity of BMS-790052 when given to CByB6F1 hybrid mice for 2 weeks and to provide data to assist in dose selection for a carcinogenicity study in Tg.rasH2 mice. BMS-790052 was given orally at doses of 0 (vehicle), 40, 400, or 1500 mg/kg/day to groups of either 6 or 33 mice per sex.

Mean Week 2 AUC values increased in a dose proportional manner across all doses, were comparable in males and females, and were similar (0.7 to 1.5×) to those observed on Day 1 (see below).

BMS-790052 was clinically well tolerated when given orally to CByB6F1 hybrid mice for 2 weeks at 40 and 400 mg/kg/day. Findings in the nonglandular stomach at 1500: white discoloration of the nonglandular stomach (1 male) that was associated with focal mild subacute inflammation with focal slight hyperplasia and mild hyperkeratosis of the squamous mucosa.

Based on histopathologic findings in the nonglandular stomach at 1500 mg/kg/day, the no-observed-adverse-effect level (NOAEL) was considered to be 400 mg/kg/day.

Table 12 - TK Summary, CBYB6F1 Mice, Study DM10043

Parameter	Period	BMS-790052 Dose					
		40 mg/kg/day		400 mg/kg/day		1500 mg/kg/day	
		M	F	M	F	M	F
C _{max} (µg/mL)	Day 1	20.2	18.7	59.4	58.5	155	154
	Week 2	18.1	20.2	71.0	67.2	111	136
AUC(0-24h) (µg•h/mL) ^a	Day 1	99.6	80.6	526	522	2,430	2,480
	Week 2	68.0	63.3	715	769	2,130	2,640

^a Mean combined-sex AUC values in Week 2 were 65.7, 742, and 2385 µg•h/mL for the doses of 40, 400, and 1500 mg/kg/day, respectively.

Study title: 28-DAY EXPLORATORY ORAL TOXICITY STUDY IN CBYB6F1 HYBRID MICE WITH A PRELIMINARY 5-DAY RANGE-FINDING TOXICITY STUDY

Study no.:	DS08152
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	28 Oct 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BMS-790052, Batch 7M23810, 90.5% pure

Key Study Findings

This study had 2 parts – 1) a 5-day range finding study and 2) a 28-day toxicology study.

In the 5-day range-finding study (0, 30, 100, 300, 1000, 1500 mg/kg), there were no clinical indications of toxicity at ≤ 300 mg/kg. At doses ≥ 1000 mg/kg, clinical signs included cowering, lethargy, and/or hunched appearance. All animals survived to scheduled sacrifice.

In the 28-day exploratory toxicity study in CByB6F1 mice, the doses were (0, 100, 300, 600, and 1000 mg/kg) based on the toxicity noted in the 5-day study. Similar findings were noted at 1000 mg/kg in the 28-day study that were noted in the 5-day study (cowering, lethargy, hunched posture).

- Body weight loss and deaths were noted. The deaths included 1 TK male at 600 mg/kg and 2 mice at 1000 mg/kg (1 male, and 1 female).
- At doses of 600 and 1000 mg/kg, minimal to mild hyperplasia of the nonglandular stomach was noted with minimal to mild focal or diffuse hyperkeratosis in 2 of 3 mice at 1000 mg/kg.
- Organ weights increased in the spleen and liver with increasing doses.
- Liver vacuolation (attributed to decreased glycogen) with increasing doses.
- Liver enzyme changes which increased with dose.
- Minimal to mild increased extramedullary hematopoiesis in the spleen was noted at all doses in females and at 1000 mg/kg/day in males, and was considered to represent the correlate for the increased spleen weights.

Based on the limited findings at 300 mg/kg (mild decrease in neutrophils in 1 sex, mild cholesterol increase in 1 sex, and lack of histological correlates) and more severe findings (including histological correlates and deaths) at 600 and 1000 mg/kg, the NOAEL for BMS-790052 was 300 mg/kg by oral gavage for 28 days in CByB6F1 Hybrid mice.

Methods

Doses: 0, 100, 300, 600, 1000 mg/kg (for final tox study). Dose of 1500 mg/kg was used in range-finding portion of the study.

Frequency of dosing: Daily

Route of administration: Oral

Dose volume: 10 ml/kg

Formulation/Vehicle: 15% PEG-400, 5% PVP, 5% TPGS and 75% 0.1 M phosphoric acid, w/w, pH ~ 3.

Species/Strain: CByB6F1 Hybrid mice from (b) (4)

Number/Sex/Group: See table below

Age: ~ 7 wks of age

Weight: male mice ranged from 22.0 to 28.1 grams
 female mice ranged from 16.3 to 22.2 grams
 Satellite groups: 2/sex/group were used for TK animals
 Unique study design: None
 Deviation from study protocol: No major deviations that would affect study outcomes.

Table 13 – Study Design: 5-Day Range Finding Study in Mice

Group	Dose levels	No. of animals/sex
Group 1 (Vehicle)	0 mg/kg	5
Group 2	30 mg/kg	5
Group 3	100 mg/kg	5
Group 4	300 mg/kg	5
Group 5	1000 mg/kg	5
Group 6	1500 mg/kg	5
Total animals		30/sex

Table 14 – Study Design: 28-Day Toxicology Study in Mice

Group	Dose Levels (mg/kg/day)	Concentration (mg/mL)	Number of Animals			
			Main Study		TK Study ^a	
			Male	Female	Male	Female
Group 1	0	0	8	8	5	5
Group 2	100	10	8	8	20	20
Group 3	300	30	8	8	20	20
Group 4	600	60	8	8	20	20
Group 5	1000	100	8	8	20	20
Total			40	40	85	85

^a Extra TK animals (2/sex) were used to ensure adequate animals for TK bleeding.

Observations and Results

The 5-day range finding portion of the study is summarized in the Key Study Findings section. The results below are for the 28-day portion of the study.

Mortality

Checked 2x daily. There were 3 deaths on study. One female (main study) at 1000 mg/kg/day was found dead on Day 8. There were no significant gross or microscopic findings in this animal. Although the cause of death for this animal was not determined, a relationship to study drug could not be excluded and it was attributed to BMS-790052. All other main study animals survived until terminal sacrifice on Day 29.

The two other deaths were from the TK animals (1 male at 600 mg/kg/day and 1000 mg/kg/day) which were found dead on Days 23 and 12, respectively. Evidence of a gavage error as the cause of deaths of these animals was not available and because a relationship to study drug could not be excluded, these deaths were attributed to BMS-790052. All other TK animals survived until scheduled sacrifice on Days 26 or 27.

Clinical Signs

Checked 2x daily.

BMS-790052-related clinical observations occurred at 1000 mg/kg/day; these included thinness, decreased motor activity, cowering, and hunched appearance in 1 female on Days 4 through 6. Thin and hunched appearance was also noted during the hands-on clinical observation in 1 female at 1000 mg/kg/day on Day 29. Similar findings were noted at 1000 mg/kg and 1500 mg/kg in the 5-day range finding animals.

Body Weights

Checked pre-dose, then 1x weekly. No changes noted.

Feed Consumption

Checked weekly. No changes noted.

Hematology

At sacrifice. BMS-790052-related hematology changes at Day 29 were limited to mild decreases in neutrophil counts at all doses. These mild decreases were not considered adverse because the values within the affected groups generally fell within the reference range for this mouse strain. Decreased neutrophil count has been observed in a study of dogs given BMS-790052, although not in rats.

Table 15 – Hematology: 28-Day Toxicology Study in Mice

Dose (mg/kg/day)	0		100		300		600		1000	
	M	F	M	F	M	F	M	F	M	F
Neutrophil Count	-	-	0.75*	-	0.64*	-	0.55*	0.41*	0.29*	0.42*

* $p < 5\%$ for absolute values. Data are expressed as treatment mean \div control mean.

Secondary changes at Day 29 suggestive of minimal fluid deficit (dehydration) occurred in males at ≥ 600 mg/kg/day. These changes were limited to non-dose-related increases in hemoglobin (1.12, and 1.04 \times) that lacked clinical or histological correlate.

Clinical Chemistry

At sacrifice. BMS-790052-related changes in serum chemistry at Day 29 were limited to increased cholesterol in both sexes and alkaline phosphatase in males.

Dose (mg/kg/day)	0		100		300		600		1000	
	M	F	M	F	M	F	M	F	M	F
<i>Cholesterol</i>	-	-	-	-	-	1.43*	1.33*	1.61*	1.31*	1.79*
<i>Alkaline Phosphatase</i>	-	-	-	-	-	-	-	-	1.74*	-

* p < 5% for absolute values. Data are expressed as treatment mean ÷ control mean

Gross Pathology

At sacrifice. No major findings noted.

Organ Weights

At sacrifice. Organ weights increased in the spleen and liver. Statistically significant weight increases were noted at doses ≥ 600 mg/kg in females and at 1000 mg/kg in males. Liver weights were significantly increased in females at doses ≥ 300 mg/kg and at 1000 mg/kg in males.

Table 16 – Organ Weight: 28-Day Toxicology Study in Mice

Dose (mg/kg/day):	100		300		600		1000	
Sex:	M	F	M	F	M	F	M	F
Liver, Absolute	-	-	-	24*	-	32*	20*	36*
Liver, Relative	-	-	-	17*	-	24*	19*	36*
Spleen, Absolute	-	20	-	29	-	67*	39*	48*
Spleen, Relative	-	20	-	22	-	57*	39*	47*

A dash (-) indicates absence of change in group; * = P ≤ 0.05; ** = P ≤ 0.01

Note: The numerical values in the table represent the respective percent increase [↑] or percent decrease [↓] from control mean value [(treated group mean - control group mean) ÷ control group mean] × 100.

Histopathology

Adequate Battery – yes.

Peer Review – no.

Histological Findings – measured at sacrifice.

BMS-790052-related histopathologic changes were noted in the stomach (males only), spleen, and liver.

Minimal to mild hyperplasia of the nonglandular stomach was noted in males at 600 and 1000 mg/kg/day and was associated with minimal to mild, focal or diffuse hyperkeratosis at the high dose. Of the 3 male mice that had hyperplasia and hyperkeratosis in the 1000 mg/kg/day dose group, 2 also had inflammation of the wall. It

is unclear if the hyperplasia with hyperkeratosis represents a primary proliferative response or represents an effect secondary to inflammation.

Minimal to mild increased extramedullary hematopoiesis was noted at all doses in females and at 1000 mg/kg/day in males, and was considered to represent the correlate for the increased spleen weights. Minimal increased extramedullary hematopoiesis was also noted in 4 vehicle control females; in BMS-790052-treated mice however, the change occurred at greater incidence and severity, was associated with weight increases, and therefore, considered drug-related. The increased incidence of extramedullary hematopoiesis in females (8/8 at 600 mg/kg/day vs. 6/8 1000 mg/kg/day) accounted for a greater increase in splenic weight at 600 mg/kg/day than at 1000 mg/kg/day. These changes in the spleen were considered to be an adaptive response.

In livers there was a minimal to mild decreased vacuolation of hepatocytes at ≥ 100 mg/kg/day that was attributed to decreased glycogen accumulation.

Table 17 – Histopathology: 28-Day Toxicology Study in Mice

	Dose (mg/kg/day):	0	100	300	600	1000
	No of Mice (M/F)	8/8	8/8	8/8	8/8	8/8
	Sex:	M/F	M/F	M/F	M/F	M/F
<u>Liver:</u>						
Decreased centrilobular vacuolation		2/0	6/3	5/6	4/3	8/7
Minimal		2/0	3/2	0/5	2/1	2/5
Mild		-	3/1	5/1	2/2	6/2
<u>Spleen:</u>						
Extramedullary hematopoiesis		0/4	0/6	0/6	1/8	5/6
Minimal		0/4	0/3	0/4	1/4	5/2
Mild		-	0/3	0/2	0/4	0/4
<u>Stomach:</u>						
Hyperplasia, non glandular (focal/diffuse)		-	-	-	1/0	3/0
Minimal		-	-	-	1/0	1/0
Mild		-	-	-	-	2/0
<u>Stomach:</u>						
Hyperkeratosis		-	-	-	-	3/0
Minimal		-	-	-	-	1/0
Mild		-	-	-	-	2/0
<u>Stomach:</u>						
Inflammation		-	-	-	-	2/0
Minimal		-	-	-	-	1/0
Mild		-	-	-	-	1/0

A dash (-) indicates absence of finding in group

Toxicokinetics

Measured on day 27 at 0.5, 1, 2, 4, 8, and 24 hours post-dose. After repeated daily dosing, increases in systemic exposures to BMS-790052 were approximately dose proportional across all doses in both male and female mice. However, nonlinearity was noted in mean T_{max} values, which tended to increase with increasing dose. Systemic exposures in males were generally similar to those in females.

Table 18 – Toxicokinetics: 28-Day Toxicology Study in Mice

Parameter	Day	BMS-790052							
		100 mg/kg/day		300 mg/kg/day		600 mg/kg/day		1000 mg/kg/day	
		Male	Female	Male	Female	Male	Female	Male	Female
C_{max} ($\mu\text{g/mL}$)	26	16.9	23.2	46.3	41.5	66.7	65.0	65.4	85.3
AUC(0-24h) ($\mu\text{g}\cdot\text{h/mL}$)	26	110	150	365	332	699	895	1170	1170
T_{max} (h)	26	0.5	0.5	4	1	4	4	8	4

Study title: TWO-WEEK ORAL EXPLORATORY TOXICITY STUDY IN RATS

Study no.: DN06068
 Study report location: EDR
 Conducting laboratory and location: BMS, Syracuse, NY
 Date of study initiation: November 20, 2006 (date of signature of Study Director on Protocol)
 GLP compliance: No
 QA statement: No
 Drug, lot #, and % purity: BMS-790052-05, Batch BMS-790052-03-005, Purity "acceptable"

Key Study Findings

BMS-790052, as a solution in 5% w/v polyvinyl pyrrolidone K30, 5% w/v D- α -tocopheryl polyethylene glycol 1000 succinate, 15% w/v polyethylene glycol 400 (PEG 400) in 75% 0.1 M H₃PO₄ (pH 3.0), was administered orally by gavage to 3 groups of rats (6/sex/group) once daily for 2 weeks at 15, 60, or 300 mg/kg. A control group of 6 rats/sex received vehicle at 10 mL/kg/day. An additional group of 6 rats/sex, given normal saline at 10 mL/kg/day, was included because of a lack of toxicity data available for the PEG-400-based vehicle used in this study.

Table 19 - TK Summary, Study DN06068

Parameter	Study Day	BMS-790052 Dose (mg/kg/day)					
		15		60		300	
		Male	Female	Male	Female	Male	Female
BMS-790052 Mean C _{max} (μ M)	1	1.7	2.9	10.7	17.8	27.0	55.9
	14	1.7	3.8	7.1	9.7	30.4	42.3
BMS-790052 Mean AUC (0-24h) (μ M·h)	1	20	22	92	111	495	899
	14	10.3	15	107	119	380	526
BMS-795853 Mean AUC (0-24h) (μ M·h)	1	0.32	0.25	1.2	1.2	5.3	7.1
	14	0.30	0.32	1.9	1.3	7.2	5.6

BMS-790052, at 60 and 300 mg/kg/day (AUC \geq 92 μ M·h), caused increased urine output and adrenal gland changes microscopically. Based on the lack of drug-related changes, the NOEL of this study was 15 mg/kg/day (AUC 10.3 to 22 μ M·h).

Study title: One-Month Oral Toxicity Study in Rats

Study no.: DS07055
 Study report location: EDR
 Conducting laboratory and location: BMS, Syracuse, NY
 Date of study initiation: March 28, 2007 (date of signature of Study Director on Protocol)
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: BMS-790052-05, Batch 7C26990, 91.0%

Key Study Findings

- No effects noted at 10 mg/kg.
- Effects at 30 and 100 mg/kg: cortical hypertrophy/hyperplasia of the adrenal gland (with associated gross findings). The adrenal findings were associated with increased urine corticosterone levels. At 100 mg/kg, there was stomach erosion and discoloration. Water consumption increased at 30 and 100 mg/kg that was associated with corresponding urine changes due to increased water consumption. Liver weights and prostate weights slightly increased at 100 mg/kg. Soiling also occurred during the last 2 weeks of the study at 100 mg/kg.

The findings at 30 and 100 mg/kg were not noted after recovery.

NOAEL was 10 mg/kg based on the cortical hyperplasia at 30 and 100 mg/kg as well as the stomach erosion and discoloration at 100 mg/kg.

Methods

Doses: 0, 10, 30, 100 mg/kg (see table below)
 Frequency of dosing: Daily
 Route of administration: Oral
 Dose volume: 10 ml/kg (see table below)
 Formulation/Vehicle: 15% polyethylene glycol 400 (PEG-400), 5% polyvinyl pyrrolidone K-30 (PVP K-30), 5% Vitamin E d- α -tocopheryl polyethylene glycol 1000 succinate (TPGS), and 75% 0.1 M phosphoric acid buffer (H₃PO₄), pH ~ 3.
 Species/Strain: Charles River Sprague Dawley (CrI: CD [SD]) rats
 Number/Sex/Group: 15/sex/group (see table below)
 Age: ~ 9 weeks old at start of study
 Weight: 264.2 to 326.6 g (males) or 178.9 to 232.4 g (females).
 Satellite groups: 9/sex/group (see table below)
 Unique study design: None
 Deviation from study protocol: 4 animals (male group 3, female group 2, and two females in group 4) had water disconnected and had a significant, transient weight loss associated with accidental water restriction. The effect was transient and did not appear to affect the study outcome.

Table 20 – Study Outline: 1 Month Rat Study

Group Number	Daily Dose		Concentration BMS-790052 (mg/mL)	Number of Animals
	BMS-790052 (mg/kg)	Volume (mL/kg)		
1 ^a	0	10	0	15 M, 15 F
2	10	10	1	15 M, 15 F
3	30	10	3	15 M, 15 F
4	100	10	10	15 M, 15 F
5 ^a	0	10	0	9 M, 9 F
6	10	10	1	9 M, 9 F
7	30	10	3	9 M, 9 F
8	100	10	10	9 M, 9 F

a: Rats in Groups 1 and 5 received the vehicle (see Section 2.1); a solution containing 15% PEG-400, 5% PVP K-30, 5% TPGS, and 75% 0.1 M H₃PO₄, pH ~ 3.

Observations and Results

Mortality

Checked 2x daily. All animals survived to necropsy.

Clinical Signs

Checked 2x daily. No clinical signs at 10 or 30 mg/kg. Soiling (head/muzzle, perigenital, limbs/paws) occurred at 100 mg/kg from day 10 through the last 2 weeks of the study. Soiling resolved during the recovery period.

Body Weights

Weighed pretest, 2x weekly during dosing, then once weekly during post-dose. No significant effect on body weights noted.

Feed and Water Consumption

Quantitative assessment 2x weekly (pretest, dosing, and post-dose). Transient decrease in food from day 1-4 at 30 and 100 mg/kg (14% and 18% decrease). After day 4, food consumption was similar between groups.

Water consumption was similar between 10 and 30 mg/kg. Water consumption dramatically increased in males and females at 100 mg/kg (ranging from 131% to 199% of controls from days 6-24). Water consumption decreased to normal during the recovery period.

Ophthalmoscopy

Pretest, then day 28. No changes noted.

Hematology and Coagulation

At sacrifice. No changes noted.

Clinical Chemistry

At sacrifice. Transient decreases in reticulocytes and platelet counts (0.81x to 0.82x and 0.78x to 0.85x) at day 7 in both sexes at 100 mg/kg – attributed to the transient decrease in food consumption. Changes resolved by day 24.

Slight increases in Triglycerides, cholesterol, bilirubin, and ALT, with a decrease in AST at 30 and 100 mg/kg (see table below).

Table 21 – Clinical Chemistry: 1 Month Rat Study

Parameter	Day	30 mg/kg/day		100 mg/kg/day	
		Male	Female	Male	Female
Triglyceride; mg/dL (Ratio)	7	48 (1.28x)	20 (1.00x)	54 (1.43x)	31 (1.58x)
	24	50 (1.41x)	32 (1.42x)	55 (1.55x)	40 (1.80x)
Cholesterol; mg/L (Ratio)	7	80 (1.35x)	75 (0.95x)	119 (2.00x)	114 (1.44x)
	24	81 (1.26x)	86 (0.97x)	103 (1.60x)	120 (1.35x)
Total Bilirubin; mg/dL (Ratio)	7	0.12 (1.09x)	0.13 (1.10x)	0.12 (1.10x)	0.15 (1.29x)
	24	0.12 (0.97x)	0.17 (1.27x)	0.12 (0.94x)	0.17 (1.27x)
Alanine aminotransferase; U/L (Ratio)	7	35 (1.00x)	33 (1.04x)	44 (1.24x)	43 (1.35x)
	24	34 (1.01x)	35 (0.99x)	40 (1.17x)	37 (1.03x)
Aspartate aminotransferase; U/L (Ratio)	7	97 (0.93x)	97 (0.92x)	86 (0.82x)	91 (0.86x)
	24	99 (0.84x)	87 (0.90x)	92 (0.78x)	72 (0.74x)

a: Ratios in the table represent the group mean relative to that of corresponding controls (treated group mean ÷ control group mean).
Ref represents the reference (control) group for ratio determinations.

Urinalysis

Week 1, 4, and 8. No effects noted at 10 and 30 mg/kg. Effects noted at 100 mg/kg (volume increase, specific gravity decrease, osmolality decrease, corticosterone/creatinine ration increase, and total corticosterone increase). See table below. It is likely that the urinalysis changes were related to the increase in water at 100 mg/kg (about a 130-200% increase) during dosing.

Table 22 – Urinalysis: 1 Month Rat Study

Parameter	Day	100 mg/kg/day	
		Male	Female
Volume; mL (Ratio)	7	47 (2.73x)	36 (3.24x)
	24	32 (2.34x)	30 (3.61x)
Specific Gravity (Ratio)	7	1.011 (0.52x)	1.011 (0.40x)
	24	1.016 (0.56x)	1.015 (0.38x)
Osmolality; milliOsm/kg (Ratio)	7	271 (0.43x)	328 (0.42x)
	24	444 (0.53x)	413 (0.40x)
Corticosterone/Creatinine (Ratio)	7	0.98 (2.80x)	1.10 (2.16x)
	24	0.49 (2.04x)	0.81 (1.84x)
Total Urine Corticosterone; ng (Ratio)	7	674.03 (2.58x)	497.12 (2.10x)
	24	436.81 (2.42x)	394.29 (2.19x)

a: Ratios in the table represent the group mean relative to that of corresponding controls (treated group mean ÷ control group mean).
Ref represents the reference (control) group for ratio determinations.

Gross Pathology

At sacrifice. Increased sizes of the adrenal gland at 30 mg/kg and 100 mg/kg. Stomach discoloration at 100 mg/kg. See table below.

Table 23 – Gross Pathology: 1 Month Rat Study

	Dose (mg/kg/day):	0	10	30	100
	Number of Rats (M/F):	10/10	10/10	10/10	10/10
	Sex:	M/F	M/F	M/F	M/F
<u>Adrenal gland:</u>					
size increased		-/1	-/1	1/5	6/7
<u>Stomach:</u>					
discoloration		-/-	-/-	-/-	4/1

A dash (-) indicates absence of finding in group. Bolded values are those considered drug-related.
M = male; F = female

Organ Weights

At sacrifice. Increases in Adrenals and Liver at 30 and 100 mg/kg. Decreases in prostate gland at 100 mg/kg. See table below.

Table 24 – Organ Weight Changes: 1 Month Rat Study

Dose (mg/kg/day):	10		30		100		
	Sex:	Male	Female	Male	Female	Male	Female
Adrenal Glands		-2	12	22	22	45*	62**
Liver		2	10	0	0	8	14*
Prostate Gland		-11	NA	-9	NA	-25*	NA

* = $P \leq 0.05$; ** = $P \leq 0.01$ for absolute values; NA = not applicable

Note: The numerical values in the table represent the respective percent increase [\uparrow] or percent decrease [\downarrow] from control mean value $[(\text{treated group mean} - \text{control group mean}) \div \text{control group mean}] \times 100$.
 Bolded values are those considered drug-related.

Histopathology

Adequate Battery – yes.

Peer Review – yes.

Histological Findings – In agreement with the gross findings and the weight changes, the histological changes were in the adrenal gland and the stomach. See table below.

Table 25 – Histopathology: 1 Month Rat Study

Dose (mg/kg/day):	0	10	30	100
No. of Rats (M/F):	10/10	10/10	10/10	10/10
Sex:	M/F	M/F	M/F	M/F
<u>Adrenal Gland:</u>				
hypertrophy/hyperplasia				
minimal	-/-	-/-	6/3	1/-
slight	-/-	-/-	-/-	-/6
mild	-/-	-/-	-/-	-/3
moderate	-/-	-/-	-/-	8/-
<u>Stomach:</u>				
erosion (minimal)	-/-	NE	NE	2/1

A dash (-) indicates absence of finding in group

M = male; F = female

NE = not examined

Special Evaluation: Safety Pharmacology Observations

Day 28 and Day 55. No changes in neurological findings or respiration were noted.

Toxicokinetics

Systemic exposure to BMS-790052 was dose-related, similar between sexes, and similar on Days 1 and 28. Increases in AUC and C_{max} for both males and females were greater than dose-proportional between 10 and 30 mg/kg/day and dose-proportional between 30 and 100 mg/kg/day. See table for BMS-790052 TK below.

Table 26 – Mean TK Parameters for BMS-790052: 1 Month Rat Study

Day	BMS-790052 (mg/kg/day)	C_{max} ($\mu\text{g/mL}$)		AUC(0-24 h) ($\mu\text{g}\cdot\text{h/mL}$) ^a	
		Male	Female	Male	Female
1	0	-	-	-	-
1	10	0.852	1.23	6.70	5.02
1	30	3.73	5.32	29.0	23.7
1	100	13.3	12.8	117	95.5
28	0	-	-	-	-
28	10	0.755	1.34	5.39	4.75
28	30	4.00	6.21	26.9	23.9
28	100	9.88	14.0	109	105

- No BMS-790052 was detected in any of the control samples.

The Sponsor also evaluated the Metabolite, BMS-805215. Systemic exposure to the metabolite was dose-related, similar between Days 1 and 28, and represented < 6% of the BMS-790052 exposures. Increases in AUC and C_{max} were generally greater than proportional to the BMS-790052 dose. Exposure to BMS-805215 was substantially greater (~ 4.3 to 17x) in males as compared with females. See table for BMS-805215 TK below.

Table 27 – Mean TK Parameters for BMS-805215 (Metabolite): 1 Month Rat Study

Day	BMS-790052 (mg/kg/day)	C_{max} ($\mu\text{g/mL}$)		AUC(0-T) ($\mu\text{g}\cdot\text{h/mL}$) ^a	
		Male	Female	Male	Female
1	0	-	-	-	-
1	10	0.0380	0.00889	0.189	0.0122
1	30	0.210	0.0408	0.794	0.0752
1	100	1.21	0.193	5.50	0.755
28	0	-	-	-	-
28	10	0.0760	0.0123	0.296	0.0176
28	30	0.292	0.0609	0.966	0.109
28	100	0.742	0.141	6.06	0.612

a: For AUC(0-T), T ranged from 2 to 24 hours postdose.

- No BMS-805215 was detected in any of the control samples.

Stability and Homogeneity

Stability of the 1- and 10-mg/mL formulations was acceptable over a 14-day period, with mean values ranging from 93.0 to 97.8% of the intended concentrations.

Study title: Six-Month Oral Toxicity Study in Rats

Study no.: DS08002
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: March 18, 2008 (date of signature of Study Director on Protocol)
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: BMS-790052
 Two batches: Batch 7B25121-01, 87.4%
 Batch 7M23810, 90.5%

Key Study Findings

- BMS-790052 was well tolerated in rats for 6 months at oral doses of 12.5 and 25 mg/kg.
- At doses of 50 mg/kg, the most significant finding was cortical hypertrophy/hyperplasia of the adrenal gland.
- Minimal APTT prolongation was noted at 25 and 50 mg/kg. The minimal prolongation was still present in the 50 mg/kg recovery group after 2 months of recovery.
- The NOAEL was 25 mg/kg based on the mild adrenal and APTT findings at 50 mg/kg (including the APTT findings after recovery at 50 mg/kg).

Methods

Doses: 0, 12.5, 25, 50 mg/kg (see table below)
 Frequency of dosing: Daily
 Route of administration: Oral
 Dose volume: 10 ml/kg (see table below)
 Formulation/Vehicle: 15% PEG-400, 5% PVP, 5% TPGS, and 75% 0.1M phosphoric acid
 Species/Strain: (b) (4) Sprague Dawley (CrI: CD [SD]) rats
 Number/Sex/Group: 20/sex/group (see table below)
 Age: ~ 6 weeks old at start of study
 Weight: males 187 to 256 g, females 122 to 192 g
 Satellite groups: 10/sex/group (see table below)
 Unique study design: None
 Deviation from study protocol: Some discrepancies were noted, some typos and edits were incorporated, and various

individuals involved in the study were amended (added or removed). None of the changes appeared to alter the study validity.

Table 28 – Study Outline: 6 Month Rat Study

Group	Dose (mg/kg/day) ^b	Number of Animals					
		Main Study ^c (Animals for End-of-Dose Necropsy)		Recovery Study ^d (Animals for Post Dose Necropsy)		Toxicokinetic Study ^e	
		Male	Female	Male	Female	Male	Female
1/ Vehicle control ^{a,f}	0	20	20	5	5	10	10
2/ BMS-790052	12.5	20	20	5	5	10	10
3/ BMS-790052	25	20	20	5	5	10	10
4/ BMS-790052	50	20	20	5	5	10	10
5/Sentinel monitoring ^g	-	6	6	-	-	-	-

Observations and Results

Mortality

Checked 2x daily. No deaths were directly associated with the drug.

Seven animals died on the study which were unrelated to drug:

Three deaths in the main study (vehicle control, and 2 animals given 25 mg/kg). The vehicle control animal and one of the dosed animals died due to gavage error. The second animal dosed 25 mg/kg that died was associated with obstructive uropathy due to calculi in both kidneys and urinary bladder. On the day prior to death, the animal was thin, moderately dehydrated, decreased activity, wet urogenital fur, and red stained fur in left periorbital area (including muzzle and paws). The animal was provided Nutragel on day 100-116 after a weight loss of 50 grams on day 117-120.

One recovery animal and 3 TK animals were found dead. The death of the recovery animal was not determined. The 3 TK animals also died of non-drug-related causes (calculus-filled urinary bladder; hemorrhage from blood sampling; aspiration of dosing formulation and associated lung findings).

Clinical Signs

Checked 2x daily. In the 25 mg/kg (males only) and 50 mg/kg (both sexes) groups, there was a drug-related increase of salivation starting on day 42. Wet fur on the lower jaw (likely due to increased salivation) was also noted. The salivation resolved after 2 months of recovery.

Body Weights

Weighed pretest, 2x weekly during dosing, then once weekly during post-dose. No changes noted.

Feed and Water Consumption

Quantitative assessment over multiple 3-4 day periods (pretest, dosing, and post-dose). No changes noted in food consumption. Water consumption increased in all groups. The increase in water consumption appeared to be dose related. See table below.

Table 29 – Mean Water Consumption Increases: 6 Month Rat Study

Dose (mg/kg/day):	12.5		25		50	
Sex:	Male	Female	Male	Female	Male	Female
Week 4	—	50%	18%	82%*	121%**	93%**
Week 12	—	98%*	39%	114%**	163%**	105%**
Week 26/27	—	—	37%	—	92%*	42%
Week 34	—	—	—	—	65%	—

a = Data are expressed as percent increase from control.

A dash (—) indicates absence of change in group.

* = $P \leq 0.05$; ** = $P \leq 0.01$ for absolute values.

Ophthalmoscopy

Pretest, then day 28. No changes noted.

Hematology and Coagulation

At sacrifice. No changes in hematology at 12.5 mg/kg. At 25 and 50 mg/kg, there was a minimal (but significant) prolongation of APTT in males and females. After recovery, there was still a minimal prolongation at 50 mg/kg.

Table 30 – APTT Prolongation in the Treatment Animals: 6 Month Rat Study

Dose (mg/kg/day):	12.5		25		50	
Sex:	Male	Female	Male	Female	Male	Female
APTT						
Week 26/27	—	—	1.12x**	1.10x*	1.12x**	1.17x**
Week 35	—	—	1.05x	—	1.09x	—

a = Data are expressed as mean of BMS-790052 dose group ÷ control mean.

A dash (—) indicates absence change in group.

* = $P \leq 0.05$; ** = $P \leq 0.01$ for absolute values.

Clinical Chemistry

At sacrifice. Changes in AST, cholesterol, triglycerides, and glucose noted. ALT was also increased in males at the high dose (1.43x), but it was attributed to a single male and was deemed to be unrelated to drug exposure. This rat had liver necrosis (by microscopic examination).

Table 31 – Clinical Chemistry Changes: 6 Month Rat Study

Dose (mg/kg/day):	12.5		25		50	
Sex:	M	F	M	F	M	F
Aspartate Aminotransferase						
Week 4	—	0.85×*	0.86×*	0.79×**	0.82×**	0.77×**
Week 12	—	—	—	—	—	0.85×
Week 26/27	—	—	—	—	—	—
Week 35	—	—	—	—	—	—
Cholesterol						
Week 4	—	—	—	—	—	—
Week 12	—	—	—	1.17×*	—	1.22×**
Week 26/27	—	—	—	1.19×*	—	1.22×**
Week 35	—	—	—	—	—	—
Triglycerides						
Week 4	—	—	—	—	—	1.63×**
Week 12	—	—	—	—	—	1.60×*
Week 26/27	—	—	—	—	1.26×	1.14×
Week 35	—	—	—	—	—	—
Glucose						
Week 4	—	—	—	—	—	—
Week 12	—	—	—	—	—	—
Week 26/27	—	—	—	—	1.28×*	—
Week 35	—	—	—	—	—	—

a = Data are expressed as mean of BMS-790052 dose group ÷ control mean.

A dash (—) indicates absence of change in group.

* = P < 0.05; ** = P < 0.01 for absolute values.

Urinalysis

Measured at weeks 1, 4, and 8. At all doses, there was an increase in urine volume and total corticosterone excretion. This increase in urine volume was associated with secondary decreases in urine specific gravity, creatinine, and osmolality, and increases in total corticosterone excretion and the corticosterone:creatinine ratio. These changes fully recovered at the end of the recovery period, except for the increase in urine volume in males given 50 mg/kg/day, which was not adverse because there were no associated effects on hydration or serum electrolytes.

Table 32 – Urinalysis: 6 Month Rat Study

Dose (mg/kg/day):	12.5		25		50	
Sex:	Male	Female	Male	Female	Male	Female
Urine Volume						
Week 4	—	1.44×	—	1.47×	1.60×**	1.64×**
Week 12	—	1.47×*	—	1.58×**	1.53×**	1.52×*
Week 26	—	1.35×	1.44×**	1.37×	1.66×**	1.48×**
Week 34	—	—	—	—	1.71×*	—
Total Corticosterone Excretion						
Week 4	—	1.06×	—	1.31×	1.60×**	1.60×**
Week 12	—	0.78×	—	1.19×	1.33×*	1.41×*
Week 26	—	—	—	—	—	—
Week 34	—	—	—	—	—	—
Corticosterone : Creatinine Ratio						
Week 4	—	—	—	—	1.81×**	1.47×**
Week 12	—	—	—	—	1.58×**	1.29×*
Week 26	—	—	—	—	—	—
Week 34	—	—	—	—	—	—

a = Data are expressed as mean of BMS-790052 dose group ÷ control mean.

A dash (—) indicates absence of change in group.

* = P < 0.05; ** = P < 0.01 for absolute values.

Gross Pathology

At sacrifice. No changes noted.

Organ Weights

At sacrifice. Concurrent with the short-term study, there was an increase in adrenal gland weight. The increases were significant in the 25 and 50 mg/kg groups.

Table 33 – Organ Weight Changes: 6 Month Rat Study

Dose (mg/kg/day):	12.5		25		50	
Sex:	Male	Female	Male	Female	Male	Female
Adrenal gland						
Absolute	+6	-4	+10	+16	+37**	+33**
% body	+8	-4	+14*	+19*	+40**	+37**

* P ≤ 0.05; ** P ≤ 0.001

The numerical values in the table represent the respective percent increase [+] or percent decrease [-] from control mean value [(mean of BMS-790052 dose group - control group mean) ÷ (control group mean × 100)].

Histopathology

Adequate Battery – yes.

Peer Review – yes.

Histological Findings – At sacrifice.

BMS-790052-related microscopic findings were noted in the adrenal glands of males and females at ≥ 12.5 mg/kg/day. Males and females at 50 mg/kg/day had minimal cortical hypertrophy/hyperplasia of the adrenal glands. Males at ≥ 12.5 mg/kg/day and females at 50 mg/kg/day had minimal cortical increased fine cytoplasmic vacuolation in the zona fasciculata of the adrenal glands.

Angiectasis (lengthening and/or dilation of blood vessels) was observed in the adrenal glands of all groups including the controls and predominantly in females (only 1 male affected), with a dose-dependent increase in both incidence and severity suggesting exacerbation by BMS-790052. The angiectasis was not considered a continuum of increased fine cytoplasmic vacuolation (minimal, diffuse, and in females found only at the high-dose), but as a separate process.

Table 34 – Histopathology: 6 Month Rat Study

Dose (mg/kg/day):	0	12.5	25	50
No. of Animals (M/F):	20/20	20/20	20/20	20/20
Sex:	M/F	M/F	M/F	M/F
<u>Adrenal gland:</u>				
Cortical hypertrophy/hyperplasia	—/—	—/—	—/—	18/13
Minimal	—/—	—/—	—/—	18/13
Increased fine cytoplasmic vacuolation in cortex	—/—	3/—	13/—	19/13
Minimal	—/—	3/—	13/—	19/13
Angiectasis	—/3	—/9	1/11	—/12
Minimal	—/3	—/7	1/9	—/8
Slight	—/—	—/2	—/2	—/3
Moderate	—/—	—/—	—/—	—/1

A dash (—) indicates absence of finding in group.

Toxicokinetics

BMS-790052 systemic exposures increased in an approximately dose-proportional manner across all doses, except in female rats. In females, the increase was slightly more than dose-proportional. BMS-790052 was highly concentrated in the liver as evidenced by the liver-to-plasma ratios. The ratios were similar at 12.5 and 25 mg/kg, but higher at 50 mg/kg. There was no sex difference in liver-to-plasma noted.

The metabolite, BMS-805215) was detected in the livers of the majority of male rats, but not in female rats (except for 1 female at 50 mg/kg), indicating a sex difference. In males, there was a general dose-related increase in the metabolite.

Table 35 – Toxicokinetic Summary for BMS-790052 in Plasma: 6 Month Rat Study

Parameter	Period	BMS-790052 Dose					
		12.5 mg/kg/day		25 mg/kg/day		50 mg/kg/day	
		Male	Female	Male	Female	Male	Female
Mean	Day 1	0.875	1.05	2.85	1.84	4.17	6.02
C_{max} ($\mu\text{g/mL}$)	Week 13	1.20	1.86	2.48	3.19	5.45	11.0
	Week 26	1.27	1.95	2.29	3.19	5.52	10.5
Mean	Day 1	5.30	5.63	14.5	10.5	30.3	33.8
AUC ₍₀₋₂₄₎ ($\mu\text{g}\cdot\text{h/mL}$)	Week 13	8.69	8.44	15.5	15.6	44.0	67.2
	Week 26	7.97	10.3	15.6	17.2	43.8	74.9

a BMS-790052 was not detected in any control plasma samples.

b The combined-gender AUC values at Week 26 were 9.14, 16.4, and 59.4 $\mu\text{g}\cdot\text{h/mL}$ for the doses of 12.5, 25, and 50 mg/kg/day, respectively.

Table 36 – Liver, Plasma, and Liver/Plasma Ratios: 6 Month Rat Study

BMS-790052 Dose (mg/kg/day)	Mean Concentration of BMS-790052					
	Liver ^a (ng/g)		Plasma ^b (ng/mL)		Mean Liver-to-Plasma Concentration Ratio ^c	
	Male	Female	Male	Female	Male	Female
12.5	339	314	36.7	20.6	9.24	15.3
25	954	1080	156	103	6.13	10.4
50	5220	3460	150	89.9	34.8	38.5

a Liver samples were collected approximately 21 to 28 hours after dosing in Week 27.

b Plasma samples were collected at 24 hours post dose in Week 26.

c Liver-to-plasma concentration ratio = mean liver concentration/mean plasma concentration.

Table 37 – Liver Concentrations of Parent and Metabolite: 6 Month Rat Study

BMS-790052 Dose (mg/kg/day)	Mean Liver Concentration (ng/g)				BMS-805215/BMS-790052	
	BMS-805215		BMS-790052		Liver Concentration Ratio	
	Male	Female	Male	Female	Male	Female
12.5	61.4	—	339	314	0.181	—
25	123	—	954	1080	0.136	—
50	462	61.0 (n=1)	5220	3460	0.092	0.005 (n=1)

—: Not applicable

Stability and Homogeneity

The study concentrations were within 20% of each other and mean concentrations were within 10% of the intended concentrations.

Study title: One-month oral toxicity study in dogs.

Study no.: DS07058
 Study report location: EDR
 Conducting laboratory and location: Bristol-Myers Squibb, Drug Safety Evaluation, Syracuse, NY.
 Date of study initiation: October 1, 2007
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: BMS-790052-05 (dihydrochloride salt), Batch 7C26990, 91.0% purity.

Key Study Findings

BMS-790052 was administered to dogs (5/sex/group) orally once daily at doses of 0 (vehicle control), 3, 15 or 100/50 mg/kg/day for one month.

- 3 mg/kg: no findings
- 15 mg/kg: mild splenic extramedullary hematopoiesis and hepatic perivascular inflammation of minimal severity, with secondary Kupffer cell hypertrophy/hyperplasia
- 100 mg/kg: Dose reduction to 50 mg/kg. Four animals in the high dose group had to be euthanized early due to poor condition (with liver and bone-marrow findings). Of the surviving animals: Bone marrow effects, liver enzyme changes, minimal/slight seminiferous tubule degeneration in the testes.
- The NOAEL for this study was 3 mg/kg/day based on the findings at 15 mg/kg and the severe toxicity at 100 mg/kg.

Methods

Doses: 0 (vehicle control), 3, 15, and 100 (50) mg/kg/day. Due to severe toxicity at 100 mg/kg/day, dosing was suspended in this group for 5 days beginning on Day 9 (females) or 10 (males), after which the dose was reduced to 50 mg/kg/day beginning on Day 14 (females) or 15 (males).

Frequency of dosing: Daily
 Route of administration: oral,

Formulation/Vehicle: 15% PEG-400, 5% PVP K-30, 5% TPGS and 75% 0.1 M H₃PO₄, pH 3.

Species/Strain: Beagle dogs.
 Number/Sex/Group: 5

Age: 9-18 months
Weight: Males (7.9 to 9.5 kg) and females (5.1 to 8.6 kg)
Satellite groups: 2/sex/group carried into the one month recovery arm. Note that reversibility of changes in the 100/50 mg/kg/day group could not be assessed due to early mortality.

Observations and Results

Mortality

Two males and 2 females were euthanized in poor condition on Day 10 (4104 and 4201, Day 11 (4102) or Day 20 (4205). Clinical signs in animals euthanized on Day 10/11 included decreased food consumption and body weight loss, salivation, thinness, dehydration, hunched posture, decreased activity, pale mucous membranes, icterus and/or fecal changes. In the animal euthanized on Day 20, clinical signs also included lesion, pain, swollen, pitting edema, and/or purulent red discharge of the hindlimb, ruptured and necrotic interdigital cyst, lameness and a reluctance to stand. The primary cause of the poor condition of these animals was considered to be liver and bone-marrow toxicity.

Mean plasma concentrations of BMS-790052 at necropsy were higher in the animals sacrificed moribund -- than for other high-dose dogs that survived until scheduled necropsies. In surviving high dose dogs, the mean liver to plasma ratio for BMS-790052 was 9, compared with early decedents whose values ranged from 0.8 to 3.8. This was also true for the metabolites, BMS-795853 and BMS-805215. This suggests a reduced ability for early death dogs to metabolize and/or eliminate BMS-790052.

Clinical Signs

Clinical signs were only seen in the high dose group and consisted of vomitus and increased incidence and/or frequency of fecal changes (liquid, white, black and/or mucous).

Body Weights

Drug-related decreases in body weight (4 to 19%) were seen only in the 4 early death animals in the high dose group.

Feed Consumption

Drug-related decreases in food consumption were seen only in the 4 early death animals in the high dose group.

Ophthalmoscopy

Unremarkable.

Cardiovascular and Respiratory Evaluation

Unremarkable except for an increase in heart rate on Day 20 in an early death animal (4205).

Hematology

Changes were seen only in high dose animals and were more severe in early death animals in that group. These changes consisted of mild to marked decreases in platelet (0.26- to 0.69-fold relative to pretest), reticulocyte (0.07- to 0.48-fold), neutrophil (0.02- to 0.61-fold), lymphocyte (0.38- to 0.64-fold), eosinophil (0.00- to 0.35-fold), and basophil (0.00- to 0.15-fold) counts and decreases in red cell mass (hemoglobin, hematocrit and red cell count, 0.72- to 0.86-fold). Mild to marked increases in fibrinogen (1.42- to 4.14-fold, relative to pretest) were also observed. Increases in activated partial thromboplastin time (1.29- to 1.67-fold) were seen only in early death animals and are considered secondary to general moribundity rather than drug-related.

Clinical Chemistry

Changes were seen only in high dose animals and were more severe in early death animals in that group. These changes included increases in total bilirubin (4.00- to 33.40-fold relative to pretest), alkaline phosphatase (3.60- to 27.08-fold), GGT (2.67- to 11.00-fold), alanine aminotransferase (1.5 to 10.64-fold), aspartate aminotransferase (2.07- to 5.06-fold), globulins (1.21- to 1.31-fold), triglycerides (3.24- to 10.40-fold), and cholesterol (1.19- to 2.26-fold and decreases in albumin (0.65- to 0.88-fold) with decrease in total protein and calcium.

Urinalysis

Unremarkable except in early death animals who exhibited findings consistent with an acute phase response.

Gross Pathology

Changes were seen only in high dose early death animals and included rough surface, mottled appearance and/or tan discoloration of the liver, dark discoloration of the splenic lymph nodes and an abscess and draining fistula/skin ulceration with secondary changes in adjacent skeletal muscle and an adjacent popliteal lymph node.

Organ Weights

Unremarkable. Decreases in prostate gland weights of 33% in high-dose males were not statistically significant.

Histopathology

Adequate Battery - yes

Peer Review - yes

Histological Findings

- Liver: hepatic perivascular inflammation of minimal to moderate severity was observed at 15 and 100/50 mg/kg/day, with secondary slight or mild Kupffer cell hypertrophy/hyperplasia and/or Kupffer cell pigmentation and minimal to mild hepatocellular degeneration was observed in the 100/50 mg/kg/day group.
- Bone marrow: moderate to marked decreases in erythroid and granulocyte components in the early death high dose animals and minimal to mild hypocellularity of the bone marrow were observed in 2 surviving high dose animals.
- Spleen/Thymus: slight to mild lymphoid depletion in early death animals only.
- Spleen: slight to mild splenic extramedullary hematopoiesis (15 and 100 mg/kg only)
- Testes: minimal to slight seminiferous tubule degeneration in 4 high dose males.

Non drug related findings:

- Prostate: slight to mild prostate gland atrophy in two early death males.
- Pancreas: minimal to slight acinar cell vacuolation in all groups (including the control group)

Special Evaluation

None.

Toxicokinetics

Systemic exposures for BMS-790052 increased in an equal to or greater than dose proportional manner. There were no sex-related differences and no evidence of drug accumulation in the low and middle dose groups. Due to the dose reduction in the high dose group, it was not possible to assess accumulation in that group. Mean AUC values for the metabolite, BMS-805215, were approximately 1.1 to 7.3% of the respective BMS-790052 values on Days 1 and 28.

Mean plasma concentrations of BMS-790052 at necropsy were higher in these animals than for other high-dose dogs that survived until scheduled necropsies. In surviving high dose dogs, the mean liver to plasma ratio for BMS-790052 was 9, compared with early decedents whose values ranged from 0.8 to 3.8. This was also true for the metabolites, BMS-805215 and BMS-795853, with ratios of 24 – 25 and 69 – 83, respectively in survivors, and ≤ 13 and ≤ 48 , respectively, in early decedents.

Table 38 - Toxicokinetic Parameters (Mean ± SD) of BMS-790052 in Plasma of Dogs Receiving Once-daily Oral Doses of 3, 15 or 100/50 mg/kg for up to 28 Days; Study DS07058

Parameter	Study Day	Dose (mg/kg/day)					
		3		15		100/50	
		Male	Female	Male	Female	Male	Female
C _{max} (µg/ml)	1	0.163	0.268	1.38	1.98	10.3	8.52
	28	0.208	0.348	2.02	2.08	9.28	5.68
AUC(0-24) (µg.h/ml)	1	1.10	1.93	15.6	19.1	174	142
	28	1.23	2.36	28.6	24.0	186	105

Table 39 - Toxicokinetic Parameters (Mean ± SD) of BMS-805215 in Plasma of Dogs Receiving Once-daily Oral Doses of 3, 15 or 100/50 mg/kg for up to 28 Days; Study DS07058

Parameter	Study Day	Dose (mg/kg/day)					
		3		15		100/50	
		Male	Female	Male	Female	Male	Female
C _{max} (µg/ml)	1	LLOQ	0.00721	0.026	0.0587	0.657	0.427
	28	LLOQ	0.0134	0.0383	0.0468	0.788	0.180
AUC(0-24) (µg.h/ml)	1	LLOQ	LLOQ	0.165	0.369	9.34	5.91
	28	LLOQ	0.0395	0.369	0.449	13.6	3.27

Study title: ONE-MONTH ORAL INVESTIGATIVE TOXICITY STUDY IN MONKEYS

Study no.: DS07214
 Study report location: EDR
 Conducting laboratory and location: BMS, 6000 Thompson Road, Syracuse, NY
 Date of study initiation: Nov 28, 2007
 GLP compliance: No. Conducted via SOPs at BMS
 QA statement: No
 Drug, lot #, and % purity: BMS-790052-05 salt (Batch 7J25449), 89.5 %

Key Study Findings

The objectives of this study were: (1) to determine the toxicity profile of BMS-790052 when given orally to cynomolgus monkeys for 1-month, (2) to determine whether liver and bone-marrow toxicities associated with BMS-790052 treatment in repeat-dose studies in dogs also occur in cynomolgus monkeys, and (3) to provide data to guide dose selection for a possible, subsequent studies in monkeys.

BMS-790052 was administered to monkeys (2/sex/group) at daily oral gavage doses of 0 (vehicle control), 10, 30, 100, or 300 mg/kg for 1 month.

Table 40 - Mean Plasma Toxicokinetic Parameters for BMS-790052, BMS-805215, and BMS-795853; Study DS07214

Analyte	Study Day	BMS-790052 Dose (mg/kg/day)							
		10		30		100		300	
		Male	Female	Male	Female	Male	Female	Male	Female
BMS-790052	1	2.63	1.29	10.1	10.2	46.7	31.6	68.5	72.5
	14	2.17	1.08	10.8	15.7	46.1	23.4	74.9	49.2
	30	2.31	1.65	14.0	13.6	38.3	21.5	54.5	88.5
BMS-805215	1	0.94	0.17	5.37	2.76	16.0	10.7	23.6	39.5
	14	0.19	0.15	2.27	2.52	9.22	4.85	25.4	14.9
	30	0.26	0.25	3.20	3.67	7.88	6.12	12.0	30.5
BMS-795853	1	NA	NA	0.06	NA	0.20	0.11	0.75	0.56
	14	NA	NA	NA	NA	0.20	0.05	0.29	0.23
	30	NA	NA	NA	NA	0.17	0.05	0.38	0.45

a: AUC(0-T) ($\mu\text{g}\cdot\text{h}/\text{mL}$)

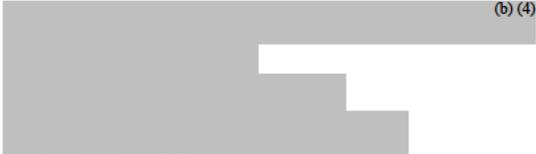
Note: N = 2 except for males at 300 mg/kg/day where N=1 on Day 30 (due to an accidental death).

NA: not applicable

BMS-790052 was clinically well tolerated by monkeys for 1 month at doses \leq 300 mg/kg/day. Drug-related findings at 30 to 300 mg/kg/day (BMS-790052 mean AUC, 10.1 to 88.5 $\mu\text{g}\cdot\text{h}/\text{mL}$; mean liver concentration, 1.1 to 82.4 $\mu\text{g}/\text{g}$) were limited to minimal perivascular inflammation in the liver which was morphologically similar (but less severe) to that observed in dogs. There was no evidence of bone marrow toxicity. The no-observed-effect-level was 10 mg/kg/day (BMS-790052 mean AUC \leq 2.6 $\mu\text{g}\cdot\text{h}/\text{mL}$ and mean liver concentration \leq 0.3 $\mu\text{g}/\text{g}$).

The following 2 studies (4 month and 9 month repeat dose toxicology studies in monkeys) were reviewed by Dr. Mark Seaton, PhD.

Study title: Four-month Oral Toxicity Study in Monkeys

Study no.: DS08039
 Study report location: EDR
 Conducting laboratory and location:  (b) (4)
 Date of study initiation: 06 May 2008
 GLP compliance: GLP compliance is stated
 QA statement: Yes
 Drug, lot #, and % purity: BMS-790052 (Batch 7M23810), 90.5%

Key Study Findings

- In males and females at 50 and 300 mg/kg/day, macroscopic changes correlated with histopathology in liver and adrenal gland. Microscopic changes were also noted in bone marrow.
- The no observed adverse effect level (NOAEL) was 15 mg/kg/day (combined-sex mean AUC = 2.31 ug.h/mL).

Methods

Doses:	0, 15, 50, 300 mg/kg
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	Solution/15% polyethylene glycol, 5% polyvinyl pyrrolidone K-30, 5% Vitamin E- δ - α -tocopheryl polyethylene glycol 1000 succinate and 75% 0.1 M phosphoric acid, w/w, pH ~3.
Species/Strain:	Cynomolgus monkey/ <i>Macaca fascicularis</i>
Number/Sex/Group:	4
Age:	2-3 years
Weight:	Males: 2.6 to 3.9 kg Females: 2.2 to 3.0 kg

Observations and Results

The following parameters were assessed: survival; BMS-790052 concentrations in plasma, liver and bile; BMS-805215 (oxidative cleavage metabolite; M2) concentrations in bile; clinical observations; body weights; qualitative food consumption; physical and ophthalmologic examinations; electrocardiography evaluations; clinical pathology evaluations (twice pretest and during Weeks 3, 4, 8, 11, and 15); evaluations of cardiac troponin I; serum amyloid A and inflammatory mediators (Week 16/17); organ weights; and gross- and microscopic-pathology analyses. Additionally, electron microscopy and immunohistochemical evaluations were performed on liver from selected control and/or high-dose monkeys. Scheduled necropsies were conducted in Week 17.

Mortality

There were no drug-related deaths.

Clinical Signs

Soft and or liquid feces was associated with vehicle, but increased with dose-related frequency at the middle and high doses (refer to sponsor's table below).

Table 41 - Incidence and Frequency of Soft/Liquid Feces During the Dosing Period

	0 mg/kg/day (Vehicle Control)		15 mg/kg/day		50 mg/kg/day		300 mg/kg/day	
	Sex: M	F	M	F	M	F	M	F
Soft Feces	58 (4)	83 (4)	24 (4)	17 (4)	79 (4)	70 (4)	125 (4)	124 (4)
Liquid Feces	19 (4)	32 (4)	3 (2)	19 (4)	50 (4)	52 (4)	99 (4)	89 (4)

a: For each entry, the first value is the frequency (total number of occurrences/group) and the 2nd number (in parentheses) is the incidence, ie, the number of animals affected; multiple observations on a given day were tallied as once per day

To control fecal changes, medical treatments (Pepto-Bismol, Tylan, Baytril, and/or dietary modifications) were periodically provided. No effect of this treatment on other study-related parameters is expected.

Body Weights

There were no drug-related effects on body weight.

Feed Consumption

There were no drug-related effects on food consumption.

Ophthalmoscopy

There were no drug-related changes in ophthalmoscopic parameters.

ECG

All available monkeys had electrocardiograms (ECG) recorded twice during the pretest period; and then at the estimated C_{max} (i.e., 2 hours (±1 hour) post dose) during Week 12.

There were no remarkable findings.

Hematology

All available monkeys had hematology evaluations twice during the pretest period and during Weeks 3, 4, 8, 11, and 15.

There were no drug-related changes in hematology parameters at doses of 15 or 50 mg/kg/day. At 300 mg/kg/day, there were mild decreases in RBC (0.72x to 0.84x), hemoglobin (0.79 to 0.91 x) and hematocrit (0.79 to 0.91x) in 1/4 females in Week 3, 2/4 females in Weeks 4, 8, 11 and 15.

Clinical Chemistry

All available monkeys had clinical biochemistry evaluations twice during the pretest period and during Weeks 3, 4, 8, 11, and 15.

At 300 mg/kg/day, there were mild to moderate increases in ALT and AST in individual males and females as described in the sponsor's table below. Increases in ALT and AST correlated with concurrent hepatocyte cytoplasmic rarefaction (likely glycogen accumulation) in two monkeys.

Table 42 - BMS-790052-related Changes in AL T and AST (Relative to Pretest) and their Incidence

Week	Sex	Alanine Aminotransferase		Aspartate Aminotransferase	
		Range of Values ^a	Number of Animals ^b	Range of Values	Number of Animals
3	Male	2.23×	1	—	—
	Female	3.56×	1	1.49×	1
4	Male	1.95 to 3.74×	2	—	—
	Female	1.83 to 5.41×	2	2.17×	1
8	Male	3.20 to 3.69×	2	—	—
	Female	1.85 to 3.03×	2	1.31×	1
11	Male	5.74 to 5.09×	2	1.84×	1
	Female	2.16 to 4.22×	3	1.31 to 1.49×	3
15	Male	6.63 to 7.33×	2	1.59 to 2.13×	2
	Female	1.76 to 3.65×	4	1.34 to 1.74×	4

a =Value represents the ratio (or range of ratios) between the value at the indicated week and the 2nd pretest value

b= Values represents the number of affected animals (of 4 per sex)

A dash (—) indicates absence of finding in group

At 300 mg/kg/day, there were mild decreases in albumin (ALB) in 2/4 males (0.86 to 0.93x) and 2/4 females (0.80 to 0.93x) at all timepoints (Weeks 3 through 15), with concurrent mild decreases in total protein (TPROT) and globulin (GLOB) in these individuals.

Following a dose during Week 16 or 17, serum amyloid A (SAA), inflammatory mediators, and cardiac troponin I levels were determined. Inflammatory mediators that were evaluated included interleukins (IL) 1 β , 2, 4, 5, 6, 8, and 12/23 p40, macrophage inflammatory proteins (MIP) 1a and 1 β , granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage-colony stimulating factor (GM-CSF), interferon (IFN)- γ , monocyte chemotactic protein-1 (MCP-1), and tumor necrosis factor (TNF)-a.

There were no changes in these parameters associated with inflammation or cardiac toxicity.

Urinalysis

Urinalysis evaluations occurred twice during the pretest period, and during Weeks 3, 4, and 12. There were no remarkable findings.

Gross Pathology

Drug-related findings were noted in the adrenal glands of males and females, and consisted of dark discoloration and/or enlargement. Dark discoloration of the adrenal glands occurred in a low incidence (1 male and 2 females) at 50 mg/kg/day and in all monkeys at 300 mg/kg/day. The adrenal dark discoloration correlated with decreased cytoplasmic vacuolation of the cortex (zona fasciculata) noted microscopically. Adrenal-gland enlargement occurred in 1 male and 1 female receiving 300 mg/kg/day; these same 2 animals had cortical hyperplasia (zona reticularis) noted microscopically, and had the highest adrenal weights.

In addition, a few of the liver macroscopic findings of pale area and/or foci correlated with microscopic findings. Pale areas in the liver with microscopic correlates were noted in 1 female at 50 mg/kg/day and 2 females and 1 male at 300 mg/kg/day. Pale foci in the liver with microscopic correlates were noted in 1 male and 2 females at 300 mg/kg/day.

Organ Weights

In males from the high dose group, an increase in mean absolute adrenal gland weight (+44%) was statistically significant. Macroscopic and microscopic changes correlated with the increased adrenal weights.

Table 43 - BMS-790052 related Organ Weight changes

Dose (mg/kg/day):	15	50	300
Sex:		Male	
Body weight	↓2	↑11	↑13
Adrenal gland			
Absolute	↓2	↑5	↑44*
% body	↑1	↓3	↑28

* $P \leq 0.01$ for absolute values.

The numerical values in the table represent the respective percent increase [↑] or percent decrease [↓] from control mean value [(treatment group mean - control group mean) ÷ (control group mean × 100)].

Histopathology

Adequate Battery
Yes

Peer Review
Yes, peer review by sponsor.

Histological Findings

In males and females at 50 and 300 mg/kg/day, the liver had minimal to slight bile duct hyperplasia (subcapsular) and Kupffer cell (centrilobular) hyperplasia/hypertrophy. Additionally, there was an increased incidence of minimal to slight centrilobular mononuclear cell infiltration in males and females at 300 mg/kg/day.

The adrenal glands had minimal to marked decreased cytoplasmic vacuolation of the cortical zona fasciculata cells in males and females at 50 and 300 mg/kg/day, which correlated with the gross finding of dark discoloration. There also was slight cortical hyperplasia of the zona reticularis in males and 1 female at 300 mg/kg/day. This latter finding correlated with the gross finding of enlargement and increased mean adrenal gland weights.

The histopathologic findings in the adrenal gland suggested involvement/activation of the hypothalamic-pituitary-adrenal axis, which often occurs with stress; however, in this study a direct or indirect drug effect cannot be excluded.

Minimal lymphoid hyperplasia (germinal center development, lymphoid follicle formation) was present in the bone marrow of the sternum and/or rib in 1 male at 50 mg/kg/day and all males at 300 mg/kg/day. This finding can occur as background change. However, the incidence in this study is indicative of a drug-related change, although there were no correlating clinical pathology changes. The toxicologic significance of the change is not known.

Table 44 - Incidence of Histopathological Findings

Dose (mg/kg/day):	0	15	50	300
No. of Monkeys (M/F):	4/4	4/4	4/4	4/4
Sex:	M/F	M/F	M/F	M/F
<u>Liver:</u>				
Bile duct hyperplasia	-/-	-/-	1/2	2/3
Minimal	-/-	-/-	1/2	1/3
Slight	-/-	-/-	-/-	1/-
Hyperplasia/hypertrophy: Kupffer cell	-/-	-/-	1/2	3/4
Minimal	-/-	-/-	1/2	3/2
Slight	-/-	-/-	-/-	-/2
Cytoplasmic rarefaction	-/-	-/-	-/2	1/1
Minimal	-/-	-/-	-/2	-/-
Moderate	-/-	-/-	-/-	1/1
Infiltration: mononuclear cell	-/1	1/-	1/1	4/4
Minimal	-/1	1/-	1/-	4/4
Slight	-/-	-/-	-/1	-/-
<u>Bone Marrow:</u>				
Lymphoid hyperplasia	-/-	-/-	1/-	4/-
Minimal	-/-	-/-	1/-	4/-
<u>Adrenal glands:</u>				
Decreased cytoplasmic vacuolation: cortex	-/-	-/-	4/2	4/4
Minimal	-/-	-/-	2/-	-/-
Slight	-/-	-/-	1/-	-/-
Moderate	-/-	-/-	-/1	-/1
Marked	-/-	-/-	1/1	4/3
Hyperplasia: cortical	-/-	-/-	-/-	2/1
Slight	-/-	-/-	-/-	2/1

An EM dash (-) indicates absence of finding in group.

Special Evaluation

Transmission electron-microscopic evaluation of liver samples from selected high-dose (300 mg/kg/day) monkeys showed amorphous granular material within distended hepatocellular endoplasmic reticulum. This material subsequently formed discrete concentric lamellae resembling geologic nodules or geodes, was phagocytized by adjacent Kupffer cells, correlating with the light microscopic finding of eosinophilic to pale material noted in hyperplastic/hypertrophied Kupffer cells, and/or are excreted into adjacent bile canaliculi. Myelinoid bodies, probably derived from altered smooth endoplasmic reticulum, also formed in hepatocytes. The origin of this granular material found in the hepatocytes, adjacent Kupffer cells and bile canaliculi was not determined

but may represent agglomerates of drug, metabolites and/or endogenous substrates that possibly contributed to the bile duct and Kupffer cell hyperplasia or hypertrophy.

Toxicokinetics

Refer to tables below (excerpted from sponsor) for toxicokinetic parameters of BMS-790052 in plasma and comparative concentrations in liver, bile and plasma.

Table 45 - Mean Toxicokinetic Parameters for BMS-790052 a,b,c

Parameter	Period	15 mg/kg/day		50 mg/kg/day		300 mg/kg/day	
		Male	Female	Male	Female	Male	Female
C _{max} (µg/mL)	Day 1	0.817	0.749	3.67	4.18	5.72	6.28
	Week 13	0.353	0.454	2.46	2.44	4.30	3.56
AUC(0-T) (µg•h/mL)	Day 1	4.47	4.30	36.3	30.0	39.6	64.8
	Week 13	2.55	2.07	19.3	25.7	48.0	34.4

a: BMS-790052 was not detected in any control plasma samples.

b: AUC(0-T) values were calculated from 0 to 24 hours post dose, except in 1 female at 15 mg/kg/day at Week 13, for which AUC(0-T) was calculated from 0 to 8 hours post dose.

c: Mean combined-sex AUC values at Week 13 were 2.31, 22.5, and 41.2 µg•h/mL for the doses of 15, 50, and 300 mg/kg/day, respectively.

Table 46 - Mean BMS-790052 Concentrations in Liver, Bile and Plasma at Necropsy

BMS-790052 Dose (mg/kg/day)	Concentration of BMS-790052			Concentration Ratio ^a		
	Liver (µg/g)	Bile (µg/mL)	Plasma (µg/mL)	Liver-to- Plasma	Bile-to- Plasma	Bile-to- Liver
15	0.478	185	0.0230	22.1	9,000	404
50	3.60	602	0.0716	47.3	9,680	227
300	40.4	1,430	0.183	277	8,870	46.6

a: Due to the absence of meaningful differences between sexes, all values represent means of male and female values; all values are rounded to 3 significant figures.

Dosing Solution Analysis

The dosing formulations were solutions; therefore, no homogeneity testing was performed. Stability of dose formulations was documented.

Study title: Nine-month Oral Toxicity Study in Monkeys

Study no.: DS08003
 Study report location: SN 115
 Conducting laboratory and location:  (b) (4)
 Date of study initiation: 17 April 2008
 GLP compliance: GLP compliance is stated
 QA statement: Yes
 Drug, lot #, and % purity: BMS-790052 (Batch 7M23810), 90.5%

Key Study Findings

- Target organs were liver and adrenal gland
- At 30 and 150 mg/kg/day, histopathology was noted in the liver (primarily Kupffer-cell hypertrophy/hyperplasia) and adrenal gland (decreased cytoplasmic vacuolation in cortex). All changes were at least partially reversible after a 2-month recovery period.
- The no observed adverse effect level (NOAEL) was defined as 15 mg/kg/day (combined-sex mean AUC = 3.26 ug•h/mL).

Methods

Doses: 0, 15, 30, 150 mg/kg
 Frequency of dosing: Once daily
 Route of administration: Oral gavage
 Dose volume: 5 mL/kg
 Formulation/Vehicle: Solution/15% polyethylene glycol, 5% polyvinyl pyrrolidone K-30, 5% Vitamin E- δ - α -tocopheryl polyethylene glycol 1000 succinate and 75% 0.1 M phosphoric acid, w/w, pH ~3.
 Species/Strain: Cynomolgus monkey/*Macaca fascicularis*
 Number/Sex/Group: 4 (+2 in recovery groups)
 Age: 2-3 years
 Weight: Males: 2.4 to 3.4 kg
 Females: 2.1 to 3.1 kg

Observations and Results

Parameters assessed in the current study included: survival, toxicokinetics of BMS-790052, measurement of BMS-805215 (oxidative cleavage metabolite; M2) concentrations in bile, clinical observations, body weights, qualitative food consumption, physical- and ophthalmologic examinations, electrocardiography evaluations, clinical pathology evaluations, immunotoxicity evaluations, organ weights, and gross- and microscopic-pathology analyses.

Mortality

One high-dose (150 mg/kg/day) male was euthanatized on Day 28 due to a poor and deteriorating condition attributed to inflammatory changes in lymphoid tissue, liver and skin. Findings in the lymphoid tissues consisted of minimal to moderate atrophy, necrosis and/or inflammation. Hepatic lesions consisted primarily of portal and periportal inflammation, with minimal hepatocellular necrosis and rare thrombosis. Findings in the skin involved both epidermal necrosis and loss (ulceration) accompanied by inflammation and crust formation, all of which varied from mild to severe. In multiple organs the inflammatory infiltrates included rare to few multinucleated giant cells. In this monkey, BMS-790052 exposure (80.3 ug.h/mL) was ~ 2x greater than the mean exposure in all other males at the same dose on Day 1, but similar to the mean exposure in females. A definitive cause for the findings in the animal could not be determined, and effects were inconsistent with findings in the remaining animals at the high-dose (see below). However, a relationship to BMS-790052 could not be excluded.

Clinical Signs

Soft and or liquid feces was associated with vehicle, but increased with dose-related frequency at the middle and high doses (refer to sponsor's table below).

Veterinarian-recommended medical treatments were administered to treat soft/liquid feces (Pepto-Bismol Extra strength, Tylan, Baytril, and Panacur), and other conditions. In addition, dietary modifications were occasionally made to treat soft/liquid feces, dehydration, inappetance, and thinness.

Table 47 - Incidence and Frequency of Soft/Liquid Feces During the Dosing Period

	0 mg/kg/day (Vehicle Control)		15 mg/kg/day		30 mg/kg/day		150 mg/kg/day		
	Sex:	M	F	M	F	M	F	M	F
Soft Feces		293 (6)	113 (6)	264 (6)	153 (6)	224 (6)	273 (6)	294 (6)	172 (6)
Liquid Feces		79 (6)	28 (6)	176 (6)	190 (6)	101 (6)	81 (6)	205 (6)	140 (6)

a: For each entry, the first value is the frequency (total number of occurrences/group) and the 2nd number (in parentheses) is the incidence, ie, the number of animals affected; multiple observations on a given day were tallied as once per day

Table 48 - Incidence and Frequency of Soft/Liquid Feces During the Recovery Period

	0 mg/kg/day (Vehicle Control)		15 mg/kg/day		30 mg/kg/day		150 mg/kg/day		
	Sex:	M	F	M	F	M	F	M	F
Soft Feces	-	1 (1)	-	2 (2)	17 (2)	-	10 (2)	-	-
Liquid Feces	-	1 (1)	-	18 (2)	8 (2)	-	18 (2)	-	-

a: For each entry, the first value is the frequency (total number of occurrences/group) and the 2nd number (in parentheses) is the incidence, ie, the number of animals affected; multiple observations on a given day were tallied as once per day.

-: No fecal changes observed.

Body Weights

There were no drug-related effects on body weight.

Feed Consumption

There were no drug-related effects on food consumption.

Ophthalmoscopy

There were no drug-related changes in ophthalmoscopic parameters.

ECG

There were no remarkable findings.

Hematology

There were no remarkable findings in hematology or coagulation parameters.

Clinical Chemistry

In high-dose (150 mg/kg/day) monkeys, there was a slight to marked increase in alanine aminotransferase compared to pretest individual values in 2 of 5 males ($\leq 6.6x$) and 4 of 6 females ($\leq 9.8x$). In two of the affected females, there was a correlating minimal increase in aspartate aminotransferase ($\leq 2.7x$). There was a slight to moderate decrease in alkaline phosphatase in 2 of 5 males and 2 of 6 females ($\leq 0.6x$). There was a moderate increase in C-reactive protein ($\leq 13.9x$) in a single male. There were no clear correlations between these clinical pathology changes and histopathologic findings.

Urinalysis

There were no remarkable findings.

Gross Pathology

Dark discoloration of the adrenal glands seen in monkeys at 150 mg/kg/day correlated microscopically with decreased cytoplasmic vacuolation of the cortex (zona fasciculata). The enlargement of the adrenal glands seen in 1 male at 30 mg/kg/day and 1 male at 150 mg/kg/day correlated with a dose-related increase in mean absolute adrenal weight in these groups. These changes seen in adrenal glands were morphologically similar to those induced by stress but the mechanism of toxicity has not been elucidated.

Pale foci observed in the liver of 5 monkeys at the high dose (1 male and all females euthanatized at the end-of-dose necropsy) correlated microscopically with slight to moderate Kupffer cell hyperplasia/hypertrophy and/or hepatocellular vacuolation. Pale areas seen in 1 additional male given 150 mg/kg/day correlated with slight bile-duct hyperplasia.

Table 49 - Incidence of BMS-790052-Related Gross Findings

Dose (mg/kg/day):	0	15	30	150
No. of monkeys (M/F):	4/4	4/4	4/4	4/4
Sex:	M/F	M/F	M/F	M/F
<u>Adrenal glands:</u>				
Dark discoloration	-/-	-/-	-/-	3/4
Enlargement	-/-	-/-	1/-	1/-
<u>Liver:</u>				
Foci pale	-/-	-/1 ^a	-/-	1/4
Area pale	-/-	-/-	-/-	1/1 ^a

a: These 2 females did not have any drug related changes in the liver. They were included in the table only for incidence purpose.

A dash (-) indicates absence of finding in group

Note: Control animals included for comparison purposes.

Organ Weights

Drug-related organ-weight changes were seen in the adrenal glands of males at 30 and 150 mg/kg/day.

Table 50 - BMS-790052-Related Organ-Weight Changes

Dose (mg/kg/day):	15^a	30	150
Sex:		Male	
Body weight	—	↑8%	↑17%
Adrenal gland			
Absolute	—	↑27%	↑40%
% body	—	↑20%	↑19%

a Weight changes seen in adrenal glands of monkeys at 15 mg/kg/day were not considered drug-related.

A dash (—) indicates absence of finding in group

Histopathology

Adequate Battery

Peer Review

Yes, peer review by sponsor.

Histological Findings

At 30 and 150 mg/kg/day, histopathology was noted in the liver (primarily Kupffer-cell hypertrophy/hyperplasia) and adrenal gland (decreased cytoplasmic vacuolation in cortex). All changes were at least partially reversible after a 2-month recovery period. Refer to incidence tables below (excerpted from sponsor).

Table 51 - Incidence of BMS-790052-Related Microscopic Findings in End-of-Dose Animals

	Dose (mg/kg/day):				
	0	15	30	150	
	No. of monkeys (M/F): 4/4				
	Sex:	M/F	M/F	M/F	M/F
<u>Liver:</u>					
Hyperplasia: bile duct	-/-	-/-	-/-	1/2	
Minimal	-/-	-/-	-/-	-/2	
Slight	-/-	-/-	-/-	1/-	
Hyperplasia/hypertrophy: Kupffer cell	-/-	-/-	3/1	3/4	
Minimal	-/-	-/-	3/1	2/1	
Slight	-/-	-/-	-/-	-/2	
Moderate	-/-	-/-	-/-	1/1	
Infiltration: mononuclear cell	-/-	-/-	-/-	1/2	
Minimal	-/-	-/-	-/-	-/1	
Slight	-/-	-/-	-/-	1/1	
Hepatocyte vacuolation	-/-	-/-	-/-	-/1	
Moderate	-/-	-/-	-/-	-/1	
<u>Adrenal glands:</u>					
Decreased cytoplasmic vacuolation: cortex	-/-	-/-	2/1	4/4	
Minimal	-/-	-/-	-/1	-/-	
Slight	-/-	-/-	1/-	1/1	
Moderate	-/-	-/-	1/-	1/1	
Marked	-/-	-/-	-/-	2/2	

A dash (-) indicates absence of finding in group

Note: Control animals included for comparison purposes

Table 52 - Incidence of BMS-790052-Related Microscopic Findings in

	Dose (mg/kg/day):				
	0	15	30	150	
	No. of monkeys (M/F): 4/4				
	Sex:	M/F	M/F	M/F	M/F
<u>Liver:</u>					
Hyperplasia/hypertrophy: Kupffer cell	-/-	-/-	-/1	2/2	
Minimal	-/-	-/-	-/1	1/-	
Slight	-/-	-/-	-/-	1/2	

A dash (-) indicates absence of finding in group

Note: Control animals included for comparison purposes.

The adrenal gland findings suggested involvement/activation of the hypothalamic-pituitary-adrenal axis. Decreased cytoplasmic vacuolation may represent

adrenocorticotropin hormone (ACTH)-induced exhaustion of corticosteroid hormone stores, or their decreased synthesis/secretion due to prolonged ACTH suppression.

Special Evaluation

The primary antibody response to a T-cell-dependent antigen (keyhole limpet hemocyanin [KLH]) was assessed in all available animals via a validated assay for KLH-specific antibodies (IgA, IgM, and IgG combined).

In females at Weeks 35 and 36, there were drug-related decreases (55 to 65% suppression based on endpoint titers) in the KLH-specific antibody response at 150 mg/kg/day. The observed decrease in antibody response dissipated with time, but that dissipation was primarily due to the waning of the KLH-specific antibody response in the control animals at the same time.

Cytokine analysis

There were no drug-related effects on serum IL-8 concentrations.

Bone marrow phenotyping

There were no drug-related changes in bone marrow cell types or distributions.

Toxicokinetics

Toxicokinetic parameters are presented in the table below (excerpted from sponsor).

Table 53 - Mean Toxicokinetic Parameters for BMS-790052 a

Parameter	Period	15 mg/kg/day		30 mg/kg/day		150 mg/kg/day	
		Male	Female	Male	Female	Male	Female
C _{max} (µg/mL)	Day 1	0.728	0.670	2.12	1.92	4.94	6.24
	Week 13	0.330	0.397	1.61	1.56	3.79	5.25
	Week 26	0.610	0.524	1.90	1.76	3.34	4.44
	Week 39	0.368	0.697	1.57	1.99	3.62	3.87
AUC(0-24h) (µg•h/mL)	Day 1	5.27	5.13	14.8	13.6	47.9	75.7
	Week 13	2.59	2.66	12.2	9.73	38.2	36.8
	Week 26	3.36	3.40	12.2	11.5	29.8	32.8
	Week 39	2.91	3.61	11.3	11.8	39.6	37.9

a: Mean combined-sex AUC values at Week 39 were 3.26, 11.6, and 38.8 µg•h/mL for the doses of 15, 30, and 150 mg/kg/day, respectively.

Dosing Solution Analysis

The dosing formulations were solutions; therefore, no homogeneity testing was performed. Concentrations of dose solutions met acceptance criteria and stability of dose formulations was documented.

COMBINATION TOXICOLOGY STUDIES

Study title: BMS-790052 and BMS-650032 ONE-MONTH ORAL COMBINATION TOXICITY STUDY IN RATS

Study no.	DS08126
Volume #, and page #	Volume 1
Conducting laboratory and location	BMS Syracuse, NY
Date of study initiation	Oct 8, 2008
GLP compliance	Yes
QA report	Yes
Drug, lot #, and % purity	BMS-790052 (DCV), batch 7M23810 BMS-650032 (ASV), batch 8A37939

Methods

Doses: (3 groups)

- 0 mg/kg/day
- 10 mg/kg/day BMS-790052 + 30 mg/kg BMS-650032
- 60 mg/kg/day BMS-790052 + 60 mg/kg BMS-650032

Species/strain: Sprague Dawley Rats

Number/sex/group or time point (main study): 10 per sex per group

Route, formulation, volume, and infusion rate: Oral gavage

Age: *not provided*. The protocol states that animals will be ~ 9 weeks old at the start of the study

Weight: *not provided*.

Results:

Mortality: No deaths noted.

Clinical signs:

- Hair/coat issues noted at both combinations in 2/10 animals (alopecia) and 3/10 animals (thin haircoat) with none noted in the control animals.
- An increase in Soiling and unformed feces was noted and was dose related. The soiling was noted more often (days 11 to day 28 in 10 of 10 animals) with the combination drugs, whereas it was only transiently noted (days 25 to 28 in 5 of 10 animals) in the control.

Body weights: No change.

Food consumption: No change.

Hematology: No major changes.

Clinical chemistry: No major changes. AST [REDACTED] (b) (4)
[REDACTED] at the 60 mg/60 mg
combination dose on day 24.

Ophthalmology: No change.

Urinalysis: 118% increase in urinary volume at 60mg/60mg combination dose for males only at day 24. Specific gravity decreased with the increase in urinary volume. Females were not different from controls at day 24.

Coagulation: No change.

Gross pathology: Alopecia increased dose-dependently.

Organ weights: Only selected organs were weighed (adrenal, brain, heart, kidney, liver, pituitary, prostate, spleen, testes, thymus, thyroid). No changes noted in the selected tissues.

Histopathology: Adequate Battery: yes (), no ()—explain
Peer review: yes (), no ()

Only two findings were notable and dose-related.

- 1) Increase in Adrenal gland vacuolation (4 of 10 males and 4 of 10 females) at the 60 mg/60mg combination dose. All of the affected females were grade 1. The males were more affected with 1 male at grade 1, 2 males at grade 2, and 1 male at grade 3.
- 2) Lung alveolar space histiocytosis (4 of 10 males and 4 of 10 females; all grade 1/mild) at the 60 mg/60mg combination dose.

Toxicokinetics:

Table 54 - TK Values for BMS-790052 and BMS-650032 in Rats Co-Administered BMS-790052 and BMS-650032 for 1 Month

Parameter	Day	BMS-790052			
		10/30 mg/kg/day (BMS-790052/BMS-650032)		60/60 mg/kg/day (BMS-790052/BMS-650032)	
		Male	Female	Male	Female
C _{max} (µg/mL)	1	0.613	0.715	5.70	5.50
	28	0.334	0.584	4.06	5.16
AUC(0-24h) (µg·h/mL)	1	4.20	4.73	49.9	43.2
	28	3.57 ^a / 3.07 ^b		34.5	35.6
T _{max} (h)	1	4.0	2.0	4.0	2.0
	28	4.0	2.0	4.0	4.0
Parameter	Day	BMS-650032			
		Male	Female	Male	Female
C _{max} (µg/mL)	1	(b) (4)			
	28				
AUC(0-24h) (µg·h/mL)	1				
	28				
T _{max} (h)	1				
	28				

^aThe 24-hour plasma sample collected from Animal 2109 on Day 28 was included in the calculation of the mean concentration and toxicokinetic parameters.

^bThe 24-hour plasma sample collected from Animal 2109 on Day 28 was excluded from the calculation of the mean concentration and toxicokinetic parameters.

Table excerpted from sponsor's application

For BMS-790052 dosed in combination with BMS-650032, AUC values on Day 28 were dose proportional between 10 and 60 mg/kg/day, similar to or slightly less than those on Day 1, and no sex differences were noted. For BMS-650032 in combination with BMS-790052, AUC values on Day 28 were greater than dose proportional between 30 and 60 mg/kg/day, similar to or slightly less than those on Day 1, and AUC values in males were similar to or slightly less than those in females.

Relative to BMS-790052 or BMS-650032 alone in previous studies in rats (figures not shown) given similar or equivalent doses, co-administration of BMS-790052 and BMS-650032 resulted in trends on Day 28 toward lower BMS-790052 AUC (~ 0.6 to 0.7x) but

(b) (4) BMS-650032 AUC (b) (4)

Table 55 - Mean Liver and Plasma Concentrations, and Liver-to-Plasma Concentration Ratios, at Necropsy for Rats Co-Administered BMS-790052 and BMS-650032 for 1 Month

Dose (mg/kg/day)	Day	Gender		BMS-790052		
				Concentration of BMS-790052 (ng/mL or g tissue)		Liver-to-Plasma Concentration Ratio
				Liver	Plasma	
10/30	29	Male	Mcan	134	29.3	6.38
			SD	121	27.6	4.36
	30	Female	Mcan	262	46.4	8.97
			SD	155	37.4	6.29
60/60	29	Male	Mcan	639	178	4.67
			SD	267	133	1.79
	30	Female	Mean	1240	297	5.35
			SD	955	267	3.26
Dose (mg/kg/day)	Day	Gender		BMS-650032		
				Concentration of BMS-650032 (ng/mL or g tissue)		Liver-to-Plasma Concentration Ratio
				Liver	Plasma	
10/30	29	Male	Mean	(b) (4)		
			SD			
	30	Female	Mean			
			SD			
60/60	29	Male	Mean			
			SD			
	30	Female	Mean			
			SD			

Table excerpted from sponsor's application

At necropsy, high liver concentrations and high liver-to-plasma ratios were observed for both BMS-790052 and BMS-650032. The liver-to-plasma ratios for both BMS-790052 and BMS-650032 were (b) (4) both combination doses with no sex difference noted.

Study title: PEGINTERFERON ALFA-2b AND RIBAVIRIN: ONE-WEEK RANGE-FINDING TOXICITY STUDY IN MONKEYS

Study no.: Study DS08147
 Study report location: EDR
 Conducting laboratory and location: BMS, Syracuse, NY
 Date of study initiation: Protocol date – 10-Oct-2008
 GLP compliance: No
 QA statement: No

Key Study Findings

The objective of this study was to determine the toxicity of subcutaneous peginterferon

alfa-2b (pIFN; every other day) in combination with oral ribavirin (RBV; daily) in monkeys after a 1-week treatment period.

Note: No experimental drugs were given in this study. It was a proof of concept study for IFN/RBV in monkeys prior to combining with other experimental drugs from BMS.

There were no drug-related mortalities. Subcutaneous pIFN at doses of 7.5, 15, or 30 µg/kg/ in combination with oral RBV at doses of ~ 50 to 75 mg/kg/day was clinically well tolerated in monkeys for 1 week. At all doses, the mostly-expected findings were limited to transient decreases in feeding behavior and minimal to mild decreased red cell mass, neutrophil count, and albumin, and increased monocyte counts.

Table 56 - TK Summary of pIFN and RBV in Monkeys, Study 08147

Analyte	Parameter	Day	Peginterferon alfa-2b and Ribavirin ^a		
			Peginterferon alfa-2b		
			7.5 µg/kg/qod	15 µg/kg/qod	30 µg/kg/qod
			Male	Male	Male
Peginterferon alfa-2b	AUC(0-48h) (ng•h/mL)	1	481	788	1,490
		7	629	585	2,240
	Cmax (ng/mL)	1	14.1	31.3	62.3
		7	23.1	23.7	90.5
Analyte	Parameter	Day	Ribavirin		
			50 to 75 mg/kg/day		
Ribavirin	AUC(0-24h) (ng•h/mL)	1	21,700	23,500	23,600
	Cmax (ng/mL)	1	2,120	2,050	2,100

^a Doses of 7.5, 15, and 30 µg/kg/day of pegylated interferon alfa-2b were combined with ~ 50 to 75 mg/kg/day ribavirin.

Study title: BMS-790052, Pegylated Interferon Alfa-2B, and Ribavirin TWO-WEEK COMBINATION TOXICITY STUDY IN MONKEYS

Study no.: Study DS08077
 Study report location: EDR
 Conducting laboratory and location: BMS, Syracuse, NY
 Date of study initiation: Protocol date – 27 Oct 2008
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: BMS-790052, Batch 7M23810, 90.5% pure

Key Study Findings

The objectives of this study were to determine whether a toxicologic or toxicokinetic interaction would occur when BMS-790052, pegylated interferon α -2b (pIFN), and ribavirin (RBV) were administered in combination to monkeys and to provide data to support the use of this combination in humans. BMS-790052 was administered by oral gavage for 2 weeks at 0, 15, and 50 mg/kg. These doses were at the lower range of toxicity compared to other toxicity studies with BMS-790052.

Mortality (2 or 3 per group; Days 12 to 14) occurred in all groups and was generally attributed to trauma, debilitation, and tissue inflammation primarily resulting from capsule dosing of RBV and to pneumonia likely exacerbated by pIFN and/or RBV.

The dose of BMS-790052 was relatively low (compared to the single drug studies with BMS-790052 alone) and the only BMS-790052-related finding was a slight to mild decrease of cytoplasmic vacuolization of adrenocortical cells, occurred at the high dose of 50 mg/kg/day BMS-790052 with pIFN/RBV and is consistent with similar findings in a previous study with BMS-790052 alone.

There was no toxicologic or toxicokinetic interaction observed when BMS-790052 was administered in combination with pIFN/RBV. See TK summary below.

All other findings were attributed to pIFN/RBV administration and/or trauma and complications associated with the oral capsule dosing procedures. Anti-pIFN antibody development and onset of lower pIFN plasma concentrations support the adequacy of the 2-week duration for the evaluation of toxicity of this combination.

Table 57 - TK Summary of BMS-790052, pIFN, and RBV in Monkeys, Study 08077

Day	Mean AUC Values (ng•h/mL) for BMS-790052, RBV, and pIFN ^{a, b}					
	BMS-790052 0 mg/kg/day		BMS-790052 15 mg/kg/day		BMS-790052 50 mg/kg/day	
	Male	Female	Male	Female	Male	Female
1	NA	NA	5,340	4,330	33,000	49,000
14	NA	NA	3,800 ^c	3,760	27,600 ^d	36,500 ^c
RBV (all groups dosed with 50 to 100 mg/kg/day)						
1	10,100	19,400	12,400	14,200	9,180	10,200
14	32,600 ^c	45,300 ^d	36,100 ^c	33,500	25,000 ^c	21,900 ^c
pIFN (all groups dosed with 15 µg/kg/qod)						
1	812	801	723	648	806	874
13	761 ^c	985 ^d	1,210 ^c	1,120	720 ^d	1,270 ^e

^a BMS-790052 (0 [vehicle], 15, or 50 mg/kg/day) was combined with RBV (50 to 100 mg/kg/day) and pIFN (15 µg/kg/qod, qod = every other day).

^b AUC values represent t=0-24 h for BMS-790052 and RBV; t=0-48h for pIFN except where indicated

^c n = 1

^d n = 2

^e n = 1, AUC values for Animal Nos. 3202 and 3203 were calculated from 0 to 24 hours and were excluded from the mean calculation.

NA - Not applicable.

Study title: BMS-790052 and BMS-650032, ONE-MONTH ORAL COMBINATION TOXICITY STUDY IN MONKEYS

Study no.: Study DS08143
 Study report location: EDR
 Conducting laboratory and location: BMS, Syracuse, NY
 Date of study initiation: Protocol date – 10-Oct-2008
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: BMS-790052 (DCV), Batch 7M23810, 90.5% pure
 BMS-650032 (ASV), Batch 8A37929, 99.1% pure

Key Study Findings

The objectives of this study were to determine whether a toxicologic interaction (eg, additive or synergistic) occurred when BMS-790052 (DCV) and BMS-650032 (ASV) were administered orally in combination to monkeys for 1-month and to provide data to support the potential use of this combination in humans.

BMS-790052 and BMS-650032 combinations (BMS-790052/BMS-650032) were given daily by oral gavage to 3 groups of monkeys (4/sex/group) at doses of 0/0 (vehicle

controls), 15/72 mg/kg/day (low combination) or 50/129.5 mg/kg/day (high combination) for 1 month.

All animals survived to the scheduled necropsy. General findings were expected to be minor due to the low exposures (compared to prior studies at much higher doses). As expected, the findings were minor (vomiting, soiling) and were noted in prior studies. Minor histological changes (adrenal gland vacuolization and thymic involution) were also noted in prior studies.

There was no toxicological interaction observed upon co-administration of BMS-790052 and BMS-650032, but there was evidence of a toxicokinetic interaction (see below). In general, for both BMS-790052 and BMS-650032, AUC values were higher on Day 1 than on Day 28, suggesting altered clearance and/or absorption with repeated dosing, and were increased in a greater than dose-proportional fashion on Day 28. Across dose levels and days, there were no sex-related differences.

Table 58 - TK Summary of BMS-790052 and BMS-650032, Study 8143

Analyte	Day	BMS-790052/BMS-650032 ^a 15/72 mg/kg/day		BMS-790052/BMS-650032 ^a 50/129.5 mg/kg/day	
		Male	Female	Male	Female
BMS-790052	1	6.51	5.02	68.7	50.8
	28	4.78	5.43	36.8	35.2
BMS-650032	1	10.4	6.04	201	144
	28	4.57	7.77	73.9	60.2

a: BMS-790052 and BMS-650032 were not detected in any control plasma samples.

b: The sex-combined AUCs for BMS-790052 and BMS-650032 on Day 28 were 5.11 and 36 $\mu\text{g}\cdot\text{h}/\text{mL}$ for the low and high dose of BMS-790052, respectively; (b) (4) for the low and high dose of BMS-650032, respectively.

Concurrent daily oral administration of BMS-790052 and BMS-650032 to monkeys at ≤ 50 mg/kg/day of BMS-790052 (AUC ≤ 68.7 $\mu\text{g}\cdot\text{h}/\text{mL}$) and at (b) (4) of BMS-650032 (AUC (b) (4)) for 1 month revealed no toxicologic interaction. All drug-related findings were minor and were consistent with the findings from previous single-agent studies.

The NOAELs were the high doses (50 and 129.5 mg/kg/day) for both BMS-790052 and BMS-650032, respectively.

Study title: BMS-790052 and BMS-650032, THREE-MONTH ORAL COMBINATION TOXICITY STUDY IN MONKEYS

Study no.:	Study DS09008
Study report location:	EDR
Conducting laboratory and location:	BMS, Syracuse, NY
Date of study initiation:	Protocol date – 17-Feb-2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BMS-790052 (DCV), Batch 7M23810, 90.5% pure BMS-650032 (ASV), Batch 8A37929, 99.1% pure

Key Study Findings

The objectives of this study were to determine whether a toxicologic interaction occurred when BMS-790052 (DCV) and BMS-650032 (ASV) were administered in combination to monkeys for 3 months and to provide data to support the potential use of this combination in humans.

BMS-790052 and BMS-650032 (as separate formulations given sequentially) were administered once daily by oral gavage to 2 groups of 4 monkeys/sex at 15/45 or 50/80 mg/kg/day, respectively.

AUC values for BMS-790052 (DCV) in this study were consistent with the AUC values observed when BMS-790052 was administered alone; whereas AUC values for BMS-650032 (ASV) were (b) (4)

There were no toxicologically significant drug-related effects or toxicologic interactions. All drug-related findings were minor and were consistent with the findings from previous single-agent studies.

Table 59 - TK Summary of BMS-790052 (DCV) and BMS-650032 (ASV), Study 9008

Parameter	Period	BMS-790052/BMS-650032 Dose			
		15/45 mg/kg/day		50/80 mg/kg/day	
		Male	Female	Male	Female
BMS-790052					
Mean AUC(0-24h) ($\mu\text{g}\cdot\text{h}/\text{mL}$)	Day 1	4.07	3.67	29.3	32.7
	Week 4	3.56	4.56	24.4	28.4
	Week 13	4.19	3.69	29.4	27.9
BMS-650032					
Mean AUC(0-T) ^a ($\mu\text{g}\cdot\text{h}/\text{mL}$)	Day 1	(b) (4)			
	Week 4	(b) (4)			
	Week 13	(b) (4)			
a	(b) (4)				

7 Genetic Toxicology

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

Study title: EXPLORATORY MINI AMES REVERSE-MUTATION STUDY IN *Salmonella typhimurium*

Study no.: DS06143
 Study report location: EDR
 Conducting laboratory and location: BMS
 Date of study initiation: June 26, 2006
 GLP compliance: No
 QA statement: No

Key Study Findings

At concentrations up to 1000 μg bulk drug/well, BMS-790052 was negative.

Study title: EXPLORATORY MINI AMES REVERSE-MUTATION STUDY IN *Salmonella typhimurium*

Study no.: DS06219
 Study report location: EDR
 Conducting laboratory and location: BMS
 Date of study initiation: Oct 19, 2006
 GLP compliance: No
 QA statement: No

Key Study Findings

At concentrations up to 5000 μg active drug/plate, BMS-790052 was negative.

Study title: BMS-790052: AMES REVERSE-MUTATION STUDY IN Salmonella typhimurium and Escherichia coli

Study no.: DS07070
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 27 April, 2007
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BMS-790052, 91.3%, Batch 7C26990

Key Study Findings

BMS-790052 was not mutagenic in the *S. typhimurium* and *E. coli* bacterial strains when tested to cytotoxicity or precipitating concentrations up to 5000 µg/plate, the maximum concentration(s) recommended by international guidelines.

Study title: A QUALIFYING AMES REVERSE-MUTATION STUDY IN SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

Study no.: 964256
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 05 January 2012
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BMS-790052-05, 88.4% pure, Lot 91375-092-01

Key Study Findings

BMS-790052-05 containing 8 structurally-related impurities (250 µg/plate individually or 2000 µg/plate) did not show evidence of genotoxic activity in this *in vitro* mutagenicity assay, when tested in accordance with regulatory guidelines.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: **CYTOGENETICS STUDY IN CHINESE HAMSTER OVARY CELLS**

Study no.: DS07064
Study report location: EDR
Conducting laboratory and location: BMS
Date of study initiation: April 6, 2007
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BMS-790052-05, 91.3% pure, Batch 7C26990

Key Study Findings

There was no statistically significant increase in the frequency of cells with structural or numerical (polyploidy or endoreduplication) aberrations at the concentrations evaluated in any of the assays with or without S9 metabolic activation. BMS-790052 was not clastogenic in CHO cells when tested to the maximum concentrations recommended by international guidelines for *in vitro* cytogenetic studies.

Study title: **Exploratory *In Vitro* Micronucleus Assay in Chinese Hamster Ovary Cells**

Study no.: DS06195
Study report location: EDR
Conducting laboratory and location: BMS
Date of study initiation: 9/12/2006
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: BMS-790052-05, 91.3% pure, Batch 7C26990

Key Study Findings

BMS-790052-02-004 and form 03-004 were not clastogenic in CHO cells when tested either in the presence or absence of S9 metabolic activation at concentrations up to 69.4 µg/ml (93.9 µM) and 21.5 µg/ml (29.1 µM) respectively.

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

Study title: **THREE-DAY ORAL MICRONUCLEUS STUDY IN MALE RATS**

Study no.: DS08011
Study report location: EDR
Conducting laboratory and location: BMS
Date of study initiation: 10 Jan 2008 (protocol date)
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: BMS-790052-05, 91.3% pure, Batch 7C26990

Key Study Findings

BMS-790052 was evaluated in the rat bone-marrow erythrocyte micronucleus assay to determine its genotoxic potential. BMS-790052 was administered to groups of 5 male rats by oral gavage for 3 consecutive days at doses of 0 (vehicle), 500, 1000, or 2000 mg/kg/day.

Increases in C_{max} and AUC_[0-24 h] values for BMS-790052 were dose-related, but less than proportional across all doses tested. BMS-805215 AUC values were 0.05x to 0.07x of the parent drug across the doses, and were similar at doses of 1000 and 2000 mg/kg/day.

Table 60- Toxicokinetic Parameters of BMS-790052 and BMS-805215 in Male Rats, Study DS08011

Analyte	Parameter	BMS-790052 (mg/kg/day)		
		500	1000	2000
BMS-790052	C_{max} ($\mu\text{g/mL}$)	40.4	54.3	78.1
	AUC(0-24 h) ($\mu\text{g}\cdot\text{h/mL}$)	830	1,120	1,430
BMS-805215	C_{max} ($\mu\text{g/mL}$)	2.42	5.60	4.97
	AUC(0-24 h) ($\mu\text{g}\cdot\text{h/mL}$)	38.9	81.8	83.2

BMS-790052 was not genotoxic in the *in vivo* rat bone-marrow micronucleus test at the maximum dose level (2000 mg/kg/day, mean AUC \leq 1430 $\mu\text{g}\cdot\text{h/mL}$) recommended by international regulatory guidelines.

7.4 Other Genetic Toxicity Studies

Please see Dr. Mark Powley's review of the impurities (Appendix A)

8 Carcinogenicity

Study title: BMS-790052: 2-Year Oral Carcinogenicity Study in Rats

Study no.: DN11082
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: May 17, 2011
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Batches 7M23810 and 7L23964
Drug purity: 89.8% and 90.4% for the
batches, respectively.
CAC concurrence: Yes

Key Study Findings

- BMS-790052 was clinically well tolerated at all doses with no BMS-790052-related effects on body weight or food consumption. BMS-790052-related clinical signs were limited to an increased incidence of salivation in males and females at 50 mg/kg/day.
- There were no BMS-790052-related effects on the incidence, distribution, or nature of neoplastic changes.
- Nonneoplastic findings attributed to BMS-790052 were limited to increased incidences of pale discoloration and enlargement of the adrenal glands with microscopic correlate of increased incidence of fine vacuolation and/or cytoplasmic rarefaction of adrenal cortical cells in males and females at 50 mg/kg/day.
- BMS-790052 was not carcinogenic in Sprague-Dawley rats following daily oral administration at ≤ 50 mg/kg/day (mean combined-sex AUC ≤ 70.3 $\mu\text{g}\cdot\text{h}/\text{mL}$).

Adequacy of Carcinogenicity Study

The study was adequate.

Appropriateness of Test Models

The model was appropriate.

Evaluation of Tumor Findings

Methods

Doses: 0, 0, 5, 15, 50 (males)
0, 0, 5, 15, 50 (females)
Frequency of dosing: Daily
Dose volume: 5 mL/kg
Route of administration: Oral

Formulation/Vehicle: 60% polyethylene glycol 400 (PEG-400) and 40% Vitamin E-d- α -tocopheryl polyethylene glycol 1000 succinate (TPGS)

Basis of dose selection: AUC

Species/Strain: SD Rat

Number/Sex/Group: 65/sex/group (main) and 20/sex/group (TK)

Age: ~ 6 wks at start of study

Animal housing: Individual housed

Paradigm for dietary restriction: None.

Dual control employed: Yes

Interim sacrifice: No

Satellite groups: Yes (TK)

Deviation from study protocol: Early termination. End of Study -- Males: Week 94. Females: Week 92.

Table 61 - Experimental Design, Study DN11082

Group No.	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Concentration (mg/mL)	No. of Animals			
				Main Study ^a		Toxicokinetic Study ^b	
				Males	Females	Males	Females
1/ Water Control	0	10	0	1001 to 1065	1501 to 1565	1066 to 1083	1566 to 1583
2/ Vehicle Control	0	10	0	2001 to 2065	2501 to 2565	2066 to 2083	2566 to 2583
3/ BMS-790052	5	10	0.5	3001 to 3065	3501 to 3565	3066 to 3083	3566 to 3583
4/ BMS-790052	15	10	1.5	4001 to 4065	4501 to 4565	4066 to 4083	4566 to 4583
5/ BMS-790052	50	10	5	5001 to 5065	5501 to 5565	5066 to 5083	5566 to 5583
6/ Health Screen ^c	-	-	-	6001 to 6010 ^d	6501 to 6510 ^d	-	-
7/ Sentinel ^d	-	-	-	7001 to 7025	7501 to 7525	-	-

- = Not applicable.

^a Male animals were necropsied during Week 94 and female animals were necropsied during Week 92.

Observations and Results

Mortality

Monitored 2x daily. There were no BMS-790052-related effects on survival or on any specific cause of death. Preterminal deaths in both control (water and vehicle) and BMS-790052-treated groups were attributed to incidental neoplasms (pituitary adenoma/adenocarcinoma, hemolymphoid neoplasia, and mammary gland tumors) and chronic progressive nephropathy.

It should be noted that the study was halted early. The early mortalities did not appear to be attributed to vehicle as the water control had a similar curve as the vehicle control. End of Study was Week 94 for Males and Week 92 for Females.

Table 62 - Survival, Study DN11082

Dose (mg/kg/day)	Males					Females				
	0 ^a	0 ^b	5	15	50	0 ^a	0 ^b	5	15	50
Number of rats	65	65	65	65	65	65	65	65	65	65
Number of deaths prior to termination	45	45	42	50	46	43	45	35	35	41
Number of survivors at termination	20	20	23	15	19	22	20	30	30	24
Percent survival (%)	31	31	35	23	29	34	31	46	46	37

These numbers included male animals that were found dead on and after Day 651 and female animals that were found dead on and after Day 637. End of Study: Males: Week 94; Females: Week 92.

^a Water control

^b Vehicle control

Figure 3 - Survival Curves (Male), Study DN11082

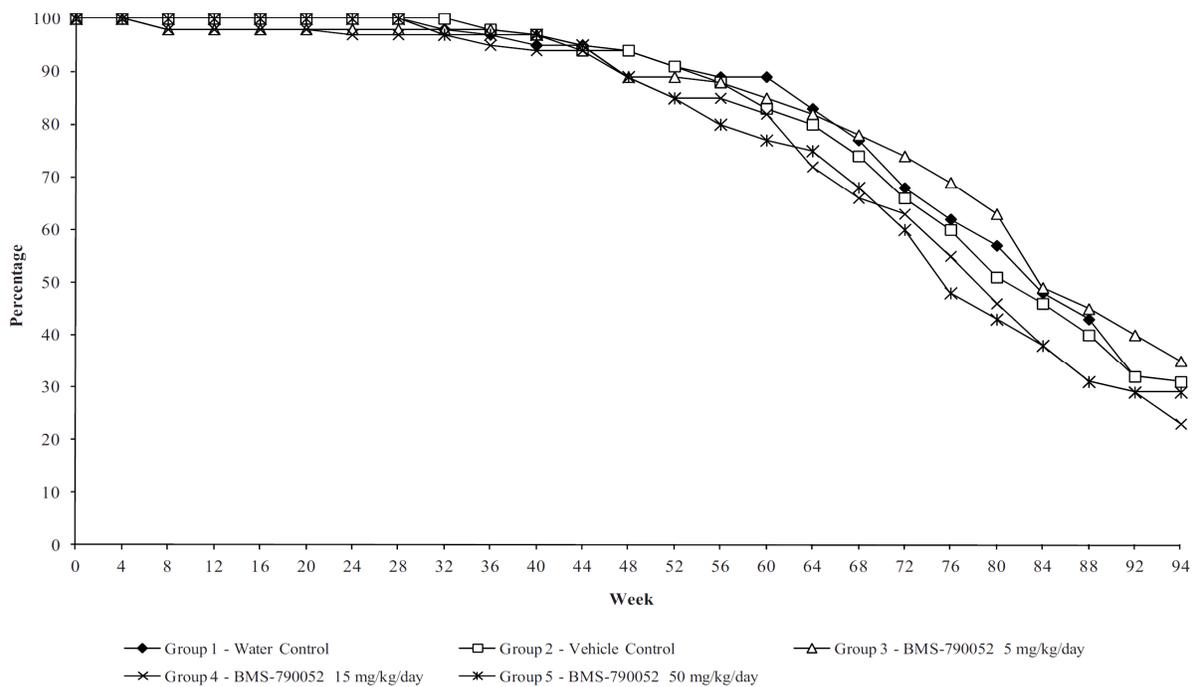
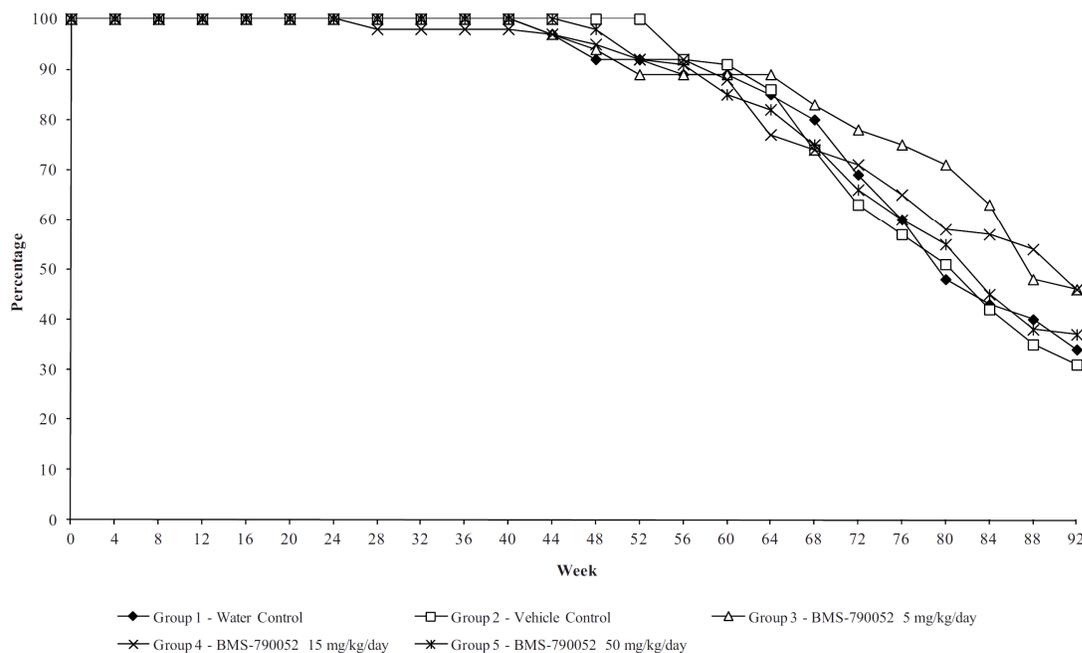


Figure 4 -Survival Curves (Female), Study DN11082



The most frequent cause of early death/euthanasia was the presence of spontaneously occurring neoplastic change in the pituitary pars distalis. The Sponsor stated that this was typical for SD rats. The incidence of principal causes of early death/euthanasia for each group is shown in the following table:

Table 63 - Cause of Death, Study DN11082

Group	Males					Females				
	1	2	3	4	5	1	2	6	7	8
	0 ^a	0 ^b	5	15	50	0 ^a	0 ^b	5	15	50
Number of Animals Examined	45	47	42	50	46	43	45	35	35	41
Pituitary adenoma/adenocarcinoma	19	24	21	26	17	33	34	26	27	32
Hemolymphoid neoplasia	4	4	1	1	1	1	1	0	0	1
Mammary gland neoplasia	0	0	1	0	0	5	4	3	4	0
Other neoplastic change	5	6	3	8	2	2	0	4	2	4
Chronic progressive nephropathy	4	2	3	4	8	0	0	2	1	1
Other non-neoplastic change	3	4	3	5	8	2	2	0	0	0
Undetermined	10	7	10	6	10	0	4	0	1	3

^a Water control
^b Vehicle control

Clinical Signs

Monitored 2x daily. Limited to salivation in 50 mg/kg animals (m/f). See below

Table 64 - Clinical Observations, Study DN11082

Dose (mg/kg/day):	0 ^a		0 ^b		5		15		50	
Sex:	M	F	M	F	M	F	M	F	M	F
Salivation	7(3)	3(3)	8(7)	2(2)	3(3)	4(4)	13(7)	2(2)	23(20)	6(6)

^a Water control

^b Vehicle control

Data are expressed as the total number of occurrences/group (number of animals affected).

Body Weights

Recorded weekly. No drug-related effects were noted.

Feed Consumption

Recorded weekly. No drug-related effects were noted.

Gross Pathology

At sacrifice. An increased incidence of pale discoloration and enlargement of the adrenal glands was recorded in both male and female animals receiving 50 mg/kg/day of BMS-790052. All other gross observations recorded at necropsy were within the normally expected range.

Table 65 - Incidence of BMS-790052-Related Gross Findings, Study DN11082

Dose (mg/kg/day):	0		5		15		50	
No. of Rats (M/F):	65/65		65/65		65/65		65/65	
Sex:	M/F	M/F	M/F	M/F	M/F	M/F	M/F	
Adrenal glands:								
Discoloration: pale	5/5		3/1		2/1		9/6	
Enlargement	5/17		5/20		6/21		5/22	

Histopathology

Peer Review

The pathology evaluation was peer reviewed.

Neoplastic

No significant effects were noted. Trends were noted, but were not concerning.

- 1) Benign Squamous Skin neoplasms were noted at 2, 0, 2, 4 for vehicle, 5, 15, and 50 mg/kg in males.
- 2) Benign granular cell tumors in the cervix were noted at 0, 0, 0, 2 in females for vehicle, 5, 15, and 50 mg/kg.
- 3) Skin/subcutis fibroma + fibrosarcoma in females were noted at 3, 2, 2, 6 for vehicle, 5, 15, and 50 mg/kg.

Non Neoplastic

Treatment related non neoplastic lesions were seen in the adrenal glands at the high dose level

Table 66 - Non-Neoplastic Findings. Study DN11082

	Dose (mg/kg/day):		0	0	5	15	50
	No. of Rats (M/F):		65/65	65/65	65/65	65/65	65/65
	Sex:		M/F	M/F	M/F	M/F	M/F
<u>Adrenal glands:</u>							
Vacuolation/rarefaction: cortical			4/1	3/0	7/0	7/2	29/4
Minimal			3/0	3/0	6/0	3/1	9/1
Slight			1/1	-	1/0	3/1	12/2
Moderate			-	-	-	1/0	7/1
Marked			-	-	-	-	1/0

Toxicokinetics

In Weeks 4 and 26, BMS-790052 AUC_{0-24h} values increased in a greater than dose-proportional manner over the dose range of 5 to 50 mg/kg/day with no sex-related differences in exposure and slight accumulation with repeated dosing. This was consistent with prior studies.

Table 67 - TK Summary, Study DN11082

Parameter	Period	BMS-790052 Dose (mg/kg/day)					
		5		15		50	
		M	F	M	F	M	F
AUC(0-24h)	Week 4	2.71	2.07	9.09	9.24	56.5	46.3
($\mu\text{g}\cdot\text{h}/\text{mL}$) ^a	Week 26	3.19	4.00	10.8	12.6	70.3	70.3

^a Mean combined-sex AUC values at Week 26 were 3.60, 11.7, and 70.3 $\mu\text{g}\cdot\text{h}/\text{mL}$ for the doses of 5, 15, and 50 mg/kg/day, respectively.

Dosing Solution Analysis

The dosing formulations were solutions; therefore, no homogeneity analyses were conducted.

However, test item/article content of the dose formulations used over the course of the study was determined.

Study title: 26-WEEK ORAL CARCINOGENICITY STUDY IN CByB6F1-Tg(HRAS)2Jic HEMIZYGOUS MICE

Study no.:	DN11083
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	13-Oct-2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BMS-790052, Batch 9M38078, Purity 95 to 105%
CAC concurrence:	Yes

Key Study Findings

- BMS-790052 was clinically well tolerated at all doses. There was no BMS-790052-related mortality, and clinical signs were limited to non-dose dependent instances of rough coat primarily in males.
- There were no BMS-790052-related macroscopic lesions. Occasional skin papillomas at similar incidence in control (water and vehicle) and BMS-790052 groups were noted on the right pinna (associated with the metal ear tag) with an increased incidence in NMU-treated mice.
- Tumor incidences in the water- and vehicle-control groups were similar and there were no BMS-790052-related neoplastic microscopic findings at any dose.
- Non-neoplastic findings were limited to minor increased incidences of splenic extramedullary hematopoiesis (EMH) in females at ≤ 100 mg/kg/day.
- BMS-790052 was not carcinogenic in CByB6F1/Tg rasH2 hemizygous mice following daily oral administration for 6 months at doses ≤ 300 mg/kg/day (combined-sex mean AUC ≤ 131 $\mu\text{g}\cdot\text{h}/\text{mL}$ at Week 26).

Adequacy of Carcinogenicity Study

The study was adequate.

Appropriateness of Test Models

The model was appropriate.

Evaluation of Tumor Findings

Methods

Doses:	0,0,30, 100, 300 (oral) and 75 IP (for both males and females)
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral
Formulation/Vehicle:	60% polyethylene glycol 400 (PEG-400) and 40% Vitamin E-d- α -tocopheryl polyethylene glycol 1000 succinate (TPGS)
Basis of dose selection:	MTD (clinical signs and mortality in the 28 day study)
Species/Strain:	CByB6F1-Tg(HRAS)2Jic HEMIZYGOUS MICE
Number/Sex/Group:	25/sex/group (main) and 36/sex/group (TK) 15/sex/group for IP injection (see below)
Age:	~ 8 wks at start of study
Animal housing:	Individual housed
Paradigm for dietary restriction:	None.
Dual control employed:	Yes
Interim sacrifice:	No
Satellite groups:	Yes (TK)
Deviation from study protocol:	No significant deviations.

Table 68 - Experimental Design, Study DN11083

Group Number	Dose Route	Daily Dose		Concentration (mg/mL)	Number of Animals	
		Dose Level (mg/kg/day)	Volume (mL/kg)		Dosing Period (Tg)	Toxicokinetic Period (non-Tg)
1	Oral	0	10	0	25 M, 25 F	-
2	Oral	0	10	0	25 M, 25 F	36 M, 36 F
3	Oral	30	10	3	25 M, 25 F	36 M, 36 F
4	Oral	100	10	10	25 M, 25 F	36 M, 36 F
5	Oral	300	10	30	25 M, 25 F	36 M, 36 F
6	Intraperitoneal Injection ^a	75	10	7.5	15 M, 15 F	-

M = male; F = female.

^a Single administration on Day 1.

Observations and Results

Mortality

There were no BMS-790052-related preterminal deaths during the course of the study. NMU-related preterminal mortality in the positive control group was observed

Table 69 - Survival Summary, Study DN11083

	Males						Females					
	0 ^a	0 ^b	30	100	300	NMU ^c	0 ^a	0 ^b	30	100	300	NMU ^c
Dose (mg/kg/day)	0 ^a	0 ^b	30	100	300	NMU ^c	0 ^a	0 ^b	30	100	300	NMU ^c
No. of Mice	25	25	25	25	25	15	25	25	25	25	25	15
No. of Deaths Prior to Termination	3	1	3	1	2	14	1	1	0	0	1	12
No. of Survival at Termination	22	24	22	24	23	1	24	24	25	25	24	3
Percent Survivorship (%)	88	96	88	96	92	7	96	96	100	100	96	20

^a Milli-Q A10 water control

^b Vehicle.

^c Positive control (N-Nitrosomethylurea).

Clinical Signs

2x daily. BMS-790052-related clinical observations were limited to non-dose-dependent incidences of rough coat observed occasionally for surviving animals across all BMS-790052-treated groups

Body Weights

Weekly. No significant changes noted. However, for the 300 mg/kg/day males, there was a slight reduction in mean body weights (-8% by Week 25 and ending at -7% on Week 27 vs. vehicle controls) and overall mean body weight gain (-24% relative to vehicle controls from Weeks 1 to 27). On the contrary, for the 300 mg/kg/day females, there was an increase in mean body weights (up to 8% by Week 16 and ending at 6.7% on Week 27 vs. vehicle controls) and overall mean body weight gain (+43% relative to vehicle controls from Weeks 1 to 27). NMU treated mice had a decrease in body weight gain, as expected.

Feed Consumption

Weekly. No significant changes noted. NMU treated mice had a decrease in food consumption, as expected.

Gross Pathology

At sacrifice. There were no BMS-790052-related gross lesions.

Table 70 - Incidence of Raise Area (Papilloma) of the Right Pinna, Study DN11083

Dose (mg/kg/day):	0 (WC)	0 (VC)	30	100	300	75 PC/NMU
No. of Mice (M/F):	25/25	25/25	25/25	25/25	25/25	15/15
Sex:	M/F	M/F	M/F	M/F	M/F	M/F
Right pinna:						
Raised area (Papilloma)	-/1	-/-	1/-	-/1	2/1	4/5

PC = Positive control; NMU = N-Nitrosomethylurea; VC = Vehicle control; WC = Water control;

A dash (-) indicates absence of finding in group

Histopathology

The few macroscopic findings in BMS-790052-treated animals were considered to be incidental and/or spontaneous in nature.

Peer Review - yes

Neoplastic

There were no BMS-790052-related neoplastic findings. All neoplastic findings were considered unrelated to treatment due to the absence of any dose relationship or statistical significance. NMU produced the expected increased incidence of lymphoma in both sexes (73% in males and 93% in females) compared with vehicle-control mice (0% in males and 12% in females).

Non Neoplastic

BMS-790052-related non-neoplastic findings were confined to increased extramedullary hematopoiesis (EMH) in the spleen in female mice at 100 and 300 mg/kg/day. This was consistent with findings in a previous 28-day range-finder study in female CByB6F1(hybrid) mice treated with BMS-790052 at 100 mg/kg/day.

Table 71 - Incidence of BMS-790052-related Microscopic Non-Neoplastic Findings, Study DN11083

Dose (mg/kg/day):	0 (WC)	0 (VC)	30	100	300	75 PC/NMU
No. of Mice:	25	25	25	25	25	14
Sex:	F	F	F	F	F	F
<u>Spleen:</u>						
Hematopoiesis, increased, extramedullary	6	2	4	14	19	1

NMU = N-Nitrosomethylurea; PC = Positive control; VC = Vehicle control; WC = Water control.

Toxicokinetics

At Weeks 4 and 26, mean BMS-790052 AUC[0-24h] were similar, generally dose proportional across doses, and there were no meaningful sex differences.

Table 72 - TK Summary, Study DN11083

Parameter	Week	BMS-790052 Dose (mg/kg/day)					
		30		100		300	
		M	F	M	F	M	F
AUC(0-24h)	4	9.59	12.6	39.9	44	78.6	123
($\mu\text{g}\cdot\text{h}/\text{mL}$)	26	15.7	13.1	50.7	50.2	117	144

^a Mean combined-sex AUC values at Week 26 were 14.4, 50.5, and 131 $\mu\text{g}\cdot\text{h}/\text{mL}$ for the doses of 30, 100, and 300 mg/kg/day, respectively.

Dosing Solution Analysis

For BMS-790052 content verification, all samples met acceptance criteria. Vehicles did not have BMS-790052 present.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: BMS-790052: Oral Study of Fertility and Early Embryonic Development in Rats

Study no.: DN08034
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: Sept 17, 2008
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: BMS-790052; 7M23810; 90.5%

Key Study Findings

BMS-790052 caused clinical findings in dams at 50 and 200 mg/kg/day with no effects on the offspring. In Males, reproductive effects (reduced prostate/seminal vesicle weights and minimally increased dysmorphic sperm) were noted at 200 mg/kg. This dose also produced overt toxicity. The NOAEL for reproductive toxicity was 50 mg/kg (AUC 51.8 $\mu\text{g}\cdot\text{h}/\text{mL}$) in males and 200 mg/kg/day (AUC 267 $\mu\text{g}\cdot\text{h}/\text{mL}$) in females.

Methods

Doses: 0, 15, 50, 200 mg/kg/day
 Frequency of dosing: Daily
 Dose volume: 10 ml/kg/day

Route of administration: Oral gavage
 Formulation/Vehicle: 15% PEG-400, 5% PVP K-30, 5% TPGS, and 75% 0.1 M phosphoric acid buffer (H₃PO₄) at pH~3.
 Species/Strain: CRL:CD(SD) rats
 Number/Sex/Group: 100 male and 160 female
 Satellite groups: See below
 Study design: See below
 Deviation from study protocol: None that would affect the study interpretation or outcome.

Table 73 - Experimental Design, Study DN08034

Group Number/ Treatment	Dose Level (mg/kg/day)	Concentration (mg/mL)	Dose Volume (mL/kg/day)	Number of Treated Males	Number of Treated Females
1/ Control	0	0	10	25 ^a	25 ^b + 10 ^c
2/ BMS-790052	15	1.5	10	25 ^a	25 ^b + 10 ^c
3/ BMS-790052	50	5	10	25 ^a	25 ^b + 10 ^c
4/ BMS-790052	200	20	10	25 ^a	25 ^b + 10 ^c

a - Assigned to fertility and early embryonic development evaluations; toxicokinetic samples were collected from the same cohort.

b - Assigned to fertility and early embryonic development evaluations.

c - Satellite females, assigned to toxicokinetic evaluation. These rats were euthanized after final blood collection.

Observations and Results

Mortality

2x daily. BMS-790052 did not cause any animal deaths. One female at 200 mg/kg died due to blood collection, but it was not drug related.

Clinical Signs

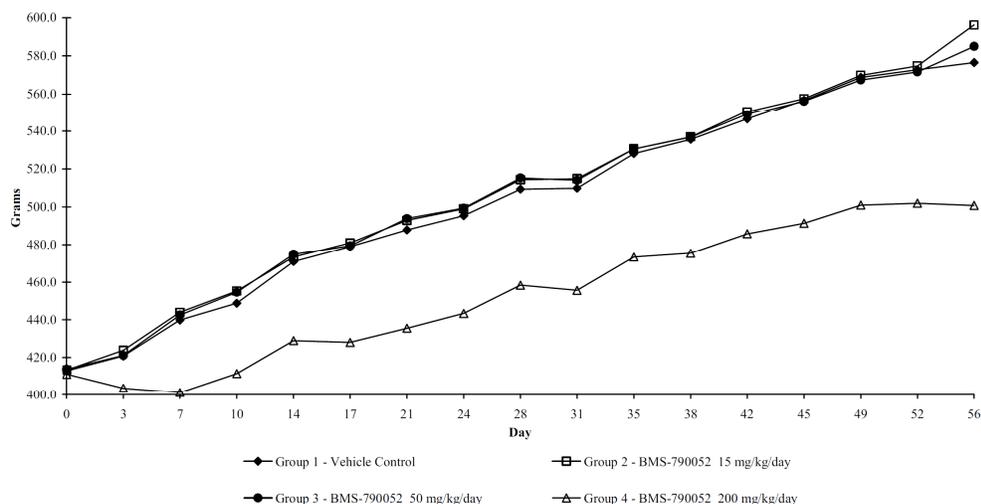
2x daily. Drug related clinical signs in both sexes at all doses. Males: excess salivation (with wet fur), red/brown fur stains (muzzle, lower jaw, forepaws) by day 6 at 50 and 200 mg/kg then at 15 mg/kg on day 12. Females had similar findings at 50 and 200 mg/kg on day 1 or 2 onward. Minor effects were noted at 15 mg/kg in females.

Firm abdominal structure in 11 females at 200 mg/kg during pre-mating and gestation periods. No pathological correlates at necropsy.

Body Weight

2x weekly. No changed in males up to 50 mg/kg. Significant decreased body weight gain at 200 mg/kg in males only. No changes in female body weights.

Figure 5 - Group mean body weights (males), Study DN08034



Feed Consumption

1x weekly. Reduced food consumption at 200 mg/kg in males and females during the first weeks of dosing.

Toxicokinetics

Systemic exposures in males were 1.1 to 1.3x those in females. Systemic exposures to BMS-790052 were greater than dose proportional. No major sex differences were noted.

Table 74 - TK Summary, Study DN08034

Parameter	Day	BMS-790052					
		15 mg/kg/day		50 mg/kg/day		200 mg/kg/day	
		Male	Female	Male	Female	Male	Female
AUC(0-24 h) (µg•h/mL)	14	9.40	8.34	51.8	39.7	290	267
Cmax (µg/mL)	14	1.39	2.07	6.20	5.80	23.3	23.7
Tmax (h)	14	2.0	1.0	2.0	2.0	4.0	4.0

Dosing Solution Analysis

BMS-790052 solutions were stable for 14 days after preparation. Results from the analysis of the solutions were within 6% of the target concentrations. Vehicle was absent of drug.

Necropsy

Gross effects were similar to prior repeat-dose toxicology studies. Primary effects were adrenal discoloration and enlargement. Also, small prostrate and seminal vesicles were observed in 1 male at 200 mg/kg.

Table 75 - Incidence of Drug-Related Gross Findings, Study DN08034

Dose (mg/kg/day):	0	15	50	200
No. of Rat Examined (M/F):	25/25	25/25	25/25	25/25
Sex:	M/F	M/F	M/F	M/F
Adrenal				
Discoloration pale	—	—	1/-	23/1
Enlargement	—	—	—	25/11
Stomach				
Area raised	—	1/1	—	13/2

A dash (-) indicates absence of finding in group.

Fertility Parameters:

- No drug-related effects on the estrous cycle.
- No drug-related effects on mating in either sex.
- No drug-related effects on caesarian-section parameters among litters.
- Mean pre-implantation loss (10.6%) in litters sired by treated males at 200 mg/kg increased compared to controls (5.6%).
- Sperm effects noted at 200 mg/kg. Increases in percentages of dysmorphic spermatozoa (11.8% compared to 6.1%) as well as misshapen head. One 200 mg/kg male had no or low sperm counts from the left cauda epididymis and vas deferens.

9.2 Embryonic Fetal Development

Study title: THIRTEEN-DAY ORAL RANGE-FINDING STUDY IN PREGNANT RABBITS

Study no.:	DN07051
Study report location:	EDR
Conducting laboratory and location:	Bristol Myers Squibb Drug Safety Evaluation One Squibb Drive, New Brunswick, New Jersey USA
Date of study initiation:	25 Oct 2007 (first day of dosing)
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	BMS-790052; 7C26990, purity 90.3%

Key Study Findings

This study was a range finding study in pregnant rabbits from GD7 – GD19. The study would also determine maternal systemic exposures to BMS-790052 and its metabolite, BMS-805215. Additionally, the study was extended to evaluate the tolerability and exposures of higher doses of BMS-790052 in non-pregnant rabbits.

BMS-790052 was administered by oral gavage at doses of 0 (vehicle), 5, 10, 25, or 50 mg/kg/day to groups of 6 pregnant rabbits from GDs 7 to 19. In the extension study, BMS-790052 was administered by oral gavage at doses of 0 (vehicle), 150 or 300 mg/kg/day to groups of 2 rabbits for 5 days.

BMS-790052-related effects (decreased food consumption) were noted only at the top 2 doses in non-pregnant rabbits of 150 and 300 mg/kg/day ($AUC \geq 633 \mu\text{g}\cdot\text{h}/\text{mL}$) and enlarged adrenals were noted only at the top dose of 300 mg/kg/day ($AUC \geq 1780 \mu\text{g}\cdot\text{h}/\text{mL}$).

Based on these results, the no adverse effect level (NOAEL) for maternal and developmental toxicity was 50 mg/kg/day ($AUC 392 \mu\text{g}\cdot\text{h} / \text{mL}$).

Table 76 - TK (Main Study), Study 07051

Parameter	Gestation Day	BMS-790052			
		5 mg/kg	10 mg/kg	25 mg/kg	50 mg/kg
C _{max} (µg/mL)	19	1.75	6.68	32.1	50.2
AUC(0-24 h) (µg•h/mL)	19	12.7	38.5	418	392
Parameter	Gestation Day	BMS-805215			
		5 mg/kg	10 mg/kg	25 mg/kg	50 mg/kg
C _{max} (µg/mL)	19	0.0986	0.451	2.08	5.86
AUC(0-T) (µg•h/mL)	19	0.637	2.92	24.4	48.9

Table 77 - TK (Extension Study), Study 07051

Parameter	Study Day	BMS-790052	
		150 mg/kg	300 mg/kg
C _{max} (µg/mL)	5	54.1	130
AUC(0-24 h) (µg•h/mL)	5	633	1780

The following EFD study in rabbits was reviewed by Dr. Mark Seaton.

Study title: Oral Study of Embryo-fetal Development in Rabbits

Study no.: DN08012
 Study report location: EDR
 Conducting laboratory and location: Bristol Myers Squibb
 Date of study initiation: 25-February-2008
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: BMS-790052; 7M23810; 90.5%

Key Study Findings

- Maternal toxicity included mortality, adverse clinical signs, and decrements in body weight and food consumption.
- Developmental toxicity consisted of increased embryo-fetal lethality, reduced fetal body weights, and increased incidences of fetal malformations of the ribs and variations, notably affecting the developing head and skull.

- The no-adverse effect dose (NOAEL) for both maternal and fetal effects was 40/20 mg/kg/day (AUC exposure of 245 ug*h/mL)

Methods

Doses:	0, 40, 200 or 750 mg/kg/day for 3-6 doses/ Dose reduction to 0, 20, 99 and 370 mg/kg/day
Frequency of dosing:	Once daily on day of gestation (DG) 7 to 19
Dose volume:	6.7 mL/kg were reduced to 3.3 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	15% polyethylene glycol 400, 5% polyvinyl pyrrolidone K-30, 5% Vitamin E d- α -tocopheryl polyethylene glycol 1000 succinate, and 75% 0.1 M phosphoric acid buffer, pH ~3)
Species/Strain:	New Zealand white rabbits
Number/Sex/Group:	27
Satellite groups:	Five animals in each group were designated for toxicokinetic parameters on DG19 (at 0.5, 1, 2, 4, 8 and 24 hrs after dose administration). However, due to moribundity and early sacrifice/deaths in the high dose group, a full toxicokinetic analysis was only conducted on the 200/99 and 40/20 mg/kg/day dose groups.
Study design:	See below
Deviation from study protocol:	Deviations noted did not invalidate study findings.

Dosed from DG7 through DG9, 10, 11 or 12:

Group Number	Daily Dose		Concentration BMS-790052 (mg/mL)	Number of Female Rabbits Assigned to Study
	BMS-790052 (mg/kg/day)	Volume (mL/kg)		
<u>Maternal and Developmental Toxicity Evaluations</u>				
1 (Control)	0 (Vehicle)	6.7	0	22
2	40	6.7	6	22
3	200	6.7	30	22
4	750	6.7	112	22
<u>Maternal Toxicokinetic Evaluation</u>				
5 (Control)	0 (Vehicle)	6.7	0	5
6	40	6.7	6	5
7	200	6.7	30	5
8	750	6.7	112	5

Dosed from DGs10, 11, 12 or 13 through DG 19:

Group Number	Daily Dose		Concentration BMS-790052 (mg/mL)	Number of Female Rabbits Assigned to Study
	BMS-790052 (mg/kg/day)	Volume (mL/kg)*		
<u>Maternal and Developmental Toxicity Evaluations</u>				
1 (Control)	0 (Vehicle)	3.3	0	22
2	20	3.3	6	22
3	99	3.3	30	22
4	370	3.3	112	22
<u>Maternal Toxicokinetic Evaluation</u>				
5 (Control)	0 (Vehicle)	3.3	0	5
6	20	3.3	6	5
7	99	3.3	30	5
8	370	3.3	112	5

* Adjusted dose volume

Observations and Results

Rabbits were evaluated for toxicokinetics, survival, clinical observations, body weight, food consumption, and euthanatized and cesarean sectioned on DG29 for fetal evaluations. Fetuses of these rabbits were weighed, sexed, and examined for external, soft tissue, and skeletal alterations.

Mortality

1 doe treated with 200/99 mg/kg/day was euthanatized in moribund condition on DG17 and 7 does were euthanatized after they aborted their litters between DG17 and DG24.

Mortality at 750/370 mg/kg/day included 2 does found dead on DG11 and DG12; 13 does euthanatized in moribund condition between DG 12 and DG15; and 7 does euthanatized between DG12 and DG15. Clinical signs in the moribund animals included lost righting reflex, ataxia, labored respiration, reduced activity and cool to the touch.

Table 78 – Mortality (EFD study in rabbits)

BMS-790052 Dose (mg/kg/day):	0	40/20	200/99	750/370
Found Dead	-	-	-	2 (DG11 and 12)
Euthanatized Moribund	-	-	1 (DG17)	13 (DG 12 to 15)
Euthanatized Humane	-	-	-	7 (DG 12 to 15)
Euthanatized after Abortion	1 (DG23)	-	7 (DG17 to 24)	-

A dash (-) indicates absence of finding in group

Data are expressed as the total number of occurrences/group (number of animals affected)

Clinical Signs

Clinical observations at all doses, including control animals, that were attributed to vehicle toxicity included scant or absent feces, as well as excessive unformed or liquid feces. Clinical signs in the moribund animals included lost righting reflex, ataxia, labored respiration, reduced activity and cool to the touch.

Body Weight

At 750/370 mg/kg, maternal body weight loss was dramatic (0.45 kg, compared to 0.08 kg weight gain in control animals during the same dosing period), due to embryo-fetal body weight loss. At 200/99 mg/kg, pregnant rabbits also lost weight, 0.05 kg, compared to 0.17 kg weight gain in control animals over the same dosing period.

Feed Consumption

However treatment at 750/370 and 200/99 mg/kg/day resulted in reductions of food consumption by 93% and 49% respectively, relative to control values, for the duration of the dosing period (DG7 to 15 and DG7 to 20, respectively).

Toxicokinetics

Table 79 - TK (EFD study in rabbits)

Toxicokinetic Summary ^a		
Parameter	BMS-790052	
	40/20 mg/kg ^b	200/99 mg/kg ^c
Gestation Day	19	19
N	5	5
C _{max} (µg/mL)	25.4	148
AUC(0-24 h) (µg·h/mL)	245	1,080

^a Only the 200/99 and 40/20 mg/kg/day dose groups that survived to DG19 are summarized.

^b Animals in satellite toxicokinetic Group 6 were dosed at 40 mg/kg/day on DG7 through 9, followed by 20 mg/kg/day on DG10 through 19.

^c Animals in satellite toxicokinetic Group 7 were dosed at 200 mg/kg/day on DG7 through 9, followed by 99 mg/kg/day on DG10 through 19.

Necropsy

All necropsy findings were seen only in rabbits that died (found dead or euthanized) prior to scheduled sacrifice. Findings in rabbits treated at 750/370 mg/kg/day included blood clots in the aorta (5 rabbits), thin or discolored stomach lining with colored foci (14 rabbits), mottled or discolored lungs (3 rabbits), small or discolored spleen (6 rabbits), black foci on ovaries (3 rabbits); and single instances of each of the following; red vascularized large intestine, white foci on gallbladder and enlarged adrenals. Findings in rabbits treated at 200/99 mg/kg/day included mottled or discolored lungs, enlarged gallbladders, enlarged adrenals and stomach lining with colored foci.

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

At 200/99 mg/kg/day fetal body weights were reduced by 11%, relative to group mean control values; all other cesarean section parameters were unaffected. No rabbits treated with BMS-790052 at 750/370 mg/kg/day survived to scheduled necropsy and only 6 of the 22 does had viable conceptuses.

Offspring (Malformations, Variations, etc.)

At 40/20 and 200/99 mg/kg/day, increased fetal and litter incidences of skeletal variations of the vertebral column were observed; fetal incidences were 4% and 7.2%, respectively, and litter incidences were 24% and 35%, respectively. Control fetal and litter incidences were 1% and 4.3%, respectively.

40/20 mg/kg: The findings below were in low dose fetuses. At the low dose, fetal and litter incidences of skeletal variations of the vertebral column were 4% and 24%, respectively, compared to 1% (fetal incidence) and 4.3% (litter incidence) in controls.

The sponsor stated that despite the increased incidence, the fetal skeletal variations are not considered adverse because they are common findings that are reversible during postnatal growth and are not associated with long-term consequences.

- Sternebrae: 2nd and 3rd, asymmetric; 4th, bifid
- Fontanel: Anterior, enlarged
- Sternebrae: 6th, bifid
- Sternebrae: 6th, bifid
- Sternebrae: 6th, bifid
- Nasals: Both, one supernumerary bone present.
- Frontals: Both, fused.
- Frontals and Parietals: Right, two supernumerary bones present, left, one supernumerary bone present.
- Clavicles: Both, bent.
- Sternebrae: 1st and 2nd and 3rd thru 5th, hyperplastic; 6th, bifid.
- Ribs: Left, 4th, absent.
- Vertebrae: Thoracic, 4th, right, hemivertebrae; centra, 3rd and 5th, irregularly-shaped.

200/99 mg/kg: increased incidences of rib malformations were observed; the incidences of these findings were 2% and 11.8% affected fetuses and litters, respectively, while there were none observed in the control group. Also seen were skeletal alterations affecting the skull; the incidences of these findings were 12.4% and 41% affected fetuses and litters, respectively, relative to control values of 2.4% and 13%, respectively. Observations included supernumerary bones, hypoplastic parietals and frontals and enlarged anterior fontanels.

Study title: TEN-DAY ORAL RANGE-FINDING STUDY IN PREGNANT RATS

Study no.: DN07054
Study report location: EDR
Conducting laboratory and location: Bristol Myers Squibb
Drug Safety Evaluation
One Squibb Drive, New Brunswick, New
Jersey USA
Date of study initiation: 3 Nov 2007
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: BMS-790052; batch 7J25449, purity
89.5%

Key Study Findings

This was a range findings study for BMS-790052 orally dosed in pregnant rats. The study would also determine maternal systemic exposures to BMS-790052 and its metabolite, BMS-805215.

BMS-790052 was administered by oral gavage at doses of 0 (vehicle), 10, 30, 100, or 200 mg/kg/day to groups of 8 pregnant rats from Gestation Days (GD) 6 to 15.

BMS-790052 was well tolerated at all doses. There were no differences in maternal necropsy observations or caesarean-section parameters at any dose evaluated.

10 or 30 mg/kg: no effects noted

100 and 200 mg/kg: dose-dependent increases in peri-oral/nasal substance and salivation, transient decreases in food consumption, elevated serum cholesterol.

200 mg/kg only: transient decrease in maternal body weight gain but rebounding after GD12, decreases in some serum chemistry (hemoglobin, hematocrit, retic count, platelets, total protein, and albumin) attributed to food consumption decreases. Adrenal gland weight increase (noted in prior studies in rodents).

Study title: ORAL STUDY OF EMBRYO-FETAL DEVELOPMENT IN RATS

Study no.: DN08011
Study report location: EDR
Conducting laboratory and location: BMS, Brunswick, NJ
Date of study initiation: 2 April 2008
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BMS-790052, Batch 7J25449, purity 89.5%)

Key Study Findings

BMS-790052 did not produce development effects at a dose that did not produce maternal toxicity (50 mg/kg). Findings among dams at doses \geq 200 mg/kg/day (AUC \geq 364 $\mu\text{g}\cdot\text{h}/\text{mL}$) included mortality, adverse clinical signs, body-weight losses, and reduced food consumption. In the offspring at 200 and 1000 mg/kg, fetal malformations were observed. Additionally, the dose of 1000 mg/kg/day was associated with profound embryoletality and fetal body-weight decrements. Based on these results, the NOAEL for maternal and developmental toxicity was 50 mg/kg/day (maternal AUC = 70.1 $\mu\text{g}\cdot\text{h}/\text{mL}$).

Methods

Doses: 0, 50, 200, 1000 mg/kg/day
Frequency of dosing: Daily
Dose volume: 10 ml/kg
Route of administration: Oral gavage
Formulation/Vehicle: 15% polyethylene glycol 400 (PEG-400), 5% polyvinyl pyrrolidone K-30 (PVP K-30), 5% vitamin E d- α -tocopheryl polyethylene glycol 1000 succinate (TPGS), and 75% 0.1 M phosphoric acid buffer (H₃PO₄), pH~3 (w/w).
Species/Strain: CRL:CD[SD] -- nulliparous time-mated female rats
Number/Sex/Group: See below
Satellite groups: See below
Study design: See below
Deviation from study protocol: No significant deviations that would affect study interpretation.

Table 80 - Experimental Design, Study DN08011

Group Number	Daily Dose		Concentration BMS-790052 (mg/mL)	Number of Females Assigned to Study
	BMS-790052 (mg/kg/day)	Volume (mL/kg)		
<u>Maternal and Developmental Toxicity Evaluations</u>				
1 (Control)	0 (Vehicle)	10	0	22
2	50	10	5	22
3	200	10	20	22
4	1000	10	100	22
<u>Maternal Toxicokinetic Evaluation</u>				
5 (Control)	0 (Vehicle)	10	0	10
6	50	10	5	10
7	200	10	20	10
8	1000	10	100	10

Observations and Results

Mortality

1x daily. No mortalities at 50 mg/kg. One (1) dam at 200 mg/kg and 1 dam at 1000 mg/kg were euthanized on days 12 and 14, respectively. Deaths were drug-related.

Clinical signs in the 2 euthanized dams: 20% body-weight losses, decreased food consumption, scant/unformed feces, red-stained fur, and/or red perioral/nasal substance. The uterine findings revealed 3 early resorptions and 3 implantation sites (200 mg/kg female) and 15 early resorptions and 15 implantation sites (1000 mg/kg female). Dark red lungs were noted at necropsy for both rats, as well as white foam-like substance in the trachea (1000 mg/kg female only).

Clinical Signs

1x Daily. No effects noted at 50 mg/kg.

At doses \geq 200 mg/kg/day, BMS-790052-related clinical observations in the rats were generally dose-related in incidence. Effects included: scant/absent feces, perivaginal substance (red, brown, or black), perioral/-nasal substance (red and/or brown), red-stained fur (head, jaw, or neck), and urine-stained coat. Peak incidence was generally observed during the 10-day dosing period. Fecal effects were observed primarily during the first week of dosing. Perivaginal substance generally appeared later in gestation and was observed in rats with significant postimplantation loss in most cases. Two rats at 1000 mg/kg/day were described as cachectic (physical loss of body weight and muscle) at unscheduled veterinary examination on GD13 and 14.

Body Weight

Predose, then daily. No effects at 50 mg/kg.

At 200 and 1000 mg/kg: Body weight losses occurred around GD 6-7. By GD 12, maximal weight loss occurred at 200 mg/kg and then rebounded to normal. However, the 1000 mg/kg animals never recovered their body weight loss.

Feed Consumption

2x weekly. No effects at 50 mg/kg.

At 200 and 1000 mg/kg/day, BMS-790052-related reductions in food consumption during the dosing period (28 and 47% less than control values, respectively) with peak reductions noted during the first days of dosing (GD 6 to 9).

Some recovery at 200 and 1000 mg/kg was evident as dosing continued; decrements relative to control values for the interval of GD 6 through GD 16 were 28% and 47%, respectively. There were also increases in food consumption relative to control values at 200 and 1000 mg/kg/day following the end of dosing from Gestation Days 16 to 21 (12 to 16% greater than vehicle controls), corresponding to increased body-weight gains at 200 mg/kg/day.

Toxicokinetics

On GD 15, BMS-790052 exposures (AUC_{0-24h}) were generally greater than dose proportional between 50 and 200 mg/kg/day and less than dose proportional between 200 and 1000 mg/kg/day. AUC_{0-24h} exposures on GD 15 were generally similar to those following the first dose on GD 6.

Table 81 - TK Values for BMS-790052, Study DN08011

Parameter	Gestation Day	BMS-790052		
		50 mg/kg	200 mg/kg	1000 mg/kg
C _{max} (µg/mL)	6	10.3	22.8	55.2
	15	7.26	24.8	51.6
AUC(0-24 h) (µg•h/mL)	6	81.1	364	930
	15	70.1	375	788

Dosing Solution Analysis

The dosing solution was analyzed and met acceptance criteria. Vehicle controls were absent of test article.

Necropsy

No significant effects on the dams at 50 mg/kg. Enlarged adrenals were noted at 200 and 1000 mg/kg (noted in prior repeat-dose studies). Also, Enlarged spleen was noted in 11/22 rats at 1000 mg/kg.

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

50 mg/kg: No effects noted.

200 and 1000 mg/kg/day: reductions in group mean fetal body weights relative to control values (5 to 34% less than controls, respectively). The fetal body weights at 200 mg/kg/day were not considered adverse.

1000 mg/kg only: increased embryoletality with associated decreases in litter size (postimplantation loss of 88% relative to 8% in controls; 71% of dams with no viable conceptuses relative to 0% in controls).

Offspring (Malformations, Variations, etc.)

50 mg/kg: No effects noted.

200 and 1000 mg/kg/day: fetal malformations of small and misshapen cerebrum, dilated cerebral ventricles, shortened lower jaw, supernumerary phalanges (hindlimbs), misshapen and/or fused sternebrae, and small eye sockets. Additional variations noted at 200 and 1000 mg/kg/day were limited to the skull and included incomplete ossification of the parietals and frontals; enlarged fontanel (anterior and/or posterior); and supernumerary bone in the nasals/frontals.

1000 mg/kg only: additional fetal external, visceral, and skeletal malformations were noted, particularly in the head/skull. External and visceral findings included absent or small and malpositioned eyes (with associated visceral findings: small, displaced, and/or malpositioned eyes and skeletal findings: small eye socket); dilated olfactory bulbs; imperforate or absent nasal openings (with associated visceral finding narrow nasal turbinates); exencephaly; shortened upper jaw; cleft lip and palate; and polydactyly of forelimb and hindlimb (with associated skeletal findings of supernumerary phalanges). Additional skeletal malformations in the skull (short nasals, short premaxillae, fused nasals and premaxillae, and misshapen tympanic annuli); pectoral girdle (misshapen clavicae and scapulae); sternebrae (malpositioned and/or cleaved); vertebrae (small thoracic arches and misshapen thoracic centra); and ribs (fused). Further skeletal variations representing incomplete ossification of skull (including nasals, premaxillae, squamosals, interparietals, supraoccipitals, and zygomas); sternebrae; vertebrae (caudal, thoracic, lumbar, and sacral centra); pubes; and forepaws/hindpaws (including metacarpals, metatarsals, and phalanges).

9.3 Prenatal and Postnatal Development

The following study was reviewed by Dr. Mark Seaton, PhD.

Study title: BMS-790052: Oral Study of Pre- and Postnatal Development in Rats

Study no.:	Test Facility Study No. 902465 BMS Reference No. DN12003
Study report location:	EDR
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	03 January 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BMS-790052 (BMS-790052-05_B) Batch (Lot) Number 9M38078 Description White powder "As is" purity 90%

Key Study Findings

- BMS-790052 produced changes in the offspring (increased stillborn pups, decreased body weight) at the maternally toxic dose of 100 mg/kg/day.
- The no-observed-adverse-effect level (NOAEL) for both maternal and developmental toxicity in rats was 50 mg/kg/day (maternal AUC value of 39.5 µg·h/mL).

Methods

Doses:	0, 25, 50 100 mg/kg
Frequency of dosing:	Once daily on GD 6 through LD 20
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Suspension; 15% polyethylene glycol 400, 5% polyvinyl pyrrolidone K-30, 5% Vitamin E d-α-tocopheryl polyethylene glycol 1000 succinate, and 75% 0.1M phosphoric acid buffer, pH~3
Species/Strain:	Rat/Crl:CD(SD)
Number/Sex/Group:	25
Satellite groups:	Toxicokinetic
Study design:	See below

BEST AVAILABLE COPY

Group No. Identification	Dose Level (mg/kg/day)	Concentration (mg/mL)	Dose Volume (mL/kg/day)	F0-Female Identification (Main Study / Satellite)
1/ Vehicle control	0	0	10	151-175 / 176-179
2/ BMS-790052	25	2.5	10	251-275 / 276-283
3/ BMS-790052	50	5	10	351-375 / 376-383
4/ BMS-790052	100	10	10	451-475 / 476-483

Terminal Procedures - F₁ Generation (Birth to PND 21)

Group Number	Targeted Number of Litters	Scheduled Euthanasia Day	Necropsy Procedures			Histology/ Histopathology
			Necropsy	Tissue Collection	Organ Weights	
Pups Culled on PND 4 ^{a,b}		PND 4	-	-	-	-
1	4 ^a	PND 10	-	-	-	-
2	8 ^a					
3	8 ^a					
4	8 ^a					
1	c	PND 21	X	X	-	-
2	25 ^b					
3	25 ^b					
4	25 ^b					
Unscheduled Death/Euthanasia			X	X	-	-

X = Procedure to be conducted; - = Not applicable; PND = Postnatal Day

^a Satellite litters

^b Main study litters

^c Pups not selected for study continuation or transferred to PCS-MTL Study 902466 (BMS Study DN12004)

Terminal Procedures - F₁ Generation (PND 22 and onward)

Group Number	Targeted Number of Rats	Scheduled Euthanasia Day	Necropsy Procedures			Histology/ Histopathology	
			Ovarian/ Uterine Examination	Necropsy	Tissue Collection		Organ Weights
1	25 F	GD 15	X	X	X	X	-
2	25 F						
3	25 F						
4	25 F						
F: Mating undetected but pregnant ^a			X	X	X	-	-
F: Unscheduled Death/Euthanasia			X ^b	X	X	-	-
1	25 M	c	-	X	X	X	-
2	25 M						
3	25 M						
4	25 M						
M: Unscheduled Death/Euthanasia			-	X	X	-	-

X = Procedure to be conducted; - = Not applicable; GD = Gestation Day; F = Female; M = Male

^a Females without a confirmed mating date may be euthanized when abdominal palpation indicates pregnancy.

^b Performed (as per Section 21.4.1) when unscheduled death/euthanasia occurs after initiation of cohabitation

^c After pregnancy status of the F₁ females is confirmed

Observations and Results

Criteria for evaluation in the dams (F0 generation) included survival, clinical signs, body weight, food consumption, gestation length, parturition observations, maternal behavior, adrenal weights, and gross pathology. Offspring of these dams (F1 generation) were

evaluated for viability, clinical signs, body weight, food consumption, sexual maturation, motor activity, auditory startle habituation, water maze learning and retention, estrous cyclicity, reproductive capacity, organ weights, and gross pathology. Additionally, BMS-790052 maternal plasma (for toxicokinetics) and milk concentrations as well as pup plasma concentrations were measured on LD 10 in corresponding satellite groups of 4 to 10 dams/litters at each dose level.

F0 Dams

Survival: BMS-790052 treatment was associated with death of 1 dam at 100 mg/kg/day. The dam was found dead during parturition on Gestation Day (GD) 21. Necropsy revealed enlarged adrenals and a dark foci in the thymus.

One dam at 100 mg/kg/day had no surviving pups and was euthanatized on Lactation Day (LD) 1 as required by the protocol. Gross changes in adrenals (enlargement and pale discoloration) and thymus (small) were identified at necropsy of this dam.

Clinical signs: Increased incidences of red-stained fur were observed at all doses during gestation and lactation. Additional drug-related clinical signs included wet fur at ≥ 50 mg/kg/day during gestation and lactation, and salivation with suspected dehydration in a few dams at 100 mg/kg/day during gestation.

Body weight: BMS-790052 treatment at ≤ 50 mg/kg/day had no effects on gestation or lactation body weights. At 100 mg/kg/day, decreased body-weight gains (including a transient body weight loss after the initial dose) were noted in dams during the first 6 days of dosing (GDs 6 to 12). As a result, maternal body weights were decreased relative to controls during gestation, with maximum decrements of 7% on GDs 12 to 14. No effects on maternal body weights were observed during the lactation period.

Feed consumption: BMS-790052 treatment at ≤ 50 mg/kg/day had no effects on gestation or lactation food consumption. At 100 mg/kg/day, persistent reductions in food consumption (16% to 30% less than controls), correlated with decreased maternal weight gains, were seen during the first 8 days of dosing (GDs 6 to 14).

Uterine content: In litters of dams treated at 100 mg/kg/day, there were increases in stillbirths (dead pups at birth; 0.8% per litter compared to 0% in control) with associated decreases in live birth index (87.3% compared to 91.3% in control).

Necropsy observation: At 50 and 100 mg/kg/day, there were increased adrenal weights, absolute (1.16× and 1.54× control, respectively) and relative to body weight (1.15× and 1.56×control, respectively).

Table: Summary of BMS-790052-Related Gross Findings in F0 Females

BMS-790052 Dose (mg/kg/day):	0	25	50	100
No. of F0 Females Evaluated:	25	25	26 ^a	25 ^b
Adrenal:				
Pale discoloration	0	1	1	7
Enlargement ^c	1	2	4	21 ^b

^a Includes one non-pregnant TK satellite female (No. 381) euthanatized on Postcoital Day 25.

^b Includes one non-pregnant female (No. 464) euthanatized on Postcoital Day 25.

^c Bilateral adrenal enlargement, except for one female at 25 mg/kg/day (No. 251) whose left adrenal was enlarged but right adrenal was small.

Toxicokinetics:

Table 82 - Toxicokinetic Summary in Dams (LD 10)

Parameter	BMS-790052 Doses (mg/kg/day)		
	25	50	100
C _{max} (ng/mL)	3,360	4,890	8,930
AUC(0-24h) (ng·h/mL)	21,900	39,500	71,300
T _{max} (h)	1.0	1.0	1.0

Table 83 - BMS-790052 in Maternal Plasma and Milk and Pup Plasma at 2 Hours Postdose on LD 10

Dose (mg/kg/day)	Concentrations of BMS-790052 (ng/mL)				Mean BMS-790052 Concentration Ratios		
	Plasma			Dam Milk	Pup Plasma/Dam Plasma	Maternal Milk/ Plasma	
	Male Pups	Female Pups	Combined Sex Pup				
25	46.3	48.5	47.4	2,740	5,350	0.02	2.0
50	119	112	116	4,790	8,720	0.02	1.8
100	305	302	304	8,880	15,100	0.03	1.7

Dosing Solution

Analysis For content verification, all samples met acceptance criteria (individual concentrations were within 100 to 120% of each other and the mean concentration was within 90 to 110% of the intended concentration).

The absence of BMS-790052 in the vehicle prepared for use on First Preparation and Last Preparation was also confirmed (0 mg/mL) or the result was below the lower limit quantification for the test article.

F₁ Generation

Survival: Increased pup mortality occurred at 100 mg/kg/day during the first 4 postnatal days. As a result, the litter size (11.1 pups) and viability index (91.2%) on PND 4 in F1 generation at 100 mg/kg/day were decreased compared to controls (12.4 and 98.9%, respectively).

Clinical signs: At 100 mg/kg/day, there were decreased activity, decreased respiratory rate, labored breathing, lying on side, and empty stomach. These signs were observed on PND 0 and restricted to litters with neonatal pup mortality

Body weight: Body weights were reduced in pups at 100 mg/kg/day during the preweaning period ($\geq 8\%$ less than controls).

At 100 mg/kg/day, bodyweight gains were decreased relative to controls during the first 6 postweaning weeks (PNDs 21 to 56). These changes were a continuation of body-weight effect seen prior to weaning. As a result, body weights were 5 to 10% lower than controls from PND 21 to scheduled necropsy (PND 118) in males and to the end of pre-mating period (PND 87) in females. No effects on body-weight parameters were evident in F1 females during the gestation period.

Feed consumption: BMS-790052 had no effects on food consumption in F1-generation rats at any dose tested.

Physical development: There were no BMS-790052-related changes in the age of sexual maturation (preputial separation in males and vaginal patency in females) in F1 generation at any dose tested.

There were no BMS-790052-related changes in reproductive organ weights in F1-generation males. All male reproductive organ weights (absolute and relative) were comparable between control and BMS-790052-treated groups.

Neurological assessment: There were no BMS-790052-related effects on motor activity, acoustic startle habituation, or water maze learning/retention.

Reproduction: There were no BMS-790052-related effects on estrous cyclicity, mating, or fertility in F1-generation females. BMS-790052 had no effect on caesarean-section parameters in F1-generation females or their litters. Values for corpora lutea, implantation sites, live/dead embryos, and resorptions (including pre- and post-implantation losses) were comparable for all groups.

10 Special Toxicology Studies

Study title: ORAL TOXICOKINETIC AND TOLERABILITY STUDY IN JUVENILE RATS

Study no.:	DN11038
Study report location:	EDR
Conducting laboratory and location:	BMS
Date of study initiation:	13-June-2011
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	BMS-790052, batch 7M23810, purity 89.7%.

Key Study Findings

This study was conducted as a range finding study in juvenile rats.

BMS-790052 was administered by oral gavage to CrI:CD(SD) rats (9/sex/group) from Postnatal Days (PNDs) 21 to 42 at doses of 0 (vehicle), 25, 75, or 150 mg/kg/day. All doses were administered at 10 mL/kg in a vehicle

In general, increases in BMS-790052 AUC values were greater than dose-proportional between 25 and 75 mg/kg/day, but dose-proportional between 75 to 150 mg/kg/day. There were no sex differences in AUC values. However, AUC values on PND 35 were

0.2 to 0.6× those on PND 21, which likely reflected maturation of metabolic capacity with development.

Table 84 - TK in Juvenile Rats, Study DN11038

AUC(0-24h) ($\mu\text{g}\cdot\text{h}/\text{mL}$)	BMS-790052 Dose					
	25 mg/kg/day		75 mg/kg/day		150 mg/kg/day	
	M	F	M	F	M	F
PND 21	39	21.4 ^b	81.6	91.3	203	181
PND 35 ^a	7.57 ^b	8.27	46.9	54.3	111	111

a - The combined-sex AUC values at 25, 75, and 150 mg/kg/day were 7.92, 50.6, and 111 $\mu\text{g}\cdot\text{h}/\text{mL}$, respectively.

b - Value of AUC(0-8h) because BMS-790052 concentrations in samples collected at 24 hours postdose were below the detectable level of the bioanalytical assay.

BMS-790052 was clinically tolerated by juvenile rats at doses \leq 150 mg/kg/day (combined-sex AUC 111 $\mu\text{g}\cdot\text{h}/\text{mL}$) for approximately 3 weeks. There were no novel toxicities, and the profile of BMS-790052-related changes in juvenile rats was similar to that observed previously in adult rats.

Study title: BMS-790052: Ten-Week Oral Toxicity Study in Juvenile Rats with a One-month Recovery

Study no.: DN12004
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: 20 Jan 2012
 GLP compliance: Yes
 QA statement: Yes

Key Study Findings

This was the definitive study in juvenile rats dosed 10 weeks with BMS-790052. Rats were dosed daily by oral gavage at 0, 25, 50 or 100 mg/kg from PND 21-90.

BMS-790052 was clinically well-tolerated by juvenile rats at oral doses up to 100 mg/kg (combined-sex AUC 117.9 $\mu\text{g}\cdot\text{h}/\text{mL}$) for 10 weeks. There were no novel toxicities, and the profile of BMS-790052-related changes in juvenile rats was similar to that observed previously in adult rats. Adrenal hypertrophy/enlargement was noted at 100 mg/kg.

Based on adrenal hypertrophy/enlargement at 100 mg/kg, the no-observed-adverse-effect-level (NOAEL) for juvenile rats was considered to be 50 mg/kg/day. The combined-sex mean AUC at this dose (46.5 $\mu\text{g}\cdot\text{h}/\text{mL}$) was 2.8× to 9.2× those achieved at the NOAEL in adult rats treated with BMS-790052 for 1 or 6 months (5.1 and 16.4 $\mu\text{g}\cdot\text{h}/\text{mL}$, respectively).

Methods

Doses: 0, 25, 50 100 mg/kg
 Frequency of dosing: Once daily on GD 6 through LD 20
 Dose volume: 10 mL/kg
 Route of administration: Oral gavage
 Formulation/Vehicle: Suspension; 15% polyethylene glycol 400, 5% polyvinyl pyrrolidone K-30, 5% Vitamin E d- α -tocopheryl polyethylene glycol 1000 succinate, and 75% 0.1M phosphoric acid buffer, pH~3
 Species/Strain: Rat/Crl:CD(SD)
 Number/Sex/Group: 30/sex/group – divided into 2 subsets (see below)
 Study design: 2 subsets of rats. See table below.

Table 85 - Study Design (Juvenile Toxicology Study)

Group No.	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Concentration (mg/mL)	No. of F ₁ -Generation Rats Assigned			
				Subset 1 ^a		Subset 2 ^b	
				M	F	M	F
1/ Vehicle Control	0	10	0	15	15	15	15
2/ BMS-790052	25	10	2.5	15	15	15	15
3/ BMS-790052	50	10	5	15	15	15	15
4/ BMS-790052	100	10	10	15	15	15	15

^a Subset-1 rats were evaluated primarily for clinical/anatomic pathology, TK, and sperm parameters. These animals were euthanized at the end of the dosing period on PND 91 (10 rats/sex/group) or at the end of the recovery period on PND 120 (5 rats/sex/group).

^b Subset-2 rats were evaluated primarily for reproductive parameters. These animals were euthanized on GD 15 (females) or after pregnancy status of the females was confirmed (males).

Viability

There was no BMS-790052-related mortality during the course of this study. Six study animals did not survive to scheduled termination but the deaths were not related to drug exposure. These deaths were related to technical errors/blood collection, lack of a mate (subsequently euthanized), or unknown (3 animals).

Body Weight

25 or 50 mg/kg: no effects

100 mg/kg: body weights on PND 24 were reduced (relative to controls) by 6% in males and 9% in females. Thereafter, body weights in males generally remained 4% to 6%

lower than controls through the end of the dosing period (PND 91), while body weights in females were comparable to controls by PND 31.

Recovery: No effects noted. Body weight changes were reversible.

Feed Consumption

No effects noted.

Clinical Observations

BMS-790052 administration was associated with clinical signs of wet fur (primarily on the lower jaw and muzzle) in males at all doses and in females at ≥ 50 mg/kg/day. These occurred predominantly during Dosing Weeks 2 to 6 (PNDs 33 to 62) and likely reflected increased salivation. Excessive salivation was noted in both sexes at 100 mg/kg with higher incidences on PNDs 29 to 46. None of these clinical signs were observed following cessation of dosing.

Sexual Maturation, Estrous Cycles, Cohabitation and Mating

25 or 50 mg/kg: No effects on sexual maturation. No other effects noted.

100 mg/kg: Delays in sexual maturation were noted at 100 mg/kg/day, with the mean age of preputial separation occurring in males on PND 43.1 (versus PND 41.7 in male controls) and vaginal patency occurring in females on PND 31.9 (versus PND 31.0 in female controls). No other effects noted.

Clinical Pathology

- Serum Sodium: minimal non-adverse dose-dependent males at 50 and 100 mg/kg/day on PNDs 49 and 91 ($0.97\times$ to $0.99\times$ control mean) and females at 100 mg/kg/day on PND 91 ($0.97\times$ control mean)
- Serum Chloride: minimal non-adverse decreases in mean serum chloride in males dosed at 50 and 100 mg/kg/day on PND 49 ($0.97\times$ to $0.98\times$ control mean) and in females dosed at 100 mg/kg/day on PND 91 ($0.97\times$ control mean)
- Cholesterol: minimal increases in females at 100 mg/kg on PND 49 ($1.41\times$ control) and at 50 and 100 mg/kg on PND 91 ($1.23\times$ to $1.39\times$ control). All values returned to normal after recovery.
- Urine: volume increases in males at 100 mg/kg and females at 50 and 100 mg/kg (similar to adults, the urine volume increases paralleled increase in water consumption). Furthermore, along with urine volume increases, parameters for urine (protein, urine creatinine, urine specific gravity and/or urine osmolality) all indicated a dilution of urine. Urine changes resolved after recovery.
- Corticosterone: increases in corticosterone to creatinine ratio and total corticosterone in both sexes at 50 and 100 mg/kg on PND 49 and 100 mg/kg on PND 91. Corticosterone changes resolved during recovery.

Toxicokinetics**Table 86 - TK Parameters in Dosing Week 10 in Juvenile Rats, Study DN12004**

Parameter	BMS-790052 Doses (mg/kg/day)					
	25		50		100	
	M	F	M	F	M	F
C _{max} (µg/mL)	3.18	3.50	6.10	7.18	15.3	11.8
AUC _{0-24h} (µg•h/mL) ^a	23.3	17.5	50.9	42.0	144	91.8

^a - The combined-sex AUC values at 25, 50, and 100 mg/kg/day were 20.4, 46.5, and 117.9 µg•h/mL, respectively.

Study title: Bovine Corneal Opacity and Permeability Assay with Optional Histology.

Study no.: 07AE50.3500079
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: Jan 8, 2007
 GLP compliance: Yes
 QA statement: Yes

Key Study Findings

This assay investigated the irritation potential in a bovine corneal opacity assay. The scores: 0-25 (mild), 25.1-55 (moderate), 55+ (severe)

BMS-790052 scored 0. Therefore, it was negative in the assay for irritation.

Table 87 - Bovine Opacity Results, BMS-790052

Assay Date	IIVS Test Article Number	Sponsor's Designation	Conc. (w/v)	Exposure Time	Mean Opacity Value	Mean OD ₄₉₀ Value	<i>In Vitro</i> Score	pH
8/8/07	07AE50	BMS-790052-05	20%	4 hours	48.9	-0.003	48.8	3.0

Study title: Local Lymph Node Assay in the Mouse

Study no.: 1212/0348
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: May 21, 2007
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: BMS-790052-05, Batch 003, 95.6% pure (HPLC)

Key Study Findings

This study was performed to assess the skin sensitization potential in CBA/Ca mice following topical application to the dorsal surface of the ear. The positive control was HCA (α -Hexylcinnamaldehyde, 85%).

BMS-790052 scored above an index of 3, and so can be considered a potential sensitizer.

Table 88 - LLNA Results, BMS-790052

Concentration (% w/w) in ethanol/distilled water 7:3	Stimulation Index	Result
10	1.40	Negative
25	1.65	Negative
50	3.43	Positive

Table 89 - LLNA Results, Positive Control (HCA)

Concentration (% v/v) in ethanol/distilled water 7:3	Stimulation Index	Result
5	1.38	Negative
10	2.03	Negative
25	8.04	Positive

Study title: ACUTE DERMAL IRRITATION IN THE RABBIT

Study no.: 1212/0349
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: May 21, 2007
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BMS-790052-05, Batch 003, 95.6% pure (HPLC)

Key Study Findings

The study was performed to assess the irritancy potential of the test material to the skin of the New Zealand White rabbit. 3-minute and 1-hour semi-occluded applications of the test material to the intact skin of one rabbit produced no evidence of skin irritation. A single 4-hour, semi-occluded application of the test material to the intact skin of three rabbits produced no evidence of skin irritation.

BMS-790052 produced a primary irritation index of 0.0 and was classified as a non-irritant to rabbit skin according to the Draize classification scheme.

Study title: NINE-DAY ORAL INVESTIGATIVE TOXICITY STUDY IN DOGS

Study no.: DS07186
Study report location: EDR
Conducting laboratory and location: BMS, Syracuse, NY
Date of study initiation: Oct 12, 2007
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: BMS-790052-05, Batch 7C26990, 90.3% pure (HPLC)

Key Study Findings

The objective of this study was to investigate potential mechanism(s) of liver and bone marrow toxicities associated with BMS-790052 in dogs by determining the kinetics and nature of early clinical pathology, histologic, immunotoxicologic, and hepatic transcriptional changes. BMS-790052 was administered to male dogs (8/group) at oral gavage doses of 0 (vehicle control), 50, or 100 mg/kg/day for either 3 or 9 days. All doses were administered at 5 mL/kg.

Administration of BMS-790052 to dogs for 3 or 9 days at 50 and 100 mg/kg/day (AUC of 75.4 to 99.7 $\mu\text{g}\cdot\text{h}/\text{mL}$) recapitulated many of the previously observed bone-marrow and liver findings observed in dogs with BMS-790052.

Table 90 - Mean Plasma Toxicokinetic Parameters on Day 1, Study DS07186

BMS-790052 (mg/kg/day)	BMS-790052		BMS-805215		BMS-795853	
	C _{max} (µg/mL)	AUC _(0-24 h) (µg•h/mL)	C _{max} (µg/mL)	AUC _(0-T) ^b (µg•h/mL)	C _{max} (µg/mL)	AUC _(0-24 h) (µg•h/mL)
50	4.81	75.4	0.19	2.57	0.70	11.3
100 ^a	6.19	99.7	0.31	4.26	0.94	15.1

^aAnimals 3101, 3102, 3104 and 3108 (in 100 mg/kg/day dose group) had vomitus at 2 hours postdose on Day 1, but these animals were included in the mean toxicokinetic values.

^bFor AUC(0-T), T = 8 or 24 hours postdose.

For all 3 analytes (parent drug and metabolites), absolute plasma, liver, and bile concentrations followed the rank order of plasma < liver << bile, likely reflecting excretion into bile. There was accumulation (group mean concentrations ranging from ~1 to 7×) of parent drug and metabolites in the liver and bile on Day 10 relative to Day 4. Ratios of bile- and liver-to-plasma ratios for parent drug were 247 to 1167-fold and 6 to 12-fold, respectively.

Table 91 - Mean Concentrations in Bile, Liver, and Plasma at Scheduled Necropsy (Days 4 & 10), Study DS07186

Analyte	Study Day	BMS-790052 Dose (mg/kg/day)	Mean Concentration of Analytes (µg/mL Bile or Plasma; µg/g Liver)			Bile-to-Plasma Concentration Ratio	Liver-to-Plasma Concentration Ratio
			Bile	Liver	Plasma		
BMS-790052	4	50	1,907	18.8	1.81	1,167	12
		100	2,304	24.1	3.63	771	7
	10	50	2,406	46.8	6.61	513	6
		100	3,567	81.8	14.72	247	6
BMS-805215	4	50	129	2.3	0.07	5,223	104
		100	132	1.5	0.15	1,642	15
	10	50	298	5.0	0.37	1,462	10
		100	704	11.1	1.16	583	10
BMS-795853	4	50	263	10.5	0.09	3,271	135
		100	389	17.4	0.21	1,827	82
	10	50	375	26.3	0.36	1,417	69
		100	381	28.4	0.61	658	50

Although no mechanistic information could be derived from extensive immunotoxicological and hepatic transcriptional evaluations, clinical pathology changes identified the early onset of both the bone-marrow and liver lesions. At 100 mg/kg/day, decreased peripheral blood cells counts and increased liver-associated enzymes (ALT, AST and/or GDH) and blood biomarkers of acute inflammation (C-Reactive Protein and fibrinogen) all occurred at the earliest intervals evaluated (ie, by Day 2 or 4), while at 50

mg/kg/day, decreased peripheral blood cells only slightly preceded increases in liver-associated serum chemistry changes.

These findings support a simultaneous and independent effect on both target organs early following initiation of BMS-790052 dosing.

Study title: A Three-Month Oral Qualifying Toxicity Study in Rats

Study no.: DM11028
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: 12 May 2011
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: BMS-790052-05, Batch 7M23810, 89.7% pure (HPLC)
 Batch for impurities: (b) (4),
 (b) (4) % pure.

Key Study Findings

The objectives of this study were to determine and compare the toxicity of BMS-790052 spiked [BMS-790052 (+) impurities] with 7 BMS-790052 process impurities:

(b) (4)
 (b) (4)) with the toxicity profile of BMS-790052 containing levels of these impurities [BMS-790052 (-) impurities] similar to those used in previous toxicity studies when given orally to Sprague Dawley rats for a 3-month period.

Table 92 - TK Summary, BMS-790052, Study DM11028

Parameter	Period	BMS-790052 (50 mg/kg/day)			
		(-) Impurities		(+) Impurities ^a	
		M	F	M	F
C _{max} (µg/mL)	Day 1	6.72	10.7	7.68	8.13
	Week 13	5.93	10.8	6.36	9.30
AUC(0-24h) ^b (µg•h/mL)	Day 1	58.3	56.3	60.3	57.4
	Week 13	78.0	84.6	73.1	79.4

^a BMS-790052 was spiked with 7 impurities (As per Certificate of Analysis: (b) (4)
 (b) (4)

^b There were no meaningful differences in BMS-790052 AUC between sexes. AUC values of 76.3, and 81.3 µg•h/mL for the 50 mg/kg/day dose of BMS-790052 (+ and - impurities), respectively, are the combined mean of male and female values at Week 13.

BMS-790052 (+) impurities (combined-sex mean AUC 76.3 $\mu\text{g}\cdot\text{h}/\text{mL}$) and (-) impurities (combined-sex mean AUC 81.3 $\mu\text{g}\cdot\text{h}/\text{mL}$) were clinically well tolerated by rats for 3 months at an oral dose of 50 mg/kg/day. The primary BMS-790052-related findings in both groups were vacuolation and hypertrophy of adrenal cortical cells of the zona fasciculata that correlated with increases in urine corticosterone/creatinine ratio and total corticosterone.

All of the target organ findings were previously known for BMS-790052 in rats and there were no meaningful toxicologic or pathologic differences in these findings in rats administered BMS-790052 (\pm) impurities.

Study title: NEUTRAL RED UPTAKE PHOTOTOXICITY ASSAY OF BMS-790052 IN Balb/c 3T3 MOUSE FIBROBLASTS

Study no.:	DS07109
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	21 June 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BMS-790052-05, Batch 7C26990, 91.3% pure

Key Study Findings

The purpose of this study was to evaluate the phototoxicity potential of BMS-790052 as measured by the relative reduction in viability of Balb/c 3T3 mouse fibroblasts exposed to the test article and light (+UVA), as compared with the viability of fibroblasts exposed only to the test article (-UVA). The positive control was chlorpromazine. The study consisted of a range-finding assay and 2 definitive assays.

The mean photo effect (MPE) value in the Range-Finding Assay was 0.038. The MPE values for the Definitive Assays were 0.127 and 0.143. The positive control (chlorpromazine) was positive in the assays (MPE 0.501 and PIF 32.566). therefore the assays performed as expected.

Based on OECD Guidelines, BMS-790052 was classified as “probably phototoxic”.

Study title: BMS-790052: SINGLE-DOSE ORAL PHOTOTOXICITY STUDY OF THE EYES AND SKIN OF LONG-EVANS PIGMENTED RATS

Study no.: DS07207
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: 30 Nov 2007
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: BMS-790052-05, Batch 7C26990, 91.3% pure

Key Study Findings

The purpose of this study was to determine the potential phototoxic effects of BMS-790052 on the eyes and skin of CrI:LE (Long-Evans) pigmented rats. BMS-790052 was administered as single oral (gavage) doses of 0 (vehicle), 10, 30, or 100 mg/kg to groups of 5 male rats followed by exposure to ultraviolet and visible radiation (UVR) from a xenon lamp (to simulate sunlight). Positive control was 8-methoxypsoralen (8-MOP).

Table 93 - Mean Toxicokinetic Parameters for BMS-790052, Study DS07207

BMS-790052 Dose (mg/kg)	C _{max} (ng/mL)	AUC ^a (ng•h/mL)
0	-	-
10	1110	3860
30	4430	24300
100	14800	107000 ^b or 83300 ^c

a: AUC(0-T), T = 8 or 24 hours

b, c: The 24-hour plasma concentration from Animal 5752 was included^b or excluded^c from the toxicokinetic analysis. The BMS-790052 concentration in the 24-hour plasma sample for this animal was 6 to 10x the concentrations from other animals evaluated at the same timepoint. To evaluate the impact of this potential outlier, the toxicokinetic values were calculated with this sample included and with this sample excluded. The results of both calculations are included in the table above.

As expected, a single oral dose of the positive-control article 8-MOP resulted in time-related appearance of skin reactions and ophthalmological and histopathological findings of the eyes. There were no BMS-790052-related deaths, skin reactions, ophthalmological observations or histopathological observations indicative of phototoxicity following BMS-790052 administration and UVR exposure.

BMS-790052 was considered to be non-phototoxic after a single dose oral administration to male CrI:LE (Long-Evans) pigmented rats at doses up to 100 mg/kg (mean C_{max} and AUC values ≤ 14,800 ng/mL and ≤ 107,000 ng•h/mL, respectively).

Based on the current recommended human dose at 60 mg/day, the 100 mg/kg dose in mice (excluding animal 5752) allows for a ~9 fold safety margin for phototoxicity

11 Integrated Summary and Safety Evaluation

Daclatasvir (DCV) is component (b) (4) for the treatment of Hepatitis C Virus (HCV) infection. All nonclinical studies required to support chronic use have been performed and submitted as a part of the nonclinical assessment for DCV. No significant effects were noted that would preclude approval of DCV as an HCV nonstructural protein 5A (NS5A) inhibitor in an HCV combination drug product.

Safety Pharmacology

Cardiovascular (CV) effects were evaluated by assessing human ether-a-go-go related gene (hERG) currents and cardiac ion channels, and rabbit Purkinje fiber action potentials. CV effects were also analyzed *in vivo* with single doses of DCV in anesthetized rabbits and telemetered dogs. The early telemetry and repeat-dose toxicity evaluations were conducted in dogs because dogs had higher exposure to DCV than monkeys. No significant findings were noted. DCV inhibited hERG potassium currents by 26.2% and 50.6% at 10 and 30 μM , respectively, resulting in a 50% inhibition concentration (IC_{50}) of 29.2 μM (21.6 $\mu\text{g}/\text{mL}$). At 10 μM , DCV inhibited cardiac sodium currents by 50.5% at 1 Hz and by 59.4% at 4 Hz.³¹ In a further assessment, DCV inhibited L-type calcium currents by 31.9% and 42.6% at 10 and 30 μM , respectively. However, DCV had no effect on any of the parameters measured in the Purkinje fiber action potential. Minor effects were noted *in vivo* in rabbits with an exposure margin of 91x compared to the recommended daily human exposure at the C_{max} . No other effects were noted in telemeterized dogs in the repeat dose toxicology study. Therefore, the *in vitro* findings on cardiac channels are not concerning based on the lack of *in vitro* findings on Purkinje fibers, the high exposure margins for rabbits, and the lack of *in vivo* findings in dogs.

No independent CNS safety pharmacology study was conducted. However, endpoints were measured as part of the repeat dose toxicology studies. Furthermore, in studies conducted with pigmented Long Evans (LE) and albino Sprague Dawley (SD) rats receiving ^{14}C -DCV, there was low distribution of DCV to the brain. No effects on the CNS were noted.

No independent respiratory safety pharmacology study was conducted. However, no DCV-related changes in respiratory status (as overt changes in respiratory rate and function) were noted in single-dose studies or repeat-dose studies in any species tested.

Metabolism

DCV was highly protein bound (95.1% to 99.5% in mouse, rat, rabbit, dog, and monkey serum; 95.6% in human serum). Generally, absorption of DCV after oral administration was rapid with a T_{max} of 0.5 to 2.9 hours. The oral bioavailability of DCV was 123% in mice, 50% in rats, 144% in dogs, 38% in monkeys, and 67% in humans. Data from *in vitro* and/or *in vivo* studies indicated that DCV was a substrate for P-gp, but is not a substrate for BCRP. After IV single-dose administration, DCV was more rapidly eliminated in mice (~1 hr) than in rats, dogs, and monkeys, ($t_{1/2}$ of 4.7, 3.9, and 3.7 hours, respectively). Clearance values were 9.3, 14.8, 20.3, and 12.4 mL/min/kg in mice, rats, dogs, and monkeys, respectively. The steady-state volume of distribution for DCV (3.6, 5.4, and 2.2 L/kg in rats, dogs, and monkeys, respectively) was greater than the reported total body water volumes, indicative of extravascular distribution. The blood-to-plasma DCV concentration ratio values, which were similar in humans (0.77 to 0.82) and animals (0.56 to 1.08), suggested that DCV in blood is distributed preferentially into plasma in most species. However, it should be noted that distribution studies indicated that DCV concentrates predominantly in the liver (mice, rats, dogs, and monkeys).

DCV and DCV metabolites (BMS-805215 or BMS-795853) were evaluated *in vitro* for their ability to inhibit ligand interactions with various receptors, enzymes, and ion channels. DCV at 10 μ M inhibited binding (65% inhibition) to the sodium ion channel [site 2], whereas the DCV metabolite, BMS-805215, at 10 μ M did not show any significant binding. DCV did not have any other notable effects. Although DCV inhibited sodium channels, the DCV concentration of 10 μ M is at least 200x the free DCV C_{max} at the recommended daily human exposure, indicating a low potential for adverse effects.

CYP3A4 was the major CYP responsible for the metabolism of DCV and in the formation of BMS-805215. *In vitro* and *in vivo*, biotransformation of DCV was characterized by the production of numerous metabolites (mainly by oxidation). Unchanged parent was the most prominent drug-related circulating components in plasma from humans and mice, rats, dogs, and monkeys. *In vivo* metabolite profiles were qualitatively similar in all species and there were no unique human metabolites. DCV was the predominant radioactive component in plasma in animals (75% to 94%) and humans (97% to 100%). BMS-805215, the lone human circulating metabolite, had metabolite-to-parent ratio of <3% in human plasma, and was present in plasma from all animals.

In vitro results indicated that DCV is a weak, reversible, time-dependent inhibitor of CYP3A. In human hepatocytes, DCV was an inducer of CYP3A4, but it was not an inducer of CYP1A2 and CYP2B6 (based on hepatocyte induction data and results of basic mechanistic static model analysis).

Toxicity Studies

Single dose toxicology was assessed in mice, rats, dogs, and monkeys. Single doses up to 150 mg/kg in dogs and monkeys and up to 1000 mg/kg in mice and rats were well tolerated.

Repeat dose toxicology was assessed in rats, dogs, and monkeys.

Rats

In the 1-month oral toxicity study in rats, DCV was administered at daily doses of 0 (vehicle control), 10, 30, or 100 mg/kg. The NOAEL in this study was 10 mg/kg (AUC 5.07 $\mu\text{g}\cdot\text{h}/\text{mL}$). Target organ effects were limited to reversible adrenal cortical hypertrophy/hyperplasia at 30 and 100 mg/kg/day (AUC ≥ 25.4 $\mu\text{g}\cdot\text{h}/\text{mL}$). Other effects at doses ≥ 30 mg/kg/day included transient decreases in food consumption, slight clinical pathology changes (increases in triglycerides, cholesterol, and TBIL). At 100 mg/kg (AUC 107 $\mu\text{g}\cdot\text{h}/\text{mL}$), adrenal changes were associated with increases in urine corticosterone levels.

In the 6-month oral toxicity study in rats, DCV was administered at daily doses of 0, 12.5, 25, or 50 mg/kg. The NOAEL in this study was 25 mg/kg/day (AUC 16.4 $\mu\text{g}\cdot\text{h}/\text{mL}$). At DCV doses ≥ 12.5 mg/kg/day (AUC ≥ 9.14 $\mu\text{g}\cdot\text{h}/\text{mL}$) DCV related effects included: 1) increases in water consumption and urine volume with indications of urine dilution (decreases in urine specific gravity, creatinine concentration, and osmolality) as well as increases in total corticosterone excretion and corticosterone:creatinine ratio) 2) adrenal effects: increased fine cytoplasmic vacuolation (minimal severity) in the zona fasciculata and as well as increased incidence and severity (minimal to moderate) of angiectasis (lengthening and/or dilation of blood vessels) predominately in females (only 1 male was affected). At 25 and 50 mg/kg, additional findings were noted in the adrenals: absolute adrenal gland weights at 25 mg/kg, AUC ≥ 16.4 $\mu\text{g}\cdot\text{h}/\text{mL}$, minimal adrenal cortical hypertrophy was correlated with increased adrenal gland weight (50 mg/kg/day, AUC 59.4 $\mu\text{g}\cdot\text{h}/\text{mL}$). The adrenal effects were secondary effects and based on the lack of any significant findings were not considered adverse. The adrenal effects were reversible after cessation of dosing.

Dogs

The dog was initially used in a 1 month study as the second nonclinical species (higher AUC in dogs than monkeys). However, the monkey was selected for all other studies as the second species due to metabolic differences (higher levels of BMS-795853 than in either humans or dogs).

In the 1-month oral toxicity study in dogs, DCV was administered at initial doses of 0 (vehicle control), 3, 15, or 100 mg/kg. The NOAEL for the 1 month study was 3 mg/kg/day (AUC 1.80 $\mu\text{g}\cdot\text{h}/\text{mL}$). At 15 or 100 mg/kg (AUC ≥ 26.3 $\mu\text{g}\cdot\text{h}/\text{mL}$) there were splenic and liver effects: dose-related minimal or moderate splenic extramedullary

hematopoiesis (EMH) and hepatic perivascular inflammation, with secondary slight to mild Kupffer-cell hypertrophy/hyperplasia and/or Kupffer-cell pigmentation. Hepatic perivascular inflammation was characterized by accumulation of mononuclear cells (macrophages) and generally fewer neutrophils around central veins and, less commonly, the portal tracts. At 100 mg/kg, there was severe toxicity in the dogs, requiring a dose modification to 50 mg/kg. At the 100/50 mg/kg dose, severe DCV-related findings occurred in the dogs which were sacrificed moribund, as well as some of the surviving animals. These findings included: 1) marked changes in hematologic and clinical chemistry parameters associated with liver and bone marrow toxicity 2) minimal/mild hepatocellular degeneration 3) moderate or marked bone-marrow hypocellularity 4) slight/mild lymphoid depletion in the thymus and/or spleen 5) minimal/slight seminiferous tubule degeneration in the testes with spermatogenic cells in the duct of the epididymus 6) slight/mild prostate gland atrophy and 7) minimal/slight pancreatic acinar cell vacuolation.

Monkeys

Three repeat-dose toxicology studies were performed in monkeys: 1) an exploratory 1 month study (to confirm the findings in the dog study) 2) a 4 month repeat dose study and 3) a 9 month repeat dose study.

In the 1 month exploratory study in monkeys, DCV was administered at 0 (vehicle control), 10, 30, 100, or 300 mg/kg/day. DCV was well tolerated at doses up to 300 mg/kg/day (AUC $\geq 71.5 \mu\text{g}\cdot\text{h}/\text{mL}$). DCV-related findings at 30, 100, or 300 mg/kg (AUC $\geq 13.8 \mu\text{g}\cdot\text{h}/\text{mL}$) were limited to minimal hepatic perivascular inflammation, and the NOEL was 10 mg/kg/day (AUC $1.98 \mu\text{g}\cdot\text{h}/\text{mL}$).

In the 4-month oral toxicity study, monkeys were given DCV at 0 (vehicle control), 15, 50, or 300 mg/kg. DCV was clinically well tolerated and there were no DCV-related deaths. No effects noted at 15 mg/kg. At 50 and 300 mg/kg (AUC $\geq 22.5 \mu\text{g}\cdot\text{h}/\text{mL}$) DCV-related findings included: 1) increases in soft/liquid feces 2) liver effects (minimal/slight bile-duct hyperplasia and Kupffer-cell hyperplasia/hypertrophy and minimal/moderate rarefaction of cytoplasm in centrilobular hepatocytes) 3) adrenal effects (minimal/ marked decreased cytoplasmic vacuolation in the cortical zona fasciculate with correlating dark discoloration) and 4) bone marrow effects (minimal lymphoid hyperplasia). At 300 mg/kg/day (AUC $41.2 \mu\text{g}\cdot\text{h}/\text{mL}$), additional DCV-related findings included: 1) mild decreases in red-blood-cell count, hemoglobin, and hematocrit; 2) mild decreases in albumin (with secondary changes in total protein and globulins); 3) mild to moderate increases in alanine aminotransferase (ALT; 1.76 to 7.33x) and/or aspartate aminotransferase (AST; 1.31 to 2.17x) which correlated with rarefaction of hepatocyte cytoplasm in some monkeys; 4) increased incidence of minimal to slight mononuclear-cell infiltration in centrilobular areas of the liver; and 5) slight cortical hyperplasia in the zona reticularis of the adrenal glands (with correlating enlargement and increases in absolute weight in males). The NOAEL was 15 mg/kg based on the findings at 50 and 300 mg/kg.

In the 9-month oral toxicity study in monkeys, DCV was administered at daily doses of 0 (vehicle control), 15, 30, or 150 mg/kg. There were deaths noted at 150 mg/kg. One high dose male was euthanized due to poor and deteriorating condition on day 28. No deaths were noted at 30 or 15 mg/kg. No effects noted at 15 mg/kg. At 30 and 150 mg/kg ($AUC \geq 11.6 \mu\text{g}\cdot\text{h}/\text{mL}$), DCV-related findings included: 1) increases soft and/or liquid feces 2) adrenal effects (increases in weights -- correlating gross enlargement as well as minimal/marked decreases in cytoplasmic vacuolation in the cortical zona fasciculata with dark discoloration at 150 mg/kg/day) 3) liver effects (minimal/moderate Kupffer-cell hyperplasia/hypertrophy with an increased incidence and severity in females at the high dose of 150 mg/kg. At 150 mg/kg/day ($AUC 38.8 \mu\text{g}\cdot\text{h}/\text{mL}$) other DCV-related findings included: 1) clinical chemistry changes (increased ALT ($\leq 9.8x$) and AST ($\sim 2.7x$) and increased C-reactive protein (CRP; 13.9x) in 1 male) 2) liver effects (minimal/slight bile duct hyperplasia, usually subcapsular; minimal/slight mononuclear cell infiltration in the liver; moderate hepatocellular vacuolation in 1 female). DCV-related effects were mostly reversible during recovery. The exception of 1 female at 30 mg/kg and all monkeys at 150 mg/kg which still had minimal to slight Kupffer cell hypertrophy/hyperplasia after recovery. The NOAEL was 15 mg/kg/day ($AUC 3.26 \mu\text{g}\cdot\text{h}/\text{mL}$) based on the adrenal and liver findings at 30 and 150 mg/kg as well as the death at 150 mg/kg.

Overall the findings in the 4 month and 9 month toxicology studies were similar. No novel findings were noted in the 9 month toxicology study.

Combination Toxicity

Potential toxicologic interactions were assessed for DCV and in combination with ASV in rats, monkeys, and dogs, and with pegIFN γ /RBV in monkeys (monkeys are the relevant species for interferon). The combination toxicology studies were administered at drug concentrations that produced PK effects with little to no toxicity. Therefore, only minor PK interactions were noted. No new toxicities were noted in the combination toxicology studies.

There were mortalities associated with the combined dosing of DCV and pIFN/RBV. However, the deaths were due to technical error (esophageal perforation) during RBV dosing which was then exacerbated by pIFN in all doses (including vehicle). Therefore, the deaths were not related to DCV administration.

Genotoxicity/Carcinogenicity

DCV was not genotoxic as evaluated by the Ames assay, chromosomal aberration assay, as well as an *in vivo* rat micronucleus assay.

A 2-year carcinogenicity study in Sprague Dawley rats was conducted (based on the 6 month toxicology data) at doses of 0 (vehicle control), 5, 15, or 50 mg/kg. There was sufficient survival among all groups at study termination (92 to 94 weeks) to evaluate

the potential carcinogenicity of DCV, and there were no DCV-related effects on the incidence, distribution, or nature of neoplastic changes. DCV was not carcinogenic in the 2 year study in rats.

A 6 month carcinogenicity study in transgenic mice was conducted (based on Tg-rasH2 nontransgenic littermate study for 28 days). DCV was not carcinogenic in Tg-rasH2 mice at doses ≤ 300 mg/kg/day (AUC ≤ 131 $\mu\text{g}\cdot\text{h}/\text{mL}$). There were no DCV-related neoplastic lesions. Nonneoplastic findings were limited to minor increased incidences of splenic extramedullary hematopoiesis in females, a finding consistent with the 28-day range-finding study.

Reproductive Toxicity

No significant adverse effects were observed in a rat fertility study. Rats were dosed 0 (vehicle control), 15, 50, or 200 mg/kg. In a fertility study, male fertility parameters were affected in rats. Mean pre-implantation loss (10.6%) was noted in litters sired by treated males at increased compared to controls (5.6%). Furthermore, in males, reproductive effects (reduced prostate/seminal vesicle weights and minimally increased dysmorphic sperm) were noted only at 200 mg/kg (AUC 290 $\mu\text{g}\cdot\text{h}/\text{mL}$), a dose that produced overt toxicity (decreased food consumption and body weights, and gross changes in the adrenals and stomach). Exposure margins for male fertility parameters was 4.6-fold (rat). Female fertility parameters were not affected with exposure margins at the recommended daily human exposure of 24-fold. DCV caused clinical findings (salivation, wet/stained fur, decreases in food consumption and weight gain) in females at ≥ 15 mg/kg/day (AUC ≥ 8.34 $\mu\text{g}\cdot\text{h}/\text{mL}$) mg/kg with no effects on reproductive function or early embryonic development. The NOAEL for reproductive toxicity was 50 mg/kg/day (AUC 51.8 $\mu\text{g}\cdot\text{h}/\text{mL}$) in males and 200 mg/kg (AUC 267 $\mu\text{g}\cdot\text{h}/\text{mL}$) in females.

In an EFD (embryo-fetal development) study in pregnant rats, DCV was administered at doses of 0 (vehicle control), 50, 200, or 1000 mg/kg during the period of organogenesis (GD 6 to 15). DCV did not exhibit selective teratogenicity at any doses. At doses of 200 and 1000 mg/kg (AUC ≥ 364 $\mu\text{g}\cdot\text{h}/\text{mL}$), DCV induced mortality, adverse clinical signs, body-weight losses, and reduced food consumption in dams. In their offspring, malformations of the fetal brain, skull, eyes, ears, nose, lip, palate, or limbs were observed at doses 200 and 1000 mg/kg. However, these fetal findings were associated with maternal toxicity. Furthermore, the dose of 1000 mg/kg was associated with profound embryoletality and lower fetal body weight. Based on these results, the NOAEL for maternal and developmental toxicity was 50 mg/kg (maternal AUC 70.1 $\mu\text{g}\cdot\text{h}/\text{mL}$).

In pregnant Sprague Dawley rats given oral ^{14}C -DCV, there was rapid distribution of radioactivity into maternal and placental tissues. Radioactivity was detected in the fetal liver only at 4 hours, with a fetal liver-to-maternal plasma ratio of 0.19. In all other fetal tissues examined, radioactivity was either not detected or was BLQ. These results indicate that DCV and/or its metabolites cross the placenta in rats, but the distribution of radioactivity in fetal tissues was limited.

In nursing rats which were exposed to ^{14}C -DCV orally, radioactivity was detected in rat milk at 4 hours after dosing. The milk-to-maternal plasma concentration ratios based on C_{max} and AUC were 1.55 and 1.27, respectively.

In an EFD study in pregnant rabbits, DCV was initially administered at doses of 0 (vehicle control), 40, 200, or 750 mg/kg during the period of organogenesis (GD 6 to 18). However, due to vehicle toxicity after 3 to 6 doses, all dose volumes were reduced by roughly half. The modified DCV doses were 20, 99, and 370 mg/kg, respectively. DCV was not a selective developmental toxicant in rabbits at the modified doses of 20 or 99 mg/kg ($\text{AUC} \leq 1,080 \mu\text{g}\cdot\text{h}/\text{mL}$). At the modified dose of 370 mg/kg, DCV caused morbidity and early sacrifice/death. Mortality at 370 mg/kg included 2 rabbits found dead on GD 11 and GD 12. Thirteen 13 females were euthanatized (moribund) between GD 12 and GD 15. Lastly, 7 does euthanatized (due to profound losses in body weight and food consumption) between GD 12 and GD 15. At the modified dose of 99 mg/kg ($\text{AUC} 1,080 \mu\text{g}\cdot\text{h}/\text{mL}$), maternal toxicity as well as developmental toxicity occurred. Maternal toxicity included: mortality, adverse clinical signs, and severe reductions in body weight and food consumption. Developmental toxicity at 99 mg/kg: increased embryo-fetal lethality, reduced fetal body weights (11%), and increased incidences of fetal malformations of the ribs as well as head and skull. The NOAEL for both maternal and fetal effects was 40/20 mg/kg ($\text{AUC} 245 \mu\text{g}\cdot\text{h}/\text{mL}$).

In a study of pre- and postnatal development in rats, DCV was administered at 0 (vehicle control), 25, 50, or 100 mg/kg from GD 6 through GD 20. DCV was excreted into the milk of lactating rats with concentrations 1.7 to 2x those in maternal plasma. There was neither maternal nor developmental toxicity at 25 or 50 mg/kg ($\text{AUC} \leq 39.5 \mu\text{g}\cdot\text{h}/\text{mL}$). At 100 mg/kg ($\text{AUC} 71.3 \mu\text{g}\cdot\text{h}/\text{mL}$), maternal mortality was observed. The females had dystocia (obstructed labor) as well as slight reductions in offspring viability and birth weight. The NOAEL for both maternal and developmental toxicity in rats was 50 mg/kg (maternal $\text{AUC} 39.5 \mu\text{g}\cdot\text{h}/\text{mL}$).

In a juvenile toxicity study in rats, there were no novel toxicities, and the toxicologic profile in juvenile rats was similar to that observed previously in adult rats. The primary findings were adrenal and liver with an increase in water consumption and urine output. Based on adrenal hypertrophy/enlargement at 100 mg/kg, the no-observed-adverse-effect-level (NOAEL) for juvenile rats was considered to be 50 mg/kg/day ($\text{AUC} 46.5 \mu\text{g}\cdot\text{h}/\text{mL}$).

Local Tolerance and Special Studies

The Sponsor completed several local tolerance studies (ocular irritation, dermal irritation/sensitization, phototoxicity). DCV was negative for irritation, but was positive as a potential sensitizer. Also of note, the phototoxicity evaluation was initially concerning. The initial distribution data suggested that DCV distributed to the uveal tract of Long Evans rats. Further *in vitro* data indicated that DCV absorbed UV, which increased concerns for phototoxicity. However, a follow up phototoxicity study (single oral dose) in Long Evans rats was negative. Furthermore, no ocular or other photo-related toxicity was noted in any of the repeat dose toxicity.

Impurities

The proposed specifications for the drug impurities are acceptable. *Please see Appendix A for review of the impurities by Dr. Mark Powley.*

Conclusion

In summary, the major nonclinical target organs of toxicity were the liver and adrenal gland. No significant findings were associated with the adrenal changes. The liver changes were reversible and monitorable in the clinic.

The safety margins (see table below) were not substantial however, clinical risk has been defined and the toxicities are monitorable.

The overall nonclinical program of DCV was considered adequate to support the safety of DCV 60 mg tablets administered daily.

Table 94 – Exposure Margins for Oral Toxicity Studies.

Species	Study	NOAEL (mg/kg)	AUC (µg*h/ml)	Exposure Multiple AUC*
Rat	4-week	10 mg/kg	5.07	0.5
Rat	6 Month	25 mg/kg	16.4	1.5
Dog	4-week	3 mg/kg	1.80	0.2
Monkey	4-week	10 mg/kg	1.98	0.2
Monkey	4 Month	15 mg/kg	2.31	0.2
Monkey	9 Month	15 mg/kg	3.26	0.3
Rat	Fertility	Paternal 50 mg/kg Maternal 200 mg/kg	51.8 267	5 24
Rabbit	Embryo-fetal	Maternal 40/20 mg/kg Fetal 40/20 mg/kg	245 245	22 22
Rat	Embryo-fetal	Maternal 50 mg/kg Fetal 50 mg/kg	70.1 70.1	6 6

Rat	Peri-, Post-natal	Maternal 50 mg/kg Fetal 50 mg/kg	39.5 39.5	3.5 3.5
Rat	Juvenile	M 50 mg/kg F 50 mg/kg Combined	50.9 42.0 46.5	4.5 4 4
Rat	2-yr Carcino- genicity	M 50 mg/kg F 50 mg/kg	70.2 70.3	6 6
Mouse	6 month Transgenic	M 300 mg/kg F 300 mg/kg	131 131	12 12
Mouse	Phototoxicity	100 mg/kg	107	9

**AUC in human: 60 mg QD in HCV patients (geometric mean pop PK parameters) AUC 11.322 $\mu\text{g}^*\text{h/mL}$*

12 Appendix/Attachments

See Appendix A for Dr. Mark Powley's review of the impurities for NDA 206-843.

Appendix A – QSAR Review by Dr. Mark Powley

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: 206-843

Supporting document/s:	Supporting Document	Sponsor Submission Date	CDER Received Date
	1	2/28/14	2/28/14
	14	6/30/14	6/30/14

Product: Daclatasvir (BMS-790052)

Indication: chronic hepatitis C infection in adults in combination with

(b) (4)

Applicant: Bristol-Myers Squibb Co. (BMS)

Review Division: Division of Antiviral Products

Reviewer: Mark W. Powley, Ph.D.

Supervisor/Team Leader: Hanan Ghantous, Ph.D., DABT

Division Director: Debra B. Birnkrant, M.D

Project Manager: Sohail Mosaddegh, Pharm.D.

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 206-843 are owned by BMS or are data for which BMS has obtained a written right of reference.

Any information or data necessary for approval of NDA 206-843 that BMS does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 206-843.

TABLE OF CONTENTS

1	EXECUTIVE SUMMARY	135
1.1	INTRODUCTION	135
2	QUALIFICATION OF DACLATASVIR DRUG SUBSTANCE.....	135
2.1	IMPURITIES	135
2.1.1	SPECIFIED IMPURITIES.....	135
2.1.2	UNSPECIFIED IMPURITIES.....	137
2.2	RESIDUAL SOLVENTS.....	137
APPENDIX.....		138
	(Q)SAR EVALUATION	138
	<i>IN VITRO</i> REVERSE MUTATION ASSAY IN BACTERIAL CELLS (AMES)	139

Table of Tables

Table 1. Proposed daclatasvir drug substance impurity specifications.....	135
Table 2. Proposed daclatasvir drug substance residual solvent specifications	135
Table 3. Summary of daclatasvir specified impurity general toxicology qualification	136

1 Executive Summary

1.1 Introduction

BMS has submitted an NDA to support the combination therapy of daclatasvir (NS5A replication complex inhibitor) + asunaprevir (HCV NS3 protease inhibitor) for treating chronic hepatitis C (b) (4)

The proposed clinical dose regimen includes 60 mg/day daclatasvir (b) (4)

This review focuses on qualification of daclatasvir impurities and residual solvent. Regulatory decision making utilizes information presented in ICH guidelines M7 “Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk”, Q3A(R2) “Impurities in New Drug Substances”, and Q3C(R5) “Impurities: Guideline for Residual Solvents”.

Overall, the proposed daclatasvir specifications summarized below are considered acceptable from a pharmacology/toxicology perspective.

Table 95. Proposed daclatasvir drug substance impurity specifications

Impurity	Specification
BM (b) (4)	(b) (4) %
BM	%
BM	%
BM	%
BM	%
BM	%
individual unspecified impurities	%

Table 96. Proposed daclatasvir drug substance residual solvent specifications

Solvent	Specification
	(b) (4)

2 Qualification of Daclatasvir Drug Substance

2.1 Impurities

2.1.1 Specified Impurities

Because the proposed specifications for BM (b) (4), BM (b) (4), and (b) (4) exceed the ICH Q3A(R2) qualification threshold (i.e., 0.15%), data was submitted to address both genetic toxicology and general toxicology.

General Toxicology – Specified impurities were present in the drug lots tested in 3-month and 6-month studies in rats (Study no. DM11028 and DS08002; see detailed pharmacology/toxicology review by Dr. Peyton Myers). The qualified level of BM (b)(4) is established using the NOAEL from the 6-month study. The remaining impurities are qualified based on a 3-month study in rats using daclatasvir spiked with BM (b)(4), BM (b)(4), BM (b)(4), BM (b)(4), and BM (b)(4). As results for 50 mg/kg/day of daclatasvir spiked with impurities are similar to 50 mg/kg/day daclatasvir alone, this dose is used to calculate qualified levels. Overall, the qualified levels of impurities summarized in Table 3 are deemed adequate to support the proposed specifications.

Table 97. Summary of daclatasvir specified impurity general toxicology qualification

Solvent	Study	Toxicology Study Content	Non-Clinical Dose	Qualified Level ^a	Proposed Specification
BM (b)(4)	6-month rat (DS08002)	(b)(4)%	25 mg/kg/day ^b	(b)(4)%	(b)(4)%
BM (b)(4)	3-month rat (DM11028)	%	50 mg/kg/day ^c	%	%
BM (b)(4)	3-month rat (DM11028)	%	50 mg/kg/day ^c	%	%
BM (b)(4)	3-month rat (DM11028)	%	50 mg/kg/day ^c	%	%
BM (b)(4)	3-month rat (DM11028)	%	50 mg/kg/day ^c	%	%
BM (b)(4)	3-month rat (DM11028)	%	50 mg/kg/day ^c	%	%

^a qualified level = (% impurity x non-clinical dose) / (body surface area conversion factor x maximum clinical dose)

^b NOAEL

^c only dose tested; no differences in toxicity profile for daclatasvir spiked with impurity vs. daclatasvir alone

Mutagenicity – BM (b)(4) is a daclatasvir diastereomer and therefore qualified by the Ames negative API. An Ames assay was also conducted using daclatasvir spiked with impurities including BM (b)(4), BM (b)(4), BM (b)(4), BM (b)(4), BM (b)(4), and BM (b)(4) (Study no. 964256; see detailed pharmacology/toxicology review by Dr. Peyton Myers). The assay results indicate that daclatasvir spiked with (b)(4)/plate of each impurity was not mutagenic. Note that while (b)(4) demonstrated that (b)(4)% of Ames positive compounds are detectable at doses (b)(4)/plate, the authors discuss potential confounding for testing API + spiked impurity vs. testing of neat impurity.

An additional evaluation of mutagenicity was performed using (Q)SAR. The Sponsor's (Q)SAR assessment relies on predictions from an expert rule-based system (i.e., Derek for Windows or Derek Nexus). Although ICH M7 recommends the use of both expert rule-based and statistically-based systems, Sponsors do not have to use 2 systems during the guideline implementation period. Therefore, the assessment is consistent with regulatory recommendations provided in the guideline. Results from the (Q)SAR evaluations are provided in the Appendix.

Based on the totality of evidence provided, the specified impurities are deemed to lack mutagenic potential.

(b)(4)

2.1.2 Unspecified Impurities

(Q)SAR was also used to evaluate the mutagenic potential of a number of impurities unspecified in the drug substance. The Sponsor describes the structures analyzed as "... starting materials, reagents, intermediates, and identified impurities of the drug substance ..." Results from the (Q)SAR evaluations are provided in the Appendix.

The (Q)SAR analysis identified [REDACTED] (b)(4) as potentially mutagenic based on the presence of an [REDACTED] (b)(4) group (i.e., primary [REDACTED] (b)(4)). The Sponsor tested the impurity in an empirical Ames assay and determined it is non-mutagenic. A review of this study is included in the Appendix. No additional potentially mutagenic impurities were identified by the (Q)SAR evaluation.

The known mutagenic and/or carcinogenic chemicals [REDACTED] (b)(4) are also reported by the Sponsor. Each of these impurities are effectively purged (see detailed CMC review by Dr. Chunchun Zhang) and do not pose substantial risk [e.g., potential exposures below the appropriate threshold of toxicologic concern (TTC) described in ICH M7].

The following comment was sent to the Sponsor on July 29, 2014:

[REDACTED] (b)(4) appears to be effectively purged from the daclatasvir drug substance; however, please note that [REDACTED] (b)(4) is mutagenic and not considered a routine impurity under ICH Q3A(R2). If necessary to support a proposed specification in future applications, results of in vivo genotoxicity testing with [REDACTED] (b)(4) could be used to justify limits exceeding the default TTC described in ICH M7. This comment is for your information and requires no additional follow-up.

2.2 Residual Solvents

The proposed specification for [REDACTED] (b)(4) (i.e., [REDACTED] (b)(4) %) is consistent with the recommended Option (b)(4) limit for [REDACTED] (b)(4) described in ICH Q3C [REDACTED] (b)(4) (i.e., [REDACTED] (b)(4) %).

Appendix

(Q)SAR Evaluation

Evaluation of mutagenic potential was performed by BMS using Derek for Windows (DfW; v8.0.0 and v12.0.0) and Derek Nexus (DX; v3.0.1).

The (Q)SAR analysis identified a mutagenic structural alert for [REDACTED] (b) (4) agent – primary [REDACTED] (b) (4). No additional potentially mutagenic impurities were identified by the (Q)SAR evaluation. Detailed results are included in the following table (table taken from the Sponsor submission).

[REDACTED TABLE]



(b) (4)

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

Title: KAT-004286: Bacterial Reverse Mutation Assay (Study no. 1165/072331)

Study report location: EDR
Conducting laboratory and location: [redacted] (b) (4)
Date of study initiation: January 25, 2007
GLP compliance: yes
QA statement: yes
Test article, batch #, and % purity: [redacted] (b) (4), batch#0611335030, purity = [redacted] (b) (4)%

Key Findings

- [redacted] (b) (4) was negative in the bacterial reverse mutation assay.

Summary - Mutagenicity was evaluated in *Salmonella* strains TA98, TA100, TA1535, and TA1537 as well as *E. coli*. strain WP2 *uvrA* at doses $\leq 5000 \mu\text{g}/\text{plate}$ [redacted] (b) (4) with or without metabolic activation. There were no drug-related increases in mean revertants/plate.

Methods

Strains: TA98, TA100, TA1535, TA1537, and WP2 *uvrA* (pKM101)
 Concentrations in test #1: 5, 15, 50, 150, 500, 1500, and 5000 µg/plate
 Concentrations in test #2: 15, 50, 150, 500, 1500, and 5000 µg/plate
 Basis of concentration selection: limit dose of 5000 µg/plate
 Vehicle/Negative control: 1-methyl-2-pyrrolidinone
 Positive control:

Strain	S9	Control	Dose
TA98	-	2-nitrofluorene	2 µg/plate
	+	benzo[a]pyrene	5 µg/plate
TA100	-	sodium azide	2 µg/plate
	+	2-aminoanthracene	5 µg/plate
TA1535	-	sodium azide	2 µg/plate
	+	2-aminoanthracene	5 µg/plate
TA1537	-	9-aminoacridine	50 µg/plate
	+	benzo[a]pyrene	5 µg/plate
WP2 <i>uvrA</i> (pKM101)	-	4-nitroquinoline-1-oxide	2 µg/plate
	+	2-aminoanthracene	10 µg/plate

Formulation/Vehicle: 1-methyl-2-pyrrolidinone
 Incubation & sampling time: Plates were incubated for ~72 hr at 37 °C with or without metabolic activation (plate incorporation method). The metabolic activation system included S9 fraction from phenobarbital and 5,6-benzoflavone induced male Sprague-Dawley rat liver (10% in test 1; 20% in test 2).

Study Validity and Positive Response Criteria

- Spontaneous Revertants – The mean number of vehicle control revertant colonies must lie within or close to the 99% confidence limits of the historical control range.
- Titers – Tester strain cultures must contain $\geq 10^9$ cells/mL.
- Positive Control Values - Positive control articles must produce $\geq 2x$ increase in the revertant colony numbers of $\geq 2x$ [TA98, TA100, and WP2 *uvrA*(pKM101)] or $\geq 3x$ (TA1535 and TA1537) the concurrent vehicle control.
- Positive Response – The test article must produce a dose-related increase in the revertant colony numbers of $\geq 2x$ [TA98, TA100, and WP2 *uvrA*(pKM101)] or $\geq 3x$ (TA1535 and TA1537) the concurrent vehicle control.

Results

There were no test article-related increases in mean revertants/plate. Precipitate was observed in all experiments at doses ≥ 1500 µg/plate without metabolic activation. There was no evidence of toxicity in any experiment. The laboratory's criteria for a valid study were met.

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

/s/

LAINÉ P MYERS
08/27/2014

HANAN N GHANTOUS
08/28/2014

I concur with Dr. Peyton Myers on his recommendation for approval.

Comments on NDA 206843 daclatasvir

From A Jacobs

Date: 8/12/14

1. I concur that there are no outstanding pharm-tox issues for approval.
2. I concur that a black box pregnancy warning is warranted.
3. I have conveyed some minor comments to the reviewer and he will address them as appropriate.

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

/s/

ABIGAIL C JACOBS
08/12/2014