

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:
21-986 & 22-072

PHARMACOLOGY REVIEW(S)

MEMORANDUM

June 28, 2006

TO: File

FROM: Kenneth L. Hastings, Dr.P.H., D.A.B.T.

SUBJECT: NDA 22-072

I concur with the primary reviewer, Dr. Haleh Saber, and the pharmacology/toxicology supervisor, Dr. David E. Morse, that the marketing application for SPRYCEL[®] (dasatinib) is approvable based on review of submitted nonclinical data. The proposed product label is adequate. Concerning the issue of the apparently unique human metabolite of dasatinib (M20: 4-OH-chloromethylphenyl dasatinib), although present in significant quantity (13% of administered dose), no further toxicology studies are needed given the serious nature of the intended indication.

Kenneth L. Hastings, Dr.P.H., D.A.B.T.
Associate Director
Office of New Drugs

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this page is the manifestation of the electronic signature.**

/s/

Kenneth Hastings
6/28/2006 02:37:33 PM
PHARMACOLOGIST



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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 21-986 and 22-072
SERIAL NUMBER: 000
DATE RECEIVED BY CENTER: December 9, 2005
PRODUCT: SPRYCEL™ (dasatinib)
INTENDED CLINICAL POPULATION: Chronic Myeloid Leukemia (CML) and Philadelphia chromosome positive (PH+) Acute Lymphoblastic Leukemia (ALL)
SPONSOR: Bristol-Myers Squibb
DOCUMENTS REVIEWED: Non-clinical studies
REVIEW DIVISION: Division of Drug Oncology Products
PHARM/TOX REVIEWER: Haleh Saber, Ph.D.
PHARM/TOX SUPERVISOR: David E. Morse, Ph.D.
DIVISION DIRECTOR: Robert Justice, M.D.
PROJECT MANAGER: Amy Baird, Consumer Safety Officer

Date of review submission to Division File System (DFS): 6/27/06

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EXECUTIVE SUMMARY

I. Recommendations

- A. Recommendation on approvability: Based on the non-clinical studies reviewed, dasatinib is approvable for the proposed indication.
- B. Recommendation for nonclinical studies: In human, metabolite M20 (4-OH-chloromethylphenyl dasatinib) was detected in significant amount (13%) in the plasma. Rats had no detectable amounts of M20 in the plasma. In monkeys, the plasma level of M20 was 2.8% of radioactivity. Therefore, toxicology studies conducted may not have adequately assessed toxicities of M20 in humans. Additional non-clinical studies with unique human metabolites are not required at this time due to the life threatening conditions of the patients. Additional studies may be required if dasatinib is developed for other indications.
- C. Recommendations on labeling: Suggestions on the labeling are contained in a separate review.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

For detailed information see the relevant sections of this review. Also see sections 2.6.2.4.1 “Results of Safety Pharmacology Studies”, 2.6.4.1 “Pharmacokinetics/Toxicokinetics Brief Summary”, and 2.6.6.1 “Overall Toxicology Summary” for information on concentrations/ doses/ exposures at which effects were observed.

Safety Pharmacology:

Cardiovascular effects:

Based on the in vitro hERG and rabbit Purkinje fiber assays, dasatinib has the potential to cause QT prolongation. QT prolongation was not observed in the EKG evaluations in the safety pharmacology or toxicology studies conducted in monkeys. There was a tendency for increased systolic, diastolic, and arterial blood pressure in monkeys at single doses of ≥ 10 mg/kg (120 mg/m²). Cardiovascular findings in the toxicology studies included: vascular and cardiac fibrosis, cardiac hypertrophy, myocardial necrosis, hemorrhage of the valves, ventricle, and atrium, and cardiac inflammation.

Safety pharmacology studies to determine acute toxic effects of dasatinib on CNS, respiratory system, and GI tract motility were not performed.

Pharmacokinetics:

After oral administration, dasatinib was generally rapidly absorbed, with a T_{max} of 1-2 hrs in the single dose studies in rats and monkeys and in a repeat-dose study in monkeys. Larger variations were reported in rats in the repeat-dose studies, with a T_{max} of 2-8 hrs.

Maximum plasma concentrations (C_{max}) of dasatinib were generally observed between 0.5 and 6 hours (T_{max}) following oral administration in humans. The parent drug and the metabolites are distributed to several tissues, mainly by 4 hrs post-dose. Dasatinib is highly metabolized. Only involvement of CYP3A4 has been verified in vivo, which appears to play a major role in human metabolism. Elimination of dasatinib takes place mainly in the first 48 hrs post-dose. Elimination is mostly hepatic/biliary.

Acute toxicology:

Single doses of dasatinib in rats and monkeys resulted in toxicities in the GI tract (including hemorrhage and edema), lymphocytic/ hematopoietic system (e.g. lymphoid and bone marrow depletion), liver (e.g. \uparrow AST and ALT, single cell necrosis, hypertrophy), and kidneys (e.g. tubular dilatation, tubular epithelial vacuolation, \uparrow urinary RBC and WBC). Cardiotoxicity was evident in rats, presenting as ventricular necrosis, valvular/ ventricular/ atrial hemorrhage (at ≥ 100 mg/kg, 600 mg/m²), and cardiac hypertrophy (mainly in males, at ≥ 30 mg/kg, 180 mg/m²). There was a tendency for increased systolic and diastolic blood pressure in monkeys.

Thrombocytopenia was seen in rats; however, hemorrhage and bruising was more evident in monkeys. Ecchymosis was observed in monkeys over numerous sites of the body (thorax, limbs, gingiva, head, and neck).

Repeat-dose toxicology

Repeat-dose studies in rats and monkeys resulted in toxicities in multiple tissues, many of which were observed at sub-therapeutic exposures. Findings were seen in the GI tract, lymphocytic/ hematopoietic system, kidneys, heart, liver, adrenals, reproductive organs, thyroid, pancreas, lung, and bile duct. Electrolyte imbalance was also noted; which may be due to the nephrotoxicity and/or GI toxicity.

Studies in SD rats

Toxicities included:

- GI tract (throughout the tract): bloated/swollen abdomen, diarrhea, distention of the GI tract with gas/ fluid/ ingesta or digesta, edema, darkened serosa and mucosa; ulceration/perforation/hemorrhage; congestion/inflammation; squamous hyperplasia/ hyperkeratosis in stomach; villus alteration (e.g. blunting, fusion, branching) in duodenum, jejunum, and ileum; fibrosis of cecum (characterized by aggregates of hyalinized collagen); crypt ectasia/abscess/ edema of cecum
- Hematopoietic/lymphocytic system: hematopoietic cell depletion in bone marrow; \downarrow lymphocytes; lymphoid depletion in lymph nodes/ thymus/ spleen, hemorrhage/ congestion of mesenteric lymph node and thymus; \downarrow weight of thymus and spleen; reticuloendothelial hyperplasia and \uparrow medullary mast cells in mesenteric lymph node; fibrous adhesion in spleen
- Liver: hypertrophy; \uparrow triglycerides; \uparrow cholesterol; slightly \uparrow AST and ALT; inflammation
- Electrolytes: \downarrow Ca, \downarrow phosphorus, \downarrow Na, \uparrow Cl
- Heart: hypertrophy; fibrosis
- Adrenals: hypertrophy; hyperplasia, vacuolation of zona glomerulosa

- Male reproductive system: ↓size of seminal of vesicles; reduced secretion of seminal vesicles; immature sperm in epididymis
- Female reproductive system: hypertrophy of ovaries; fluid filled uterus; dilatation of uterus; ↑ corpus lutea and cyst in ovaries, ↓ incidence of acyclic ovaries; ↓squamous metaplasia of endometrial glands
- Thyroid/ parathyroid: hypertrophy, ↑colloid of thyroid
- Pancreas: acinar atrophy
- Kidney: ↑urinary volume; tubular epithelia regeneration; tubular ectasia/ proteinosis; tubular epithelial hyaline droplets; fibrosis; electrolyte imbalance may be partially attributed to renal injury.
- Lung: minimal segmental medial arteriolar hyperplasia
- Pituitary: ↓weight
- Tongue: degeneration/necrosis, hyperkeratosis, hemorrhage, cyst
- Other: ↑or↓ platelets; chromorhinorrhea; labored breathing; necrosis in salivary gland

Studies in Cynomolgus monkeys

Repeated dosing of dasatinib in Cynomolgus monkeys for 10 days, 1 month, or 9 months resulted in severe toxicities to the GI tract and lymphocytic system, regardless of the duration of the study.

Organ/tissues that were affected in the 9-month study but not in the shorter term repeat-dose studies included the following: bile duct (hyperplasia), lung (hyperplasia/hypertrophy, fibrosis, hemorrhage, and inflammation), adrenal medulla (mineralization), reproductive organs (♂: immature prostate/ seminal vesicle/ testis; ♀: mineralization and inflammation of uterus), pancreas (inflammation). Of note, multiple organs presented with vascular mineralization in the 9-month study, e.g. in heart, tongue, spleen, stomach, and pancreas.

Primary toxicologic findings observed in the monkeys included:

- GI tract: vomiting, diarrhea, gas/fluid-filled contents of the cecum and colon; red/liquid feces, ulceration/ hemorrhage, edema, inflammation, enterocyte vacuolation and villous fusion; flattening of superficial epithelium, rectal crypt abscess
- Lymphocytic system: ↓lymphocytes; thymic weight; lymphocytic depletion of thymus, spleen, and mesenteric lymph node
- Liver/ hepatobiliary: hypertrophy, slightly ↑AST and ALT, focal necrosis/ hepatocellular necrosis, ↑triglycerides; bile duct hyperplasia
- Heart: inflammation, hypertrophy, vascular mineralization
- Kidney: inflammation; degeneration of cortical tubular epithelial cells; dilatation of cortical tubules; tubular ectasia/proteinosis; cortical mineralization of tubules and glomeruli/ fibrosis; inflammation; ↑BUN
- Phosphorus: hypo-phosphatemia
- Lung: hyperplasia/hypertrophy, hemorrhage, fibrosis, inflammation
- Pancreas: inflammation

- Adrenal medulla: mineralization
- Male reproductive organs: immature prostate, seminal vesicle, and testis
- Female reproductive organ: mineralization and inflammation of uterus
- Multiple organs: vascular mineralization e.g. in heart, tongue, spleen, stomach, and pancreas

Genetic toxicology:

BMS-354825 was clastogenic to CHO cells, in the absence or presence of metabolic activation.

Dasatinib was not mutagenic in the bacterial reverse mutation assays (Ames Test) using *S. typhimurium* strains TA98, TA100, TA1535, TA1537, and for *E. coli* strain WP2 *uvrA*. BMS-354825 did not cause chromosomal damage in the rat bone marrow micronucleus test, under the conditions of the study.

No genetic toxicology studies were conducted with the unique human metabolites.

Reproductive toxicology:

The effects of dasatinib on male and female fertility have not been studied. However, results of repeat-dose toxicity studies in multiple species indicate the potential for dasatinib to impair reproductive function and fertility. Dasatinib was evaluated for embryo-fetal toxicities in rats and rabbits. Dosing in both species was from implantation (GD 6 in rats and GD 7 in rabbits) to the end of organogenesis (GD15 in rats and GD 19 in rabbits). Rats were sacrificed on GD 20 and rabbits on GD 29.

Dasatinib was teratogenic in both species. Embryo-fetal effects, i.e. lethality (rats) and/or abnormalities (rats and rabbits), were present at doses that did not cause maternal toxicities. In addition, in both studies embryo-fetal lethality and/or abnormalities were seen at sub-therapeutic exposures.

In rats, the lowest dose (2.5 mg/kg/day or 15 mg/m²/day) resulted in embryo-fetal toxicities. This dose had maternal AUC of 105 ng·hr/mL (0.3 x the human AUC in females at the recommended dose of 70 mg BID). In rabbits the lowest dose (0.5 mg/kg/day or 6 mg/m²/day) caused embryo-fetal toxicities. This dose had a maternal AUC of 44 ng·hr/mL (0.1 x the human AUC in females at the recommended dose of 70 mg BID).

Embryo-fetal toxicities included the following:

Rats:

- Embryo-lethality, starting at the LD (2.5 mg/kg or 15 mg/m²): ↑resorption and ↓mean litter size
- Fetal abnormalities starting at the LD: malformations of the scapula or humerus (bent) and reduced ossification of the sternbrae and thoracic vertebral centra (irregularly/dumbbell shaped and/or reduced ossification site counts). Additional fetal abnormalities at the LMD (5 mg/kg or 30 mg/m²) included fluid-filled thoracic and abdominal cavities, edema (body), small

liver, misshapen clavicles, bent radius and femur, wavy or nodulated ribs, and reduced ossification of the thoracic, lumbar, and sacral vertebrae (hypoplastic, not ossified, and/or incompletely ossified centra) and forepaw phalanges (reduced ossification site counts).

Rabbits:

- Fetal abnormalities starting at the LD: delays in ossification of the fetal lumbar vertebrae (bifid arches), pelvis (incomplete ossification or not ossified pubes), and possibly hyoid body (incomplete ossification or not ossified). Additional observations at the HD consisted of irregular ossification of the hyoid (angulated) and presence of 7th cervical ribs.

No embryo-fetal NOAEL was identified in either species tested.

B. Pharmacologic activity

Dasatinib (BMS-354825) is an inhibitor of multiple protein tyrosine kinases. It inhibits the following kinases at nM ranges as shown in biochemical assays: The SRC family of kinases (SRC, LCK, YES, FYN); BCR-ABL; c-KIT; EPHA2; and PDGF-R β . The IC50s ranged from 0.55 nM (c-SRC) to 28 nM (PDGF-R β).

Pharmacology studies conducted with dasatinib showed that it had anti-growth/ anti-tumor activity in CML and ALL cell lines as well as in CML tumor models. In addition, CML cell lines and tumor models resistant to imatinib were sensitive to dasatinib treatment. Over-expression of the SRC family of kinases, e.g. FYN, HCK, and LYN was detected in the imatinib-resistant cell lines produced in culture or in cell lysates obtained from imatinib-resistant CML patients (5-6 patients). This limited information suggests that in a sub-population of imatinib-resistant CML patients, increased expression of SRC kinases may take place.

Based on the Secondary Pharmacology/Pharmacodynamics, dasatinib showed inhibitory effects on bone resorption in vitro and in vivo. This effect may be due to the inhibition of the SRC family of kinases; SRC kinase has been shown to be involved in osteoclast function.

Because LCK (a member of the SRC family) may be involved in T cell signaling, the immunosuppressive potential of dasatinib to prevent graft rejection was tested in murine models of T cell proliferation and graft rejection. Dasatinib was shown to inhibit T-cell proliferation in a dose dependent manner. Moreover, oral administration of dasatinib resulted in reduced graft rejection, when dasatinib was administered continuously.

C. Nonclinical safety issues relevant to clinical use

The following toxicities as identified in the toxicity assessment of dasatinib represent those effects of greatest concern to clinical use, including: cardiovascular toxicities (e.g. \uparrow blood pressure, QT prolongation, and necrotic changes); hemorrhage (was seen in

multiple organs/tissues in the animals); electrolyte imbalance; renal toxicity; GI toxicities including ulceration. Of note, fibrosis and mineralization was observed in multiple organs/tissues in the animal studies: mineralization of the uterus, adrenal medulla, tubules and glomeruli of kidney, and vascular system (in heart, tongue, spleen, stomach, and pancreas), fibrosis in the heart, lung, spleen, kidney, and cecum. Fibrosis/mineralization may impair the function of these organs/tissues.

Dasatinib was teratogenic at sub-therapeutic exposures in both species tested.

Dasatinib has an immunosuppressive potential that differs mechanistically from that exerted by many other anti-cancer drugs. The immunosuppressive potential of dasatinib is at least partially attributed to its ability to inhibit the LCK kinase involved in T-cell signaling. Dasatinib was shown to reduce T-cell proliferation. This effect may lead to increased incidence of infection in patients.

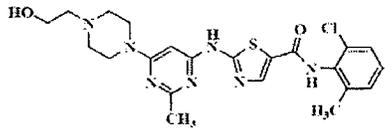
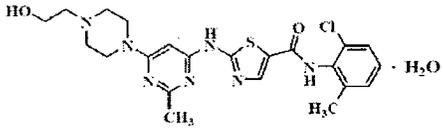
Based on the summary of non-clinical studies, dasatinib inhibited platelet aggregation in vitro, in human, monkey, and rat platelet-rich plasma at concentrations of 0.5 and 5 µg/mL. Hemorrhage reported in animals and in patients may be attributed to platelet dysfunction.

**APPEARS THIS WAY
ON ORIGINAL**

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-986 and 22-072
Review number: 1
Sequence number/date/type of submission: 000/ Dec 9, 2005/ Original NDA
Information to sponsor: Yes (X) No ()
Sponsor and/or agent: Bristol-Myers Squibb
5 Research Parkway
P.O. Box 5100, Mailstop 3SIG-5014
Wallinford, CT 06492
Manufacturer for drug substance: BMS facility
Swards Laboratories
Watery Lane
Swards, County Dublin, Ireland
US Establishment Registration # FCEI009
Reviewer name: Haleh Saber, Ph.D.
Division name: Division of Drug Oncology Products
Review completion date: 4/21/06
Drug:
Trade name: SPRYCEL™
Generic name: Dasatinib
Code name: BMS-354825; BMS-354825-01
Chemical name: N-(2-Chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5thiazolecarboxamide, monohydrate
CAS registry number: 863127-77-9
Molecular formula/molecular weight: C₂₂H₂₆ClN₇O₂S • H₂O/506.02
(monohydrate)/ 488.01 (anhydrate)
Structure:

Structural Formula	BMS Number-Form Number	Form
	BMS-354825-01	Free base. Anhydrate
	BMS-354825-03	Monohydrate

Relevant INDs:

IND 66-971 (Bristol-Myers Squibb)

Drug class: multi-tyrosine kinase inhibitor**Intended clinical population:** Chronic Myeloid Leukemia (CML) and Philadelphia chromosome positive (PH+) Acute Lymphoblastic Leukemia (ALL)**Clinical formulation:** Tablets of 20 mg, 50 mg, and 70 mg strengths
Compositions of the tablets are as follows:

Component	Compendial Reference	Function	% w/w ^a	Amount (mg/tablet)		
				20 mg	50 mg	70 mg
Dasatinib ^b	---	Active	/	20.0	50.0	70.0
Lactose Monohydrate ^c	NF, Ph. Eur.	/	/	/	/	/
Microcrystalline Cellulose	NF, Ph. Eur.	/	/	/	/	/
Hydroxypropyl Cellulose	NF, Ph. Eur.	/	/	/	/	/
Croscarmellose Sodium	NF, Ph. Eur.	/	/	/	/	/
Magnesium Stearate	NF, Ph. Eur.	/	/	/	/	/
White.	---	Film Coat	---	---	---	---
n ^c	USP, Ph. Eur.	/	---	---	---	---
Tablet Weight	---	---	---	83.2	207.0	288.4

Table provided by the sponsor.

Route of administration: Oral

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise. Parts of this review have been excerpted directly from the NDA package. Parts of this review have been excerpted from Dr. Leigh Verbois' review of the IND (#66-971; review date of 3/28/03).

Studies reviewed within this submission: See Table below

Studies not reviewed within this submission: See Table below

Title	Reviewed	
	Yes	No
PHARMACOLOGY		
Study 930003300 -- Preclinical Pharmacology of BMS-354825, a SRC Protein Tyrosine Kinase Inhibitor (930003300 v2.0)	X	

Title	Reviewed	
	Yes	No
Study 930012327 -- Further Studies on the Preclinical Pharmacology of Dasatinib (BMS-354825), a Multi- Targeted Tyrosine Kinase Inhibitor (930012327 v1.0)		X
Study 930003220 -- Activity of a Novel Tyrosine Kinase Inhibitor BMS-354825 on ST1571 Sensitive and Resistant CML Cells (930003220 v1.0)		X
Study 930011745 -- Structural Basis for Inhibition of ABL Kinase By BMS-354825 (DASATNIB), A Dual SRC/ABL Kinase Inhibitor (930011745 v1.0)		X
Study 920036261 -- Antitumor Activity of Dasatinib (BMS- 354825) in a Preclinical Model of Intracranial CML (920036261 v1.0)		X
Study 930003470 -- Effect of SRC Kinase Inhibitor BMS-354825 on Bone Resorption Both In Vitro and In Vivo (930003470 v2.0)	X	
Study 930003221 -- Identification of Genes for Predicting Activity of Compounds that Interact with SRC Tyrosine Kinases and the SRC Tyrosine Kinase Pathway in Cancer Cells (930003221 v1.0)		X
Study 920035967 -- Pharmacokinetics- and Pharmacodynamics-Guided Optimization of the Dose and Treatment Schedule for the Multi- Oncogenic Tyrosine Kinase Inhibitor Dasatinib (BMS-354825) (920035967 v1.0)		X
Study 920036247 -- Prediction of the optimal dose and exposure of novel panSRC kinase inhibitor dasatinib (BMS-354825) for phase I/II clinical studies in cancer patients using pharmacokinetic and pharmacodynamic biomarker approach (920036247 v1.0)		X
Study 910072849 -- In Vitro Human Umbilical Vein Endothelial Cell (HUVEC) Migration Assay Protocol (910072849 v1.0)		X
SAFETY PHARMACOLOGY		
Study DS03027 -- In Vitro Evaluation of Effects on Receptor and Ion-Channel Binding and Enzyme Activity (930003305 v1.0)	X	
Study 920018211 -- Effects on HERG/IKr Currents and Rabbit, Purkinje Fiber Action Potentials (920018211 v1.0)	X	
Study DS03098 -- Single-Dose Oral Cardiovascular Safety Pharmacology Study in Monkeys (930005453 v1.0)	X	
Study DS05124 -- In Vitro Evaluation of Effects on Receptor and Ion-Channel Binding and Enzyme Activity (930011274 v1.0)		X
Study DT05071 -- Effects of BMS- 573188, BMS-582691 and BMS-606181 on hERG/IKr and Rabbit Purkinje Fiber Action Potentials (930010945 v1.0)	X	
PHARMACODYNAMIC DRUG INTERACTIONS		
Study 930003300 -- submitted under Pharmacology		X*
PHARMACOKINETICS		
Analytical methods and validation		
Study DDBS008 -- Quantitative Determination of BMS-354825 in Rat K3EDTA Plasma by LC/MS/MS (930003278 v2.0)		X
Study 930010742 -- Quantitative Determination of BMS-354825 in Rabbit EDTA Plasma by LC/MS/MS (930010742 v1.0)		X
Study DDBS007 -- Quantitative Determination of BMS-354825 in Monkey K3EDTA Plasma by LC/MS/MS (930003277 v1.0)		X
Study 930011548 -- Determination of BMS-354825 and BMS-586291 in 1:1 Human Serum/Dialysis Buffer in an In Vitro Protein Binding Study (930011548 v1.0)		X

Title	Reviewed	
	Yes	No
Study 930011547 -- Partial Method Validation for the Quantitative Determination of BMS-354825 and BMS-582691 in 1:1 Human Serum/Dialysis Buffer by LC/MS/MS (930011547 v1.0)		X
Absorption		
Evaluated as part of Study MAP005 and toxicology studies	X**	
Distribution		
Study MBA00038 -- Tissue Distribution of Radioactivity in Male Long-Evans Rats Following Oral Administration of [14C]BMS-354825 (930008720 v1.0)	X	
Study 930011593 -- In Vitro Protein Binding Determination of BMS-582691 in Human Serum Using Equilibrium Dialysis (930011593 v2.0)	X	
Metabolism		
Study 930010531 -- Biotransformation of [14C]BMS- 354825 After Intravenous and Oral Administration to Bile Duct Cannulated Rats (930010531 v1.0)	X	
Study 930011324 -- Biotransformation of [14C]Dasatinib in Hepatocyte and Liver Microsomal Preparations from Rat, Monkey and Human (930011324 v1.0)		X
Study 930011323 -- Identification of Enzymes Involved in the Oxidative Metabolism of [14C]Dasatinib (BMS-354825) (930011323 v1.0)		X
Study 930011321 -- Biotransformation of [14C] Dasatinib (BMS-354825) in Rats, Monkeys, and Humans (930011321 v1.0)	X	
Study 930011322 -- In Vitro Evaluation of BMS-354825 as an Inhibitor of Human Cytochrome P450 Enzymes (930011322 v1.0)		X
Study 930011325 -- In Vitro Assessment of BMS-354825 as an Inducer of Cytochrome P450 Expression in Cultured Human Hepatocytes (930011325 v1.0)		X
Excretion		
Study MBA00096 -- Mass Balance of Radioactivity after Oral Administration of [14C]BMS- 354825 to Male Rats (930010421 v2.0)		X
Study MBA00097 -- Pharmacokinetics of Radiolabeled BMS-354825 and Excretion of Radioactivity after Oral Administration of [14C]BMS- 354825 to Male Cynomolgus Monkeys (930010419 v1.0)	X	
Study MBA00127 -- Biliary Excretion of Radioactivity After Intravenous Administration of [14C]BMS-354825 to Male Cynomolgus Monkeys (930010809 v1.0)	X	
Other Pharmacokinetic Studies		
Study MAP005 -- Preclinical Evaluation of the Pharmacokinetics and Metabolism of BMS-354825 (930003190 v1.0)		X
TOXICOLOGY		
Single-dose		
Study DS02138 -- Single-Dose Oral Toxicity Study in Rats (930003147 v3.0)	X	
Study DS02147 -- Single-Dose Oral Toxicity Study in Monkeys (930003271 v2.0)	X	
Repeat-dose		
Study 930003311 -- Toxicity Studies in Rats Administered BMS-354825 Orally with Various Doses, Interrupted Dosing Schedules and Recovery Periods (930003311 v1.0)		X
Study DS02047 -- Two-Week Oral Exploratory Study in Rats	X	

Title	Reviewed	
	Yes	No
(930002738 v1.0)		
Study DS02158 -- One-Month Intermittent Dose Oral Toxicity Study in Rats (930003258 v2.0)	X	
Study DS03072 -- BMS-354825: Six-Month Oral Toxicity Study in Rats (930011518 v1.0)	X	
Study DS02050 -- Ten-Day Oral Exploratory Toxicity Study in Dogs (930003268 v1.0)		X
Study DS02062 -- Ten-Day Oral Exploratory Toxicity Study in Monkeys (930003269 v1.0)		X
Study DS03170 -- BMS-354825: Two-Week Oral Investigative Toxicity Study in Monkeys (930008034 v1.0)		X
Study DS02159 -- One-Month Intermittent Dose Oral Toxicity Study in Monkeys (930003259 v2.0)	X	
Study DS03073 -- BMS-354825: Nine-Month Oral Toxicity Study in Cynomolgus Monkeys (930011520 v1.0)	X	
GENOTOXICITY		
Study DS01124 -- Spiral Ames Reverse-Mutation Study in Salmonella (920012131 v1.0)	X	
Study DS02066 -- Exploratory Ames Reverse-Mutation Study in Salmonella (920019681 v1.0)	X	
Study DS02193 -- Reverse-Mutation Study in Salmonella typhimurium and Escherichia coli (930003157 v2.0)	X	
Study DS03025 -- Cytogenetics Study in Chinese Hamster Ovary Cells (930004234 v1.0)	X	
Study DS02177 -- Oral Micronucleus Study in Rats (930003176 v2.0)	X	
REPRODUCTIVE AND DEVELOPMENTAL TOXICITY (Embryo-Fetal Development)		
Study DN04078 -- Oral Study of Embryo-Fetal Development in Rats (930011508 v1.0)	X	
Study DN04062 -- Thirteen-Day Oral Range-Finding Study in Pregnant Rabbits (930009355 v1.0)		X
Study DN04080 -- Oral Study of Embryo-Fetal Development in Rabbits (930010604 v1.0)	X	
SUPPLEMENTAL TOXICITY STUDIES		
Study 930003471 -- Report on Analysis of Immunosuppressive Potential of BMS-354825 (930003471 v5.0)	X	
Study 930004015 -- Effects of BMS-354825 on Platelet Function (930004015 v1.0)		X
Study 930008306 -- Effects of BMS-354825 on Bleeding Time and Ex Vivo Platelet Function (930008306 v1.0)		X
Study DS05008 -- BMS-354825: Neutral Red Uptake Phototoxicity Assay in Balb/c 3T3 Mouse Fibroblasts (930012310 v1.0)		X

* Multiple studies were submitted in the report. Studies pertaining to the Pharmacodynamic drug interaction were not reviewed.

** PK parameters were reviewed as part of the toxicology studies. Study MAP005 was not reviewed.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Dasatinib is a multi-protein tyrosine kinase inhibitor, inhibiting mainly the SRC family of kinases, BCR-ABL, c-KIT, EPHA2, and PDGFR β .

Pharmacology studies conducted with dasatinib showed that it had anti-growth/ anti-tumor activity in CML and ALL cell lines as well as in CML tumor models. In addition, CML cell lines and tumor models resistant to imatinib were sensitive to dasatinib treatment. Over-expression of the SCR family of kinases, e.g. FYN, HCK, and LYN was detected in the imatinib-resistant cell lines produced in culture or in cell lysates obtained from imatinib-resistant CML patients (5-6 patients). This limited information suggests that in a sub-population of imatinib-resistant CML patients, increased expression of SRC kinases may take place.

The proposed indications for dasatinib are:

- Treatment of adults with chronic, accelerated, or blast phase chronic myeloid leukemia with resistance or intolerance to prior therapy including imatinib.
- Treatment of adults with Philadelphia chromosome-positive acute lymphoblastic leukemia and lymphoid blast chronic myeloid leukemia with resistance or intolerance to prior therapy.

2.6.2.2 Primary pharmacodynamics

Mechanism of action:

Dasatinib (BMS-354825) is an inhibitor of multiple protein tyrosine kinases. It inhibits the following kinases at nM ranges as shown in biochemical assays: the SRC family of kinases (SRC, LCK, YES, FYN); BCR-ABL; c-KIT; EPHA2; and PDGF-R β . The IC50s ranged from 0.55 nM (c-SRC) to 28 nM (PDGF-R β).

Drug activity related to proposed indication:

- Dasatinib inhibited the proliferation of the BCR-ABL dependent CML and ALL cell lines (K562, KU-812, MEG-01, and SUP-B15) tested in culture, with IC50s of ≤ 1 nM
- Dasatinib induced erythroid differentiation of K562 CML cells, as assessed by increased expression of erythroid cell surface markers
- Dasatinib was cytotoxic to K562/STI-571/R CML cells, a cell line established in culture to be resistant to STI-571 (imatinib) treatment. Cytotoxicity was seen at dasatinib concentrations comparable to that in the parent K562 CML cells. Mechanism of resistance to imatinib in K562/STI-571/R and a few other imatinib-resistant CML cells was at least partially attributed to the over-expression of SRC family of kinases, such as FYN, LYN, and HCK.
- Dasatinib showed antitumor activity in xenograft models of CML and imatinib-resistant CML tumors

Study title: Preclinical Pharmacology of B545-354825, a SRC Protein Tyrosine Kinase Inhibitor

Key study findings:

- In the biochemical assays, BMS-354825 inhibited mainly the following kinases, with IC50s in the nM ranges: SRC family kinases (SRC, LCK, YES, FYN); BCR-ABL; c-KIT; EPHA2 receptor; and PDGF-R β
- BMS-354825 inhibited the proliferation of the BCR-ABL dependent CML and ALL cell lines (K562, KU-812, MEG-01, and SUP-B15) tested in culture, with IC50s of ≤ 1 nM
- BMS-354825 induced erythroid differentiation of K562 CML cells, as assessed by increased expression of erythroid cell surface markers
- BMS-354825 inhibited growth of K562/STI-571/R CML cells, a cell line established in culture to be resistant to STI-571 (imatinib) treatment. Growth inhibition was seen at dasatinib concentrations comparable to that in the parent K562 CML cells. Mechanism of resistance to STI-571 in K562/STI-571/R and few other STI-571 resistant CML cells was at least partially attributed to the over-expression of SRC family of kinases, such as FYN, LYN, and HCK.
- BMS-354825 showed antitumor activity in xenograft models of CML and imatinib-resistant CML tumors
- BMS-354825 showed growth inhibitory activity in non-leukemic cells, e.g. in human prostate, colon, and breast carcinoma cell lines

Report no.: 930003300

Note: The following studies submitted as part of this pharmacology report have not been reviewed:

- *Genetic determinant of sensitivity to BMS-354825 (microarrays),*
- *Pharmacodynamics of drug exposure,*
- *Effects on bone resorption and immuno-suppressive effects.*

Some of these studies may be considered the Secondary Pharmacology/ Pharmacodynamics and will be discussed in section 2.6.2.3. Only studies reviewed will be presented in this section. The article by Donato et al(1) was also reviewed; results of this article were discussed in the present study report.

Assessments:

- Kinase assay; biochemical IC50s
- Cell survival and growth inhibition
- Erythroid differentiation
- In vivo anti-tumor activity
- Activity in STI-571 resistant cells in culture and in xenograft models

Results:

IN VITRO AND CELL CULTURE STUDIES

In vitro IC50s; biochemical assays

The inhibitory activity of BMS-354825 was evaluated against human recombinant SRC kinases and a panel of protein kinases in vitro. In these in vitro biochemical assays, the main targets of BMS-354825 were defined as the following tyrosine kinases/kinase families: SRC family kinases (SRC, LCK, YES, FYN); BCR-ABL; c-KIT; EPHA2 and PDGF-R β , with IC50s ranging from 0.55 nM (c-SRC) to 28 nM (PDGF-R β). Some inhibitory effect was also seen for p38 (IC50= 103 nM).

Protein Kinase	Class	IC ₅₀ (nM)	
		BMS-354825	STI-571
c-SRC	PTK	0.55	85,300
LCK	PTK	1.1	920
YES	PTK	0.41	31,000
FYN	PTK	0.2	38,200
BCR-ABL	PTK	3.0	790
c-KIT	PTK	22	169
PDGF β receptor	PTK	28	1,590
EPHA2 receptor	PTK	17	ND
FAK	PTK	> 50,000	ND
IGF-1 receptor	PTK	> 50,000	ND
Insulin receptor	PTK	> 50,000	ND
HER1/HER2 receptors	PTK	180/710	ND
VEGF-2 receptor	PTK	> 2,000	ND
FGF receptor	PTK	880	ND
MEK kinase	PTK	1700	ND
MET	PTK	> 25,000	ND
EMT/ZAP-70	PTK	28,200	ND
SYK	PTK	< 5,000	ND
P38	Serine/threonine	103	ND
PKA	Serine/threonine	> 50,000	ND
PKC(alpha, delta, theta, zeta)	Serine/threonine	> 50,000	ND
GSK-3	Serine/threonine	> 50,000	ND
CaMKII	Serine/threonine	5,000	ND

ND: not done

Table provided by the sponsor.

Growth inhibition in cell culture assays (CML and ALL)

BMS-354825 inhibited the proliferation of the BCR-ABL dependent CML and ALL cell lines tested in culture, with IC50s of ≤ 1 nM. Under the conditions of the assays, for the 4 cell lines tested, STI-571 (imatinib), an inhibitor of BCR-ABL kinase, had IC50s of 57-350 nM.

In vitro growth inhibition of BMS-354825 in human BCR-ABL dependent leukemia cell cultures

Cell Culture	Histology	Growth Inhibition IC ₅₀ ^a (nM)		Fold Potency Difference
		BMS-354825	STI-571	BMS-354825/STI-571
K-562	CML (erythromyeloblastoid)	0.77	231	300
KU-812	CML (myeloid)	0.087	57	655
MEG-01	CML (megakaryocytic)	0.28	120	429
SUP-B15	B cell precursor ALL	1.0	350	350

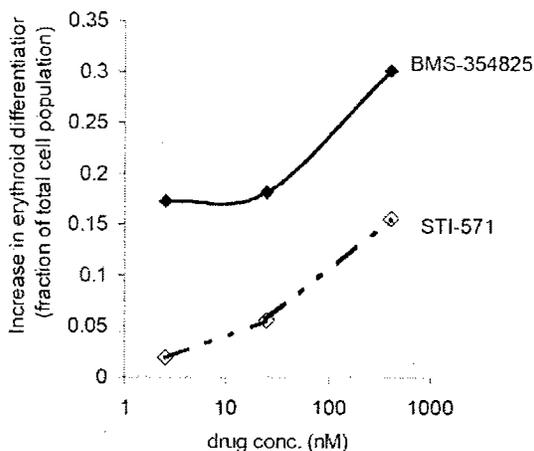
^a IC₅₀ is defined as the concentration of the agent to affect a 50% reduction of cell number at the end of the treatment period, as compared with untreated control. Results are from Studies Nos. BMSR-1227-1483

Table provided by the sponsor.

Induction of cell death and differentiation in K562 CML cell cultures

The human K562 cell line was used as a model system for the study of cell differentiation. These cells exhibit a low proportion of hemoglobin-synthesizing cells under standard cell growth conditions, but are capable of undergoing erythroid differentiation when treated with various agents. Cells were exposed to various concentrations of drugs for a total period of 24 hours and then harvested for analysis of changes in expression of erythroid cell surface markers (CD71 and Glycophorin A). Expression levels were determined by FACS analysis. Both BMS-354825 and STI-571 induced erythroid differentiation of K562 cells. Under the conditions of the experiment, lower concentrations of BMS-354825 was needed to induce comparable levels of differentiation; i.e. 2.5 nM of BMS-354825 vs 405 nM of STI-571 to achieve 0.15% to 0.2% increase in differentiation.

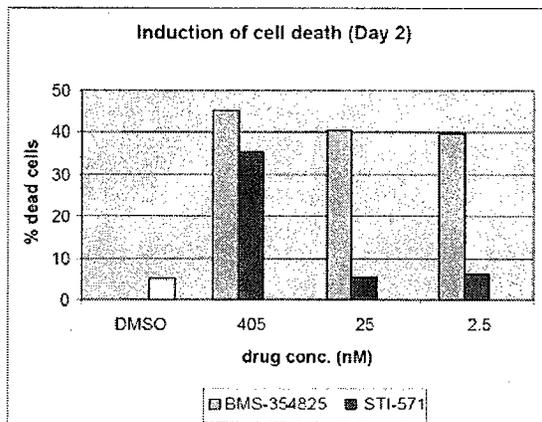
Induction of erythroid differentiation in K562 cells by BMS-354825 and STI-571 (imatinib).



Graph provided by the sponsor.

Cells were exposed to various concentrations of drugs for a total period of 48 hours and then harvested for analysis of cell death by propidium iodide (PI) staining; see graph below. The level of cell death was comparable between the 2 substances at higher concentrations (405 nM). Under conditions tested, lower concentrations, e.g. 2.5 nM of each test article, BMS-354825 yielded more cell deaths (40% vs 6%)

Induction of cell death in K562 cells by BMS-354825 and STI-571 (imatinib)



Graph provided by the sponsor.

Activity in a preclinical CML model of STI-571 resistance (K562/STI-571/R)

A STI-571 resistant variant of K562 (designated as K562/STI-571/R) was established by treatment of K562 cells with STI-571 in step-wise concentration increment, up to 1.8 µM (approximates the highest steady-state concentration achievable in patients according to the sponsor). The resulting resistant variant was isolated and cloned and was found to be 6-fold resistant to STI-571. The STI-571 resistant cell exhibited an IC50 of 1.0 nM when treated with dasatinib, which was minimally increased from that in the parent K562 cell (0.7 nM).

Sensitivity of K562 and STI-571-Resistant Subline of K562 to STI-571 and BMS-354825

	K562	K562/STI-571/R
	IC50	IC50
	(nM)	(nM)
STI-571	217	1288
BMS-354825	0.7	1.03

Table provided by the sponsor.

Mechanism of STI-571 resistance in K562/STI-571/R

Expression levels of the SRC family members and other related genes in the resistant cell line and the parental K562 cell line were assessed. Under the culture conditions that resulted in the resistant phenotypes, FYN, a member of the SRC family of kinases, was

over-expressed in the STI-571 resistant cells both at the RNA level (PCR) and the protein level (Western blot).

Over-expression of the SRC family of kinases, e.g. LYN and HCK, was also proposed by Donato et al(1) in cell culture modeling of resistance to STI-571 and based on the over-expression seen in the cell lysate of patients with accelerated phase and blast crisis CML (n=5 or 6) that failed STI-571 therapy. All patients had progressed within 3 to 6 months of STI-571 therapy. Therefore, SRC family of kinases may play a role in late-stage disease and STI-571 (imatinib) resistance, in at least a sub-population of patients with CML.

The over-expression of FYN, a SRC family member, in the STI-571-resistant cell line K562 analyzed by Taqman quantitative PCR

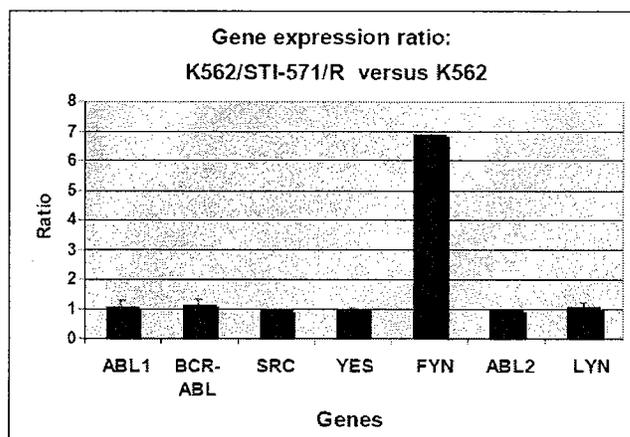


Table provided by the sponsor.

Growth inhibitory activity (cell culture)

BMS-354825 was tested for anti-proliferative activity against several cancer cell lines. The IC₅₀ ranged from 5.4 nM for the P815 murine mastocytoma to close to 1 μ M for the human colon carcinoma line —

In Vitro Growth Inhibitory Activity of BMS-354825 in a Panel of Cancer Cell Lines

Cell Cultures	Histology ^a	Growth Inhibition IC ₅₀ (nM)
		BMS-354825
P815	Mastocytoma	5.4
PC3	Prostate carcinoma	23.2
—	Prostate carcinoma	144
MDA-PCa-2b	Prostate carcinoma	125
DU145	Prostate carcinoma	103
WiDr	Colon carcinoma	257
LOVO	Colon carcinoma	22.4
SW-480	Colon carcinoma	389
—	Colon carcinoma	845
—	Colon carcinoma	> 1200
MDA-MB-231	Breast carcinoma	16.6
—	Rhabdomyosarcoma	55.8
A549	Lung carcinoma	> 1200

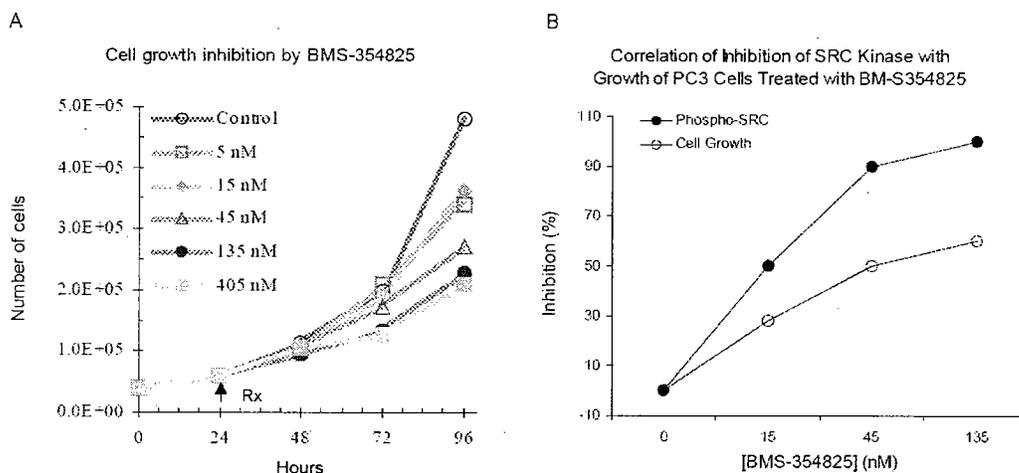
^a All cell lines are of human origin except for P815 which is a murine derived line.

Table provided by the sponsor.

Inhibition of SRC kinase phosphorylation in cells

Dasatinib inhibited the phosphorylation of SRC in the PC3 prostate carcinoma cells. Concentrations needed to inhibit phosphorylation were in the same range as those needed for inhibition of cell growth. Dasatinib also inhibited the SRC activity of several other cell lines, which were susceptible to the antiproliferative effect of the test article (WiDr, SW-480, and — colon carcinoma, and PC3, DU145 and Pca2b prostate carcinoma). See the following Figures:

- (A) Effects of treatment with various concentrations of BMS-354825 on cell growth of the human PC3 prostate carcinoma cells.
- (B) Correlation between the inhibition of the tyrosine phosphorylation of SRC and the antiproliferative activity of BMS-354825



Graphs submitted by the sponsor.

Inhibition of SCF/c-KIT mediated cellular proliferation in cell lines

BMS-354825 inhibited cellular proliferation of 3 small cell lung cancer cell lines expressing c-KIT kinase at IC50s > 100 nM.

Cell Line	IC50 (nM)
	BMS-354825
H526	220
NCI-H69	114
H187	128

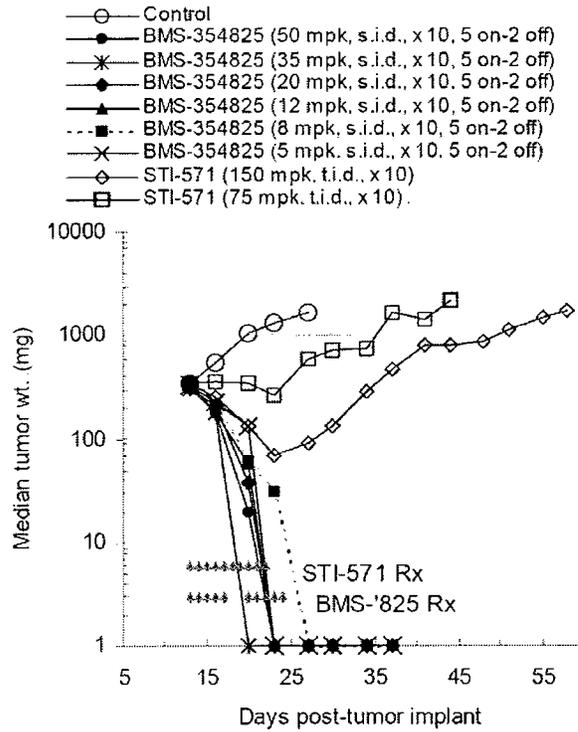
Table provided by the sponsor.

IN VIVO ANTITUMOR ACTIVITY

CML xenograft

SCID mice bearing the K562 human CML tumors were treated at the indicated doses when tumors reached ~200-500 mg. BMS-354825 was administered orally (p.o.), once a day for 10 days (QD x 10), with a 2 day break following every 5 days of treatment (5 on, 2 off). STI-571 was administered orally (p.o.), 3 times a day for 10 consecutive days (QD x 10). Each symbol represents the median tumor weight of a group of 8 mice.

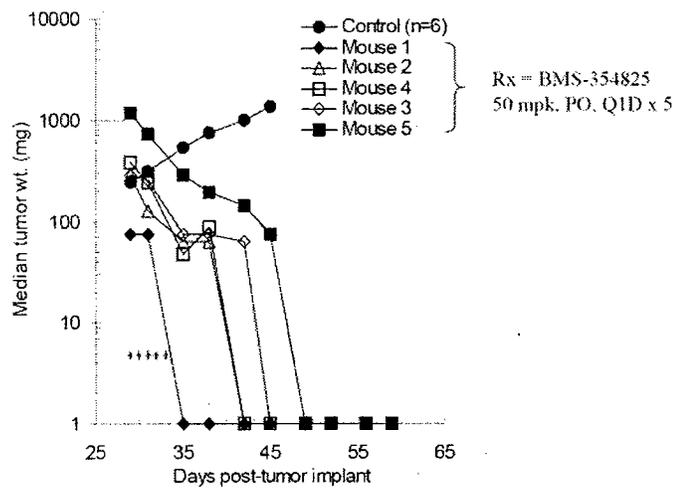
- Dasatinib administered orally, QD, at doses of 8-50 mg/kg for a total of 10 doses (5 days-on 2 days-off), resulted in complete shrinkage of tumors. At 5 mg/kg, 70% of the mice had complete shrinkage.
- All doses were well tolerated.



s.i.d: single injection per day; mpk: mg/kg
Graph provided by the sponsor.

In another study, SCID mice bearing the KU812 human CML tumors were treated with a dose of 50 mg/kg BMS-354825. BMS-354825 was administered orally (p.o.), once a day for 5 days (QD x 5). Each symbol represents the tumor weight of an individual mouse.

- The dose of 50 mg/kg dasatinib, given daily for 5 days resulted in complete shrinkage of tumor in all treated mice.

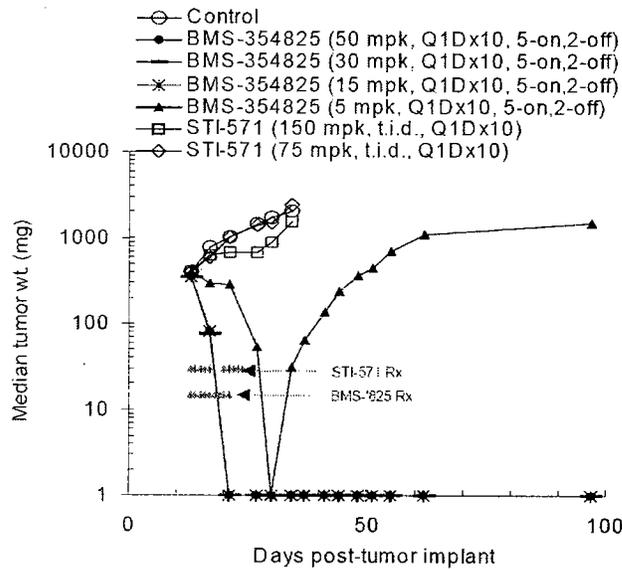


Graph provided by the sponsor.

Antitumor efficacy in an in vivo model of STI-571 resistance (K562/STI-571/R)

Mice bearing the K562/STI-571/R human CML tumors were treated at the indicated doses when tumors reached ~200-500 mg. BMS-354825 was administered orally (p.o.), once a day for 10 days (QD x 10), with a 2 day break following every 5 days of treatment (5-on, 2-off). STI-571 was administered orally (p.o.), 3 times/day for 10 consecutive days (QD x 10). Each symbol represents the median tumor weight of a group of 8 mice.

- The K562/STI-571/R was sensitive to dasatinib treatment in this xenograft model. Sensitivity was comparable to that of the parent cell K562; doses of 15-50 mg/kg resulted in complete shrinkage of tumors in all mice, when given daily for 10 days (5 days-on, 2 days-off).
- The lowest dose of 5 mg/kg resulted in complete shrinkage in 25% of the mice.
- Under the conditions of this experiment, growth of the tumors was comparable to the control when animals were treated with STI-571.



Graph provided by the sponsor

Comparative antitumor efficacy and tolerability of BMS-354825 and STI-571 in the K562/STI-571/R-resistant CML sc tumor model in SCID mice

Compound	Rx Regimen			Efficacy ^a		Tolerability	
	Dose (mg/kg)	Route	Schedule	Lck ^b	Cures ^c (%)	AWC ^d (g)	Mortality (%)
BMS-354825	50	PO	Q1D x 10:13 (5 on, 2 off)	3.4	100	0.6	0%
	30			3.4	100	0.6	0%
	15			3.4	100	0.2	0%
	5			1.8	25	1.6	0%
STI-571	150	PO	SQD x 10:13	0.4	0	-2.5	0%
	75			0.0	0	-2.3	0%

^a Each study group contained 8 mice. Results were from Study No. BMSR-1408.

^b Lck, log cell kill.

^c Cures are defined as the absence of detectable tumor at a time greater than 10X the tumor volume doubling time after the cessation of treatment.

^d AWC, average weight change.

Summary of the studies:

This pharmacology report incorporated the results of several studies. Only studies reviewed were presented above and are summarized here.

Note for BMS-354825 (dasatinib) vs STI-571 (imatinib) comparative studies:

It should be noted that results of the in vitro, cell culture, and in vivo studies may vary depending on the conditions of the experiments. In addition, the data presented for leukemic cells (sensitive to both drugs) may not necessarily reflect results of treatment in patients. Results in patients will depend on multiple factors, e.g. the plasma levels of drugs at the MTD or at the maximum recommended dose, non-specific binding (secondary pharmacology and toxicity levels), ADME data (e.g. drug with toxic metabolites, tissue retention, etc). It should be also noted that STI-571 resistant cells with increased expression of SRC kinases may represent a sub-population of the resistant phenotype.

In vitro/ cell culture studies

In the biochemical assays, BMS-354825 inhibited mainly the following kinases, with IC50s in the nM ranges:

- SRC family kinases (IC50: SRC = 0.55 nM, LCK = 1.1 nM, YES = 0.41 nM, FYN = 0.2 nM)
- BCR-ABL (3 nM)
- c-KIT (22 nM)
- EPHA2 receptor (17 nM)
- PDGF-Rβ (28 nM)

BMS-354825 showed some inhibitory effect on p38 serine/threonine kinase (IC50: 103 nM) and was less potent against other kinases, such as PTKs (FAK, IGF1 receptor,

insulin receptor, HER1/HER2 receptors, VEGF-R2, FGFR, MEK, MET, EMT/ZAP-70, SYK) and serine/threonine kinases (PKA, PKC kinases, GSK-3, CaMKII).

BMS-354825 inhibited the proliferation of the BCR-ABL dependent CML and ALL cell lines (K562, KU-812, MEG-01, and SUP-B15) tested in culture, with IC50s of ≤ 1 nM. Under the condition of the assays, for the 4 cell lines tested, STI-571 (imatinib), an inhibitor of BCR-ABL kinase, had IC50s of 57-350 nM.

Both BMS-354825 and STI-571 induced erythroid differentiation of K562 cells. Under the conditions of the experiment, lower concentrations of BMS-354825 was needed to induce comparable levels of differentiation; i.e. 2.5 nM of BMS-354825 vs 405 nM of STI-571 to achieve 0.15% to 0.2% increase in differentiation, as assessed by increase in the expression of erythroid cell surface markers. Staining of cells showed that, after 48 hrs exposure to either BMS-354825 or STI-571, percent cell death was comparable between the 2 substances at higher concentrations (405 nM). Under conditions tested, lower concentrations, e.g. 2.5 nM of each test article, BMS-354825 yielded more cell deaths (40% vs 6%).

K562/STI-571/R, a STI-571 resistant variant of K562 was established in culture, by treatment of K562 cells with STI-571 in step-wise concentration increment. The resulting cell modeled resistant variant was found to be 6-fold resistant to STI-571 under the conditions of the experiment. The STI-571 resistant cell exhibited an IC50 of 1.0 nM when treated with dasatinib, which was minimally increased from that in the parent K562 cell (0.7 nM). Mechanism of resistance to STI-571 in K562/STI-571/R and few other STI-571 resistant CML cells was at least partially attributed to the over-expression of the SRC family of kinases, such as FYN, LYN, and HCK.

In addition to the CML and ALL cell lines, growth inhibitory activity of dasatinib was shown in other cancer cells (prostate, colon, and breast carcinoma, mastocytoma, and rhabdomyosarcoma) with IC50s ranging from 5.4 nM for P815 murine mastocytoma to about 1 μ M for human colon carcinoma cell line. Growth inhibition was in part due to the inhibition of SRC phosphorylation, as tested in PC3 prostate cell line.

In Vivo antitumor activity

- Dasatinib administered p.o. to SCID mice bearing K562 CML tumor, QD, at doses of 8-50 mg/kg for a total of 10 doses (5 days-on 2 days-off), resulted in complete shrinkage of tumors. At 5 mg/kg, 70% of the mice had complete shrinkage. All doses were well tolerated.
- Dasatinib administered p.o. to SCID mice bearing KU812 CML tumor, QD x 5, at 50 mg/kg resulted in complete shrinkage of tumor in the treated mice.
- Dasatinib administered p.o. on a 5 days-on 2 days-off schedule, for a total of 10 doses to mice bearing K562/STI-571/R (STI-571 resistant CML) tumor, resulted in complete shrinkage of tumors at doses of 15-50 mg/kg. This showed that this STI-571 resistant CML tumor type was still sensitive to dasatinib treatment; sensitivity to dasatinib was comparable to that of the parent K562 tumor.

2.6.2.3 Secondary pharmacodynamics

Study Title: Effect of SRC kinase inhibitor BMS-354825 on bone resorption both in vitro and in vivo

Key study findings: Dasatinib had inhibitory effect on bone resorption, when tested in vitro or in vivo under the condition of the assays

Report no.: 930003470

Methods

Compound: BMS-354825-02

In vitro bone resorption assay:

Pregnant Sprague Dawley rats were injected s.c. with 200 μCi ^{45}Ca on Day 18 of gestation. The following day, radii and ulnae were dissected free from muscle and connective tissue. The cartilaginous ends of the bones were removed and the calcified diaphyses were cultured. The bone explants were maintained in culture for 5 days in the presence or absence of test substances. Residual ^{45}Ca was extracted from the bone explants by incubation in trichloroacetic acid (TCA) and subsequently neutralized. The amount of radioactive calcium was determined by liquid scintillation counting. Bone resorption was expressed as the percentage ^{45}Ca released by Day 5 of total amount of ^{45}Ca originally incorporated in bone explants.

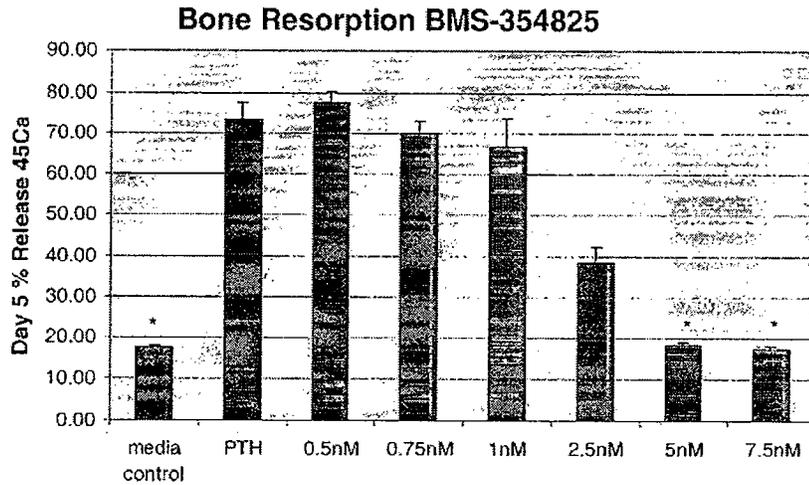
Serum Calcium in Thyro-parathyroidectomized Rats

Thyro-parathyroidectomy (TPTX) surgery was performed on male Sprague- Dawley rats (200 gram body weight). Thyro- parathyroidectomized (TPTX) rats with serum calcium values of 5-8 mg/dl were used for the study (normal reference range was considered to be 10-12 mg/dl). On the day of the study (48 hours post-surgery), blood was collected from the tail vein under anesthesia, and \sim mini-pumps (delivering 1 $\mu\text{l/hr}$) containing 0.3 $\mu\text{g/ml}$ of parathyroid hormone (PTH) were implanted subcutaneously. Immediately following implantation of the pumps, the animals were dosed IP with vehicle, 5 IU salmon calcitonin or BMS-354825 at 3 & 10 mg/kg. Blood was collected at 3, 6 8 24 hours after dosing and analyzed for serum calcium levels.

Results

In vitro bone resorption assay:

- PTH (10^{-8} M) stimulated bone resorption approximately 4-fold.
- BMS-354825 inhibited PTH-stimulated release of ^{45}Ca dose- dependently with an apparent IC_{50} of 2 nM. At 5 and 7.5 nM of BMS 354825, percent Ca release was comparable to that of the control, indicating that BMS-354825 was able to completely block PTH-stimulated bone resorption in this study.

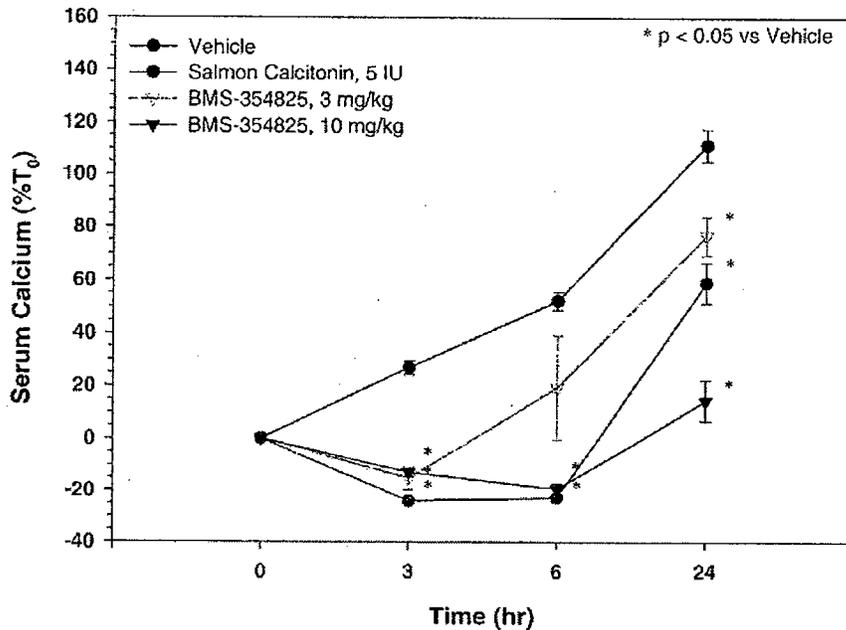


*p<0.05 vs PTH

Graph provided by the sponsor.

Serum calcium in thyro-parathyroidectomized rats

- Compared to vehicle treated animals, all treatments significantly prevented the PTH stimulated increase in serum calcium through the time course of this study.
- After 24 hours, serum calcium levels of animals treated with BMS-354825 at 10 mg/kg were significantly lower than both vehicle and calcitonin treated groups.



Treatment	N	Serum Calcium (mg/dl)			
		0 Hour	3 Hour	6 Hour	24 Hour
Vehicle, ip	5	6.56 ± 0.18	8.32 ± 0.29	9.96 ± 0.22	13.84 ± 0.48
Salmon Calcitonin, 5 IU ip	5	6.68 ± 0.21	5.06 ± 0.13 *	5.14 ± 0.15 *	10.62 ± 0.64 *
BMS-354825, 3 mg/kg ip	5	6.74 ± 0.29	5.68 ± 0.26 *	7.94 ± 1.19	11.88 ± 0.61 *
BMS-354825, 10 mg/kg ip	5	6.68 ± 0.25	5.80 ± 0.19 *	5.36 ± 0.12 *	7.70 ± 0.78 *

- p < 0.05 vs Vehicle

Graph and Table provided by the sponsor.

Summary:

Based on CGAP (<http://cgap.nci.nih.gov/>) and SwissProt (entries P12931 and P41240) databases, SRC kinase is a cytoplasmic protein tyrosine kinase, which is expressed in a variety of tissues/organs, e.g. in the digestive system (small intestine, large intestine, colon), urinary bladder, lymphoid system (T-cells), and bone and was shown to be activated in several cancer cells. c-SRC kinase is involved in a variety of cell pathways, e.g. cell-cell adhesion signaling, regulation of actin cytoskeleton, signaling through the T-cell receptor, cell signaling pathway.

Parathyroid hormone (PTH) is an enhancer and calcitonin is an inhibitor of osteoclast activity and hence of bone resorption. In the in vitro bone resorption study, BMS-354825 inhibited PTH-stimulated release of ⁴⁵Ca dose-dependently and was able to completely block PTH-stimulated bone resorption at concentrations ≥ 5 nM under the conditions of the experiment. In vivo studies with thyro-parathyroidectomized rats implanted with PTH delivering system, showed that 10 mg/kg of BMS-354825 given i.p., had inhibitory effect on bone resorption similar to or slightly higher than that observed with calcitonin (5 IU).

In addition, studies conducted with c-SRC-deficient mouse models suggest that SRC kinase is essential for osteoclast function. Mice deficient in SRC kinase had changes in their bone phenotype consisting of osteopetrosis, a syndrome of high bone mass due to impaired resorption(2). The major role of SRC in bone appears to be within osteoclasts, where it modulates vesicle transport, resulting in secretion of proteases and is involved in the cytoskeletal organization of microtubules and actin polymers. . The function of SRC in osteoclastic bone resorption appears to be mainly dependent on the protein binding domains of SRC, SH2 and SH3. Therefore, drugs binding to SH2 and SH3 may have higher inhibitory effect on bone resorption.

2.6.2.4 Safety pharmacology

Study Title: *In vitro* evaluation of effects on receptor and ion-channel binding and enzyme activity

Key study findings: dasatinib did not show a significant interaction with any receptors (n=42) or acetylcholinesterase, which were analyzed in this panel

Report number: BMS # DS03027

Study number: — #454530

Volume/ Page number: Item 5

Conducting laboratory and location: BMS, Syracuse, NY

Date of study initiation: May 23, 2001

GLP compliance: yes () no (X)

Drug, lot #, radiolabel, and % purity: BMS354825, no further information

Number of replicates: 2

Results:

- BMS354825 did not show a significant interaction with any receptors analyzed in this panel, nor did it inhibit acetylcholinesterase activity.
- All assays showed <46% inhibition at 10 μ M
- The potential for BMS-354825-02 to inhibit human-derived acetylcholinesterase activity was investigated because this activity was inhibited by a structurally similar drug candidate in this class (BMS-355942-03)

Receptor Target	% Inhibition at 10 μ M	Receptor Target	% Inhibition at 10 μ M
Adenosine A ₁ (h)	21	Sodium Channel, Site 2 (r)	43
Adenosine A _{2A} (h)	33	DHP-sensitive L-Type Ca ²⁺	-
Adrenergic α_{1A} (r)	-	Verapamil-sensitive L-Type Ca ²⁺	43
Adrenergic α_{2A} (h)	-	K ⁺	-
Adrenergic β_1 (h)	-	Cl-	-
Adrenergic β_2 (h)	-	Opiate (non-selective)	-
Glutamate, Kainate (r)	-	PCP	10
Glutamate, AMPA (r)	-	Rolipram	-
Glutamate, NMDA	-	Glucocorticoid (h)	-
Glycine (strychnine insensitive)	-	Estrogen (h)	-
GABA _A , Agonist Site (r)	12	Progesterone (h)	-
GABA _B (nonselective)	-	SST (non-selective)	-
Dopamine D ₁ (h)	29	5-HT Transporter	39
Dopamine D _{2L} (h)	16	Serotonin 5-HT _{1A} (h)	14
Dopamine Transporter	46	Serotonin 5-HT _{2B} (h)	18
CCK _A (h)	21	Serotonin (non-selective)	17
Adrenergic, Norepinephrine transporter (h)	18	Nicotinic Acetylcholine, Central (r)	-
MAO-A	-	Muscarinic Acetylcholine	26
MAO-B	-	Choline Transporter	17
		Y (nonselective)	-

Study Title: Effects on hERG/IKr Currents and Rabbit Purkinje Fiber Action Potentials**Key study findings:**

- BMS-354825 inhibited hERG currents by $6 \pm 1\%$, $36 \pm 6.3\%$ and $77 \pm 5\%$ (n=3) at 3, 10 and 30 μM , respectively. The IC_{50} was calculated to be 14.3 μM .
- APD_{50} and APD_{90} were prolonged by 26% and 11%, respectively, following 30 μM BMS354825.

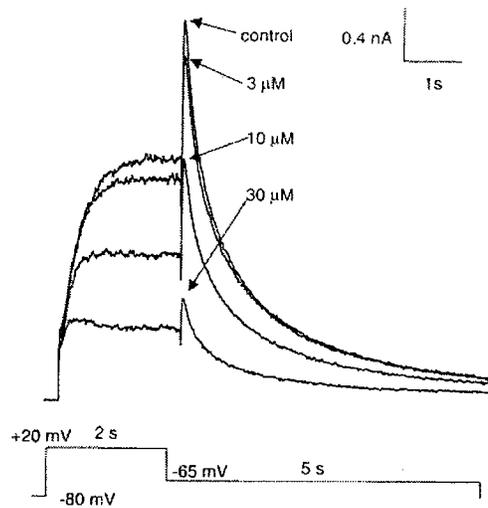
Study number: 920018211
Volume/ Page number: Item 5
Conducting laboratory and location: BMS Pharmaceutical Research Institutes,
Hopewell Biology and Drug Discovery,
New Jersey
Date of study initiation: not provided
GLP compliance: yes () no (X),
Drug, lot #, radiolabel, and % purity: not provided

Methods:

Strains/species/cell line: Transfected human embryonic kidney cells that stably express the human ether-a-go-go gene (hERG)
Concentrations of the test article: 3, 10, and 30 μM (vehicle: DMSO)
Positive controls: none
Replication: Triplicate

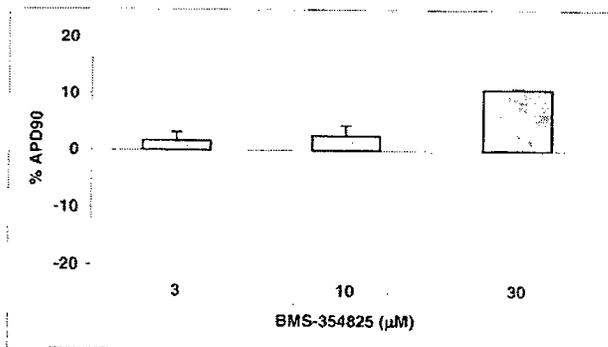
Results:

BMS-354825 inhibited hERG currents by $6.1 \pm 1.2\%$, $36.5 \pm 6.3\%$ and $76.8 \pm 4.5\%$ (n=3) at 3, 10 and 30 μM , respectively. The calculated IC_{50} was 14.3 μM . BMS-354825 prolonged APD_{50} by $26 \pm 5\%$ and APD_{90} by $11 \pm 0\%$ at 30 μM . BMS-354825 had little effect on other action potential parameters measured in the Purkinje fiber assay.



Typical hERG current records during BMS354285 application

Effects of BMS-354825 on hERG currents expressed in an HEK293 cell. Currents were elicited by voltage steps (2 sec) applied at 20 second intervals from a holding potential of - 80 mV to +20 mV. Tail currents were recorded at - 65 mV. The percent inhibition of tail currents was used to calculate the % inhibition of hERG current since there are no endogenous tail currents in plasmid-transfected control HEK293 cells.



Effects of BMS-354825 on APD90 (n=3).

Figures provided by the sponsor.

Study Title: Single-dose cardiovascular safety pharmacology study in monkeys

Key study findings: Single oral dose of BMS-354825 at 10 mg/kg resulted in 5%-15% increases in mean systolic and 8%-21% increases in the mean diastolic blood pressure for approximately 2 hrs after dose.

Report number: DS03098
Volume/ Page number: Item 5
Conducting laboratory and location: BMS; Princeton , NJ and Syracuse, NY
Date of study initiation: June 2003
GLP / QA compliance: Yes
Drug, lot #, radiolabel, and % purity: BMS-354825-03; lot # RD71123-45; pure

Methods:

Strains/species/cell line: Cynomolgus monkeys
Number of animals: 3 ♀s and 3 ♂s
Age of animals: 3-5.5 years old
Weight of animals: 3.2-5.7 kg

Note: animals were non-naïve but treatment-free for at least 4 weeks and had not previously received this test article.

Route: Oral (orogastric intubation)

Doses administered: A single oral dose of 10 mg/kg (120 mg/m²)
 The dose was selected based on the results of a single-dose oral toxicity study in monkeys that utilized doses of 15, 25, and 45 mg/kg and a 10-day study that utilized doses of 1, 10, and 15 mg/kg.

	BMS-354825 Dose (mg/kg)	Volume (ml/kg)	Concentration (mg/ml)
Treatment 1 (day 1)	0 ^a	2	0
Treatment 2 (day 3)	10	2	5

^a Vehicle (80 mM sodium citrate buffer, pH ~3.2)

Table provided by the sponsor.

Concentration verifications showed the dosing solutions had concentrations (5-5.1 mg/mL) in the accepted range.

Instrumentation: Commercial telemetry implants / — were used. The transmitter was placed in the abdominal cavity and secured to the abdominal wall. The leads of the ECG were positioned to approximate a limb Lead II electrogram with the positive lead positioned in the subcutaneous muscle over the left 10th intercostal space while the negative lead was positioned in the upper right thoracic area immediately caudal to the thoracic inlet. The pressure transducer was placed in the femoral artery.

Evaluations:

Cardiovascular: heart rate, blood pressure (systolic and diastolic), and EKG parameters (RR, PR, P width, QRS interval, and QT interval)

Clinical observations: daily for mortality and moribundity. The monkeys were observed for clinical signs prior to and approximately 4 hr after dose on treatment days, and once daily on non-treatment days.

Body weight: prior to treatment for dosing purposes

Results:

- There was no clear drug-related effect on EKG parameters, heart rates, and body temperatures.
- Systolic and diastolic blood pressures were increased shortly after treatment. Increases were remarkable 0.5-2 hrs post-dose: ↑5-15% for systolic and ↑8-21% for diastolic blood pressure.
- Increases in the systolic pressures were comparable to the vehicle control by Hour 4.5 postdose. Increases in the diastolic pressures were comparable to the control at by Hr 3 post-dose.

Hrs post-dose	Vehicle	Dasatinib	Δ*
Systolic			
0	126.77 ± 5.95	126.72 ± 4.33	-0.05
0.5	113.88 ± 5.34	120.25 ± 5.62	6.37 (↑5%)
1	108.64 ± 4.77	125.10 ± 5.67	16.46 (↑15%)
1.5	113.95 ± 5.11	123.91 ± 6.57	9.97 (↑9%)
2	112.98 ± 4.81	121.89 ± 6.12	8.91 (↑8%)
2.5	115.06 ± 4.66	118.83 ± 5.65	3.78
3	111.87 ± 4.06	116.34 ± 4.78	4.47
3.5	114.75 ± 3.90	118.04 ± 5.43	3.29
4	123.76 ± 5.26	125.67 ± 6.01	1.91
Diastolic			
0	83.32 ± 6.62	83.29 ± 5.35	-0.02
0.5	73.68 ± 5.95	79.62 ± 4.40	5.93 (↑8%)
1	69.99 ± 6.36	84.69 ± 4.02	14.70 (↑21%)
1.5	74.67 ± 5.48	82.39 ± 6.05	7.73 (↑11%)
2	72.73 ± 5.54	79.47 ± 6.36	6.74 (↑8%)
2.5	74.12 ± 6.44	75.76 ± 7.05	1.64
3	72.38 ± 5.77	72.38 ± 6.34	0.00
3.5	74.21 ± 5.44	74.20 ± 6.44	-0.01
4	80.39 ± 6.58	79.54 ± 7.48	-0.85

* Differences between dasatinib values and vehicle control (dasatinib – control).
Bolded numbers depict statistically significant differences.

Summary of the study:

Cynomolgus monkeys (3 ♂s and 3 ♀s) were administered a single oral dose of BMS-354825 at 10 mg/kg. The monkeys were fasted overnight prior to dosing and given vehicle (2 ml/kg, 80 mM sodium citrate buffer, pH ~3.2) as control 2 days prior to BMS-354825 administration. Systolic and diastolic blood pressure, heart rate, and electrocardiographic (EKG) parameters including RR, PR, P width, and QRS and QT intervals were assessed from 1 hr prior to and for approximately 22 hr after treatment with vehicle and BMS-354825. Assessments were done in conscious animals.

- There was no clear drug-related effect on EKG parameters, heart rates, and body temperatures.
- Systolic and diastolic blood pressures were increased shortly after treatment. Increases were remarkable 0.5-2 hrs post-dose.
- Since PK parameters were not evaluated, it is not known how these effects relate to the exposure and the Tmax.

Study Title: Effects of BMS-573188, BMS-582691 and BMS-606181 on hERG/IKr and Rabbit Purkinje Fiber Action Potentials

Key study findings (metabolites of dasatinib):

- BMS-573188 (metabolite M6) had minimal effect on hERG currents and on rabbit cardiac Purkinje fiber action potentials.
- BMS-582691 (metabolite M4) had moderate inhibition on hERG (24%, 72%, and 95% inhibition at 3, 10, and 30 μ M) but had minimal effects on Purkinje fiber action potential duration.
- BMS-606181 (metabolite M5; major metabolite in rat) had minimal effect on hERG currents and Purkinje fiber APD, but reduced action potential Vmax in a dose-dependent manner. Inhibition of Vmax may be suggestive of Na channel inhibition.
- None of these 3 metabolites are considered the major metabolite in humans.

Report number: DT05071
Volume/ Page number: Item 5
Conducting laboratory and location: BMS; NJ
Date of study initiation: Not provided
GLP compliance: yes () no (X)
Drug, lot #, radiolabel, and % purity: purities not provided
Metabolites of BMS-354825:

- BMS-573188 (02-001, lot)
- BMS-582691 (JL-56962-043, batch)
- BMS-606181 (JL-56962-047, batch)

Methods:

Strains/species/cell line: Human embryonic kidney (HEK293) cells stably transfected with human ether-a-go-go related gene (hERG) cDNA

Concentrations of the test article:

BMS-573188 and BMS-606181: 10 and 30 μ M (hERG)

BMS-582691: 3, 10 and 30 μ M (hERG)

BMS-573188, BMS-582691 and BMS-606181: of 3, 10 and 30 μ M (Purkinje)

Each dose of test agent was prepared individually from a DMSO stock solution just prior to use in the assays.

Vehicle: DMSO was used as vehicle in both assays. The final concentration of DMSO never exceeded 0.1% in hERG assays and 0.3% in Purkinje fiber experiments.

Positive controls: none

Replication: triplicates

Test system: Membrane current recordings were made with a Multiclamp series integrating patch-clamp amplifier () using the whole-cell variant of the patch-clamp technique. Cells expressing hERG potassium channels were placed in a plexiglass bath chamber, mounted on the stage of an inverted microscope, and perfused continuously with bath solution.

Effects of test agents on hERG were calculated by measuring inhibition of peak tail currents. Percent inhibition of tail currents was plotted as a function of test agent concentration to quantify hERG channel inhibition.

For Purkinje fiber assay, transmembrane action potentials were recorded with conventional glass microelectrodes. Action potential parameters measured included resting membrane potential, overshoot, maximal upstroke velocity (V_{max}), and action potential duration (APD) time to 50% and 90% repolarization (APD50 and APD90).

Results (metabolites of BMS-354825):

- BMS-573188 inhibited hERG currents by $6.2 \pm 2.5\%$ and $10.6 \pm 2.8\%$ ($n=3$) at 10 and $30\mu\text{M}$, respectively.
- BMS-582691 inhibited hERG currents by $23.5 \pm 3.7\%$, $72.3 \pm 3.1\%$ and $94.7 \pm 0.5\%$ ($n=3$) at 3, 10 and $30\mu\text{M}$. The calculated IC_{50} is $5.8\mu\text{M}$.
- BMS-606181 inhibited hERG by $7.9 \pm 2.1\%$ and $11.5 \pm 2.7\%$ ($n=3$) at 10 and $30\mu\text{M}$, respectively.
- In the Purkinje fiber assay, BMS-573188 did not have any significant effects on any of the action potential parameters up to a maximal concentration of $30\mu\text{M}$.
- BMS-582691 affected APD, prolonging APD50 and APD90 by $9.9 \pm 2.9\%$ and $9.1 \pm 1.4\%$ ($n=3$), respectively, at $30\mu\text{M}$.
- BMS-582691 reduced V_{max} by 11.3 ± 1.8 ($n=3$) at $30\mu\text{M}$, and had no effect on other action potential parameters up to $30\mu\text{M}$.
- BMS-606181 reduced V_{max} by $9.1 \pm 3.6\%$ and $17.7 \pm 5\%$ ($n=3$) at $10\mu\text{M}$ and $30\mu\text{M}$, respectively.
- BMS-606181 had no effect on APD or other action potential parameters up to $30\mu\text{M}$.

Inhibition of V_{max} may be suggestive of sodium channel inhibition.

Summary of the study:

BMS-573188 (M6), BMS-582691 (M4) and BMS-606181 (M5), metabolites of BMS-354825, were tested in vitro for effects on a cardiac potassium channel (hERG/IKr) and rabbit Purkinje fiber action potentials. BMS-354825 was tested in both assays previously

and was shown to inhibit the K channel (hERG inhibitor with an $IC_{50}=14.3\mu M$) and caused prolongation of action potential duration.

BMS-573188 had minimal effect on hERG currents and on rabbit cardiac Purkinje fiber action potentials. BMS-582691 inhibited hERG with moderate potency but had minimal effect on Purkinje fiber action potential duration. Because BMS-582691 reduced the V_{max} (maximum upstroke velocity) in the Purkinje fiber assay, the hERG inhibition by this metabolite may be at least partially contributed to the blockage of Na channel. BMS-606181 had minimal effect on hERG currents and Purkinje fiber APD, but reduced action potential V_{max} in a dose-dependent manner.

2.6.2.4.1 Results of safety pharmacology studies:

Neurological effects:

No safety pharmacology study was conducted to assess dasatinib-induced neurological effects. Neurological effects were evaluated as part of single- or repeat-dose toxicology studies. Except for \uparrow muscle tone that was seen in HD (45 mg/kg or 540 mg/m²) monkeys in the single dose toxicology study, all other findings appear to be secondary to the unhealthy condition of the animals and not direct effects of dasatinib. Those included: \downarrow activity, ptosis, tremor, and abnormal behavior (in one HD monkey in the single dose study).

Cardiovascular effects:

The potential for dasatinib to cause cardiovascular toxicities has been evaluated through 1) in vitro hERG/IKr potassium channel assay, 2) in vitro rabbit Purkinje fiber assay, and 3) a single-dose oral study in conscious telemetered monkeys. In addition, cardiovascular toxicities were assessed as part of the single- and repeat-dose oral toxicity studies with dasatinib in rats and monkeys. Based on the hERG and rabbit Purkinje fiber assays, dasatinib has the potential to cause QT prolongation. However, QT prolongation was not observed in the EKG evaluations in the toxicology studies conducted in monkeys. There was a tendency for increased systolic, diastolic, and arterial blood pressure in monkeys at single doses of ≥ 10 mg/kg (120 mg/m²). Vascular and cardiac fibrosis, was reported in the chronic toxicology studies. Other findings included cardiac hypertrophy, myocardial necrosis, hemorrhage of the valves, ventricle, and atrium, and cardiac inflammation.

In addition to the parent compound, 3 metabolites of dasatinib, M4, M5, and M6 were tested in the in vitro hERG/IKr potassium channel and rabbit Purkinje fiber assays. BMS-573188 (metabolite M6) had minimal effects on hERG currents and on rabbit cardiac Purkinje fiber action potentials. BMS-582691 (metabolite M4) had moderate inhibition on hERG (24%, 72%, and 95% inhibition at 3, 10, and 30 μM) but had minimal effects on Purkinje fiber action potential duration. BMS-606181 (metabolite M5; major metabolite in rat) had minimal effect on hERG currents and Purkinje fiber APD, but reduced action potential V_{max} in a dose-dependent manner, suggesting inhibition of Na channel. None of these 3 metabolites are considered the major metabolite in human.

Results of cardiovascular toxicities of dasatinib are summarized below:

- Dasatinib inhibited hERG currents dose-dependently. Inhibition was 6%, 36% and 77% at 3, 10 and 30 μM , respectively. The IC_{50} was calculated to be 14.3 μM .
- Dasatinib prolonged action potential duration (APD times to 50% and 90% repolarization) in the rabbit Purkinje fiber assay. APD_{50} and APD_{90} were prolonged by 26% and 11%, respectively at 30 μM BMS354825.
- Single oral dose telemetry of dasatinib in Cynomolgus monkeys at 10 mg/kg (or 120 mg/m²) revealed \uparrow 5%-15% in the mean systolic and \uparrow 8%-21% in the mean diastolic blood pressure for approximately 2 hrs after dose.
- Cardiotoxicity was observed in the single oral dose toxicity study in SD rats at dasatinib doses of 30, 100, or 300 mg/kg (180, 600, or 1800 mg/m²). Multifocal myocardial necrosis (ventricular) and hemorrhage (valvular, ventricular, and atrial) occurred at MD and HD and cardiac hypertrophy occurred starting at the LD.
- Single oral dose toxicity study was conducted in Cynomolgus monkeys at dasatinib doses of 15, 25, or 45 mg/kg (180, 300, or 540 mg/m²). A potential for cardiotoxicity was observed as \uparrow systolic, diastolic, and mean arterial blood pressures. Blood pressures were not attainable on Day 1. Diastolic and systolic blood pressures and mean arterial blood pressure were increased on Day 11 in LD and MD ♂ s (no data available for HD animals due to mortality). It is not clear whether dasatinib treatment contributed to the heart murmur in 1 MD ♂ on Day 14. No other changes in the cardiac parameters (heart rate, oxygen saturation, EKG parameters) were noted. Organ weights were not assessed in this study.
- One month repeat-dose toxicity study of dasatinib in rats at doses of 1, 15, and 25 mg/kg (6, 90, and 150 mg/m²) 5-days-on 2-days-off, showed cardiac hypertrophy at MD and HD at the end of the one-month treatment period (MD: C_{max} =52-58 ng/mL and AUC =700-830 ng.hr/mL; HD: C_{max} =50-64 ng/mL and AUC = 945-950 ng.hr/mL; Day 26 data) and at the end of the 2-week recovery period. Cardiac hypertrophy was more pronounced in ♀ s.
- Six-month repeat-dose toxicity study in SD rats at dasatinib doses of 1.5, 4, and 15/10/8 mg/kg/day (9, 24, and 90/60/48 mg/m²/day) revealed cardiac hypertrophy at all dose levels at the end of the 6-month treatment period and mainly at MD and HD at the end of the 4-week recovery period. In addition, based on the histopathology examination, few HD animals had fibrosis in the heart (2/19 HD ♂ s and 1/18 HD ♀) at the end of the treatment period.
- One month oral toxicity study of dasatinib in Cynomolgus monkeys at doses of 0, 1, 5 and 15 mg/kg (12, 60, and 180 mg/m²), 5-days-on 2-days-off, showed cardiac hypertrophy (HD) and chronic inflammation (LD, MD, and HD). Both findings were observed at the end of the recovery period at HD. No QT prolongation was noticed.
- 9-month repeat-dose toxicity study in monkeys did not reveal any clear sign of dasatinib-related cardiac toxicity; e.g. in unscheduled sacrifices,

degeneration/necrosis of cardiomyocytes was observed in both control (1/1 ♀) as well as treated animals (1/4 MD ♀ and 1/2 HD ♀). However, vascular mineralization was observed and was noted in multiple organs, including the heart. Vascular inflammation in the heart was noted in one (1/4 MD ♀) dasatinib-treated unscheduled death. Hyperplasia/hypertrophy of aorta was observed in 1/2 HD scheduled sacrifice (♀, end of treatment); neutrophilic/lymphohistocytic infiltrate in the heart was also reported with higher incidence in dasatinib treated groups.

Pulmonary effects:

No safety pharmacology study was conducted to assess dasatinib-induced pulmonary effect. Labored/irregular breathing was observed in the one-month (at 25 mg/kg, 150 mg/m²) and 6-month (15/10/8 mg/kg, 90/60/48 mg/m²) rat toxicology at high doses that resulted in moribund condition of the animals and appear to be secondary to the unhealthy conditions of the animals and not a direct effect of dasatinib.

Renal effects:

No safety pharmacology study was conducted to assess dasatinib-induced renal toxicities. Renal adverse effects were observed in the single- and repeat-dose toxicology studies conducted in rats and monkeys.

Gastrointestinal effects:

No safety pharmacology studies were conducted to assess GI effects. However, GI toxicities were assessed as part of the single-dose and repeat-dose toxicology studies. Studies showed that dasatinib can cause severe adverse effects in the GI tract.

Abuse liability: not assessed

2.6.2.5 Pharmacodynamic drug interactions

Combination treatment with BMS-354825 and paclitaxel were conducted and described in Report # 930003300. These studies have not been reviewed.

The following has been excerpted from the sponsor's summary as submitted in Module 2 of the NDA. Individual studies have not been reviewed. Murine BAF/3 cells engineered to be solely dependent on BCR-ABL for survival and proliferation were used in the PD interaction studies.

Nonclinical studies were conducted to evaluate the interaction between dasatinib and imatinib when these 2 agents were used in combination in the treatment of models of CML harboring the wild-type BCR-ABL protein or various mutation variants that confers varying degree of imatinib resistance. It was demonstrated that dasatinib retained its full inhibitory activities against both wild-type and mutant BCR-ABL. Activity of the 2 agents when tested in combination was greater than for either agent when tested alone at similar concentrations. Co-treatment with imatinib and dasatinib resulted in lower IC₅₀ values for dasatinib in wild-type BCR-ABL and 3 imatinib-

resistant mutants (M351T, Y253F, and E255K). Neither imatinib nor dasatinib was active against the T315I mutant (a cell with a mutated imatinib contact-residue within the drug-binding site).

2.6.3 PHARMACOLOGY TABULATED SUMMARY

The following Tables have been submitted by the sponsor. Only selected Tables are presented here.

**APPEARS THIS WAY
ON ORIGINAL**

In Vitro	Type of Study	Test System	Noteworthy Findings	Testing Facility	Report No.
Inhibitory activity against recombinant human BCR-ABL tyrosine kinase	Purified cytoplasmic/kinase domain fused to GST carrier	SRC Family Kinases: Inhibition for Dasatinib vs Imatinib	SRC IC ₅₀ = 0.6 nM vs 85,300 nM LCK IC ₅₀ = 1.1 nM vs 920 nM YES IC ₅₀ = 0.4 nM vs 31,000 nM FYN IC ₅₀ = 0.2 nM vs 38,200 nM BCR-ABL IC ₅₀ = 3.0 nM vs 790 nM c-KIT IC ₅₀ = 22 nM vs 169 nM PDGFRβ IC ₅₀ = 28 nM vs 1590 nM EPHA2 IC ₅₀ = 17 nM (imatinib not tested)	BMS	930003300
Cytotoxicity against BCR-ABL dependent human leukemic cell lines	K562 CML (erythromyeloblastoid), KU812 CML (myeloid), MEG-01 (megakaryocytic), and SUP-B15 (B cell ALL)	15 other kinases not inhibited with selectivity ratio between 100-20,000 fold	IC ₅₀ of 0.7 nM, 0.087 nM, 0.28 nM, and 1.0 nM, respectively	BMS	930003300
			Imatinib was 300-650x less active than dasatinib in these cell lines		

In Vivo Type of Study/Species/Strain	Schedule/Route/Duration of Study/ Vehicle/ Formulation	Range of Doses (mg/kg)	Gender and No. per group	Noteworthy Findings	Testing Facility	Report No.
Antileukemic activity versus CML/ mice/SCID	QD, 5 days per week for 2 weeks/PO/90 days/PG-H2O (50%/50%)	5-50	Female:8	Dasatinib was curative at a wide range of doses (5-50 mg/kg) versus K562 human CML. The level of activity was superior than imatinib administered at its MTD	BMS	930003300
Antileukemic efficacy versus CML/ mice/SCID	QD, 5 days per week for 2 weeks/PO/90 days/PG-H2O (50%/50%)	1.25-5	Female:8	Dasatinib cured 58% of mice at 5 mg/kg, and produced an active 0.7 Lck at 2.5 mg/kg versus K562 human CML	BMS	930003300
Antileukemic efficacy versus CML/ mice/SCID	QD, 5 days per week for 2 weeks/IV/90 days/PG-H2O (50%/50%)	2.5-10	Female:8	Dasatinib was curative from 2.5-10 mg/kg versus K562 human CML	BMS	930003300
Antileukemic efficacy versus CML/ mice/SCID	QDx5/PO/90 days/PG-H2O (50%/50%)	50	Female:8	Dasatinib was curative at 50 mg/kg versus KU812 human CML	BMS	930003300
Antileukemic efficacy versus CML/ mice/SCID	QD, 5 days per week for 2 weeks/PO/90 days/PG-H2O (50%/50%)	5-50	Female:8	Dasatinib was curative at a wide range of doses (5-50 mg/kg) versus the imatinib resistant human CML model K562/STI-571/R. Imatinib was inactive at all dose levels	BMS	930003300
Antileukemic efficacy versus imatinib resistant K562/ADM CML	QD x 14/PO/90 days/PG-H2O (50%/50%)	5-30	Female/6	Dasatinib was curative at 30 mg/kg and significantly active at 15 mg/kg producing growth delay of 16.7 days (P=0.0009). The dose of 5 mg/kg was	BMS	930012327

In Vivo Type of Study/Species/Strain	Schedule/Route/ Duration of Study/ Vehicle/ Formulation	Range of Doses (mg/kg)	Gender and No. per group	Noteworthy Findings	Testing Facility	Report No.
model/ mice/SCID						
Antileukemic efficacy versus K562 CML model implanted in the brain	BID x 10/PO/30 days/PG-H ₂ O (50%/50%)	5	Female/6 -8	Dasatinib significantly prolonged survival of mice (LS 175%, P<0.01). Imatinib was inactive (LS = 125%)	BMS	930012327
Antileukemic efficacy - Schedule dependency - Effects of a treatment break	BID x 10/PO/30 days/PG-H ₂ O (50%/50%)	2.5-5.0	Female/6 -8	A treatment break of 2 days out of every 5 days treatment did not significantly affect the antileukemic activity of dasatinib	BMS	930012327
Antileukemic efficacy - Schedule dependency - QD versus BID	QD and BID x 10, 5- days-on, 2-days-off/PO/90 days/PG-H ₂ O (50%/50%)	1.25-5.0	Female/8	At a fixed total daily dose, BID is more efficacious than QD. However at doses of 5 mg/kg or greater, both schedules were active (curative)	BMS	930012327
In Vivo						
Combination chemotherapy study with paclitaxel/Rabbit	BID, 5 days per week for 14 days/PO/90 days/PG-H ₂ O (50%/50%)	10	Female/8	The combined regimen produced antitumor effect that was more than additive of the individual effects of the single agent alone in the PC3 prostate cancer model	BMS	930003300
	Paclitaxel, weekly x 3/IV/90days/ Cremophor/EtOH/ H ₂ O (10/10/80%)	18		Paclitaxel (18 mg/kg) - Growth delay (22.5 d) Dasatinib (10 mg/kg) - Growth delay (4.5 d) Combination - Growth delay (37.2 d)		

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

In brief, after oral administration, dasatinib was generally rapidly absorbed, with a T_{max} of 1-2 hrs in the single dose studies in rats and monkeys and in a repeat-dose toxicology study in monkeys. Larger variations were reported in rats in the repeat-dose studies, with a T_{max} of 2-8 hrs. The parent drug and the metabolites are distributed to several organs/tissues, mainly by 4 hrs post-dose. Dasatinib is highly metabolized. Only involvement of CYP3A4 has been verified in vivo, which appears to play a major role in human metabolism. Elimination of dasatinib takes place mainly in the first 48 hrs post-dose. Elimination is mostly hepatic/biliary.

Absorption:

- Oral administration of dasatinib to rats and monkeys resulted in T_{max} mainly ranging from 1-8 hrs.
- There were no clear gender-dependent differences in the exposures in the single-dose or repeat-dose toxicology studies, when dasatinib was orally administered to rats or monkeys. Systemic exposures to dasatinib were generally dose-proportional.
- There was no obvious accumulation of the drug after repeated daily dosing to rats and monkeys.
- Based on the summary data presented, bioavailability ranged from 14% (mouse) to 34% (dogs). Bioavailability was 27% in rats and 15% in monkeys.

Distribution:

- Dasatinib was 97% protein bound in rats and monkeys and 96% protein bound in human in the in vitro studies. The N-dealkylated metabolite (BMS-582691, M4) was also highly bound (>93%) to human serum. The protein binding of dasatinib and BMS-582691 was not concentration-dependent from 100 ng/mL to 500 ng/mL.
- The steady state volume of distribution of dasatinib in mice, rats, dogs, and monkeys was greater than the volume of total body water, suggesting extravascular distribution of the drug.
- Drug-related radioactivity was distributed in the tissues in rats after oral administration of [^{14}C]dasatinib. The highest percentages of dosed radioactivity were associated with the tissues of the GI tract and liver, which was consistent with the oral route of administration and major route of excretion in the bile. Mean concentrations of radioactivity were highest in the GI tract (mainly Hrs 1-12). High levels of radioactivity were also detected in the following organs/tissues from the highest to the lowest levels of radioactivity: liver, adrenal glands, kidneys, lungs, spleen, urinary bladder, thyroid, femoral bone marrow, eyes, heart, skeletal muscle.
- T_{max} of radioactivity was 4 hrs for most organs/tissues. T_{max} for the GI tract was 1 hr. T_{max} for the eyes was 12 hrs. T_{last} for tissues ranged from 4 hrs

(blood, bone, skeletal muscle) to 168 hrs (adrenal glands, eyes, kidneys, and liver).

- The radioactivity was eliminated mainly within the 48 hrs after dosing. Only 0.7% of the radioactivity was detected at Hr 48. Total amount of radioactivity was 0.01% by Day 7.

Metabolism:

- The plasma metabolite profile of humans was most similar to the monkey profile.
- Dasatinib was highly metabolized in human (parent compound accounted for 26% of the plasma radioactivity) and monkey (parent compound accounted for 32% of the plasma radioactivity). In rat plasma, the parent drug was 53% of the radioactivity.
- Major metabolic pathways of dasatinib in human involved hydroxylation on the chloromethylphenyl ring followed by sulfation, oxidation of the hydroxyethyl moiety to the carboxylic acid, N-oxidation of the piperazine ring, and products formed by a combination of the above pathways
- In human, metabolite M20 (4-OH-chlormoethylphenyl dasatinib) and its sulfate conjugate (M21) were detected in significant amount (13% and 10%, respectively) in the plasma. Rats had no detectable amounts of M20 in the plasma. In monkeys, the amount of M20 in plasma was only 2.8% of the radioactivity. Therefore, neither species could assess toxicities of M20 in humans.
- M5 was the major metabolite in rats' plasma and M8a was the major metabolite in monkeys' plasma.
- In rats and monkeys, unchanged parent drug represented only a small fraction of the radioactivity in bile and urine, suggesting that metabolism plays a major role in the elimination of dasatinib in these species.
- Multiple enzymes were involved in the oxidative metabolism of dasatinib, when tested in vitro, including CYP3A4 and FMO3. Other enzymes capable of metabolizing dasatinib were: CYP1A1, 2A6, 1B1, 2B6, 2C8, 2C9, 2E1, and 4A11. Only the contribution of CYP3A4 has been verified in vivo. The involvement of CYP3A4 in the metabolism of dasatinib was confirmed in a clinical study where the systemic exposure of dasatinib was decreased significantly (> 80%) in healthy human subjects co-administered dasatinib after 8 days of continuous dosing with rifampin, a potent CYP3A4 inducer.

Excretion:

- Fecal excretion was the main route of excretion for dasatinib and its metabolites, when dosed orally to intact monkeys, as shown by the 77% recovery of radioactivity in feces. In the bile duct cannulated monkeys, 14% of the radioactivity was recovered in the feces. Biliary excretion was the major excretion route as indicated by the amount of total biliary radioactivity in i.v.-dosed monkeys (approximately 61-67% of the dose was detected in the bile in different studies).
- Radioactivity was excreted at a moderately rapid rate in monkeys, with >80% of the dose recovered within 48 hrs of dosing.

- Urinary excretion was low in monkeys (3-10% of radioactivity was recovered in the urine in different studies)
- Radioactivity was measurable through 8 hrs post-dose in monkeys in both the oral and i.v. dosing (Studies MBA00097 and MBA00127).
- The terminal half life following oral administration ranged from 2 to 5 hours, for mouse, rat, dog and monkey. Terminal half-life was similar across species which was comparable to that in humans.

For permeability in Caco-2 cells, p-glycoprotein studies, and studies of the CYP induction and inhibition, see the clinical pharmacology review of the NDA.

2.6.4.2 Methods of Analysis

Also see under individual study reviews

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Validated analytical methods

Species	Rat	Rabbit	Monkey	Human	Human
Matrix	Plasma	Plasma	Plasma	(Serum;dialysis buffer;1:1)	(Serum;dialysis buffer;1:1)
Analyte	dasatinib	dasatinib	dasatinib	dasatinib	BMS-582691 (M4)
Method	LC/MS/MS	LC/MS/MS	LC/MS/MS	LC/MS/MS	LC/MS/MS
Volume/Amount	0.1 mL	0.1 mL	0.1 mL	0.1 mL	0.1 mL
Standard Curve Range	2 to 2000 ng/mL	2 to 2000 ng/mL	2 to 2000 ng/mL	1 to 1000 ng/mL	1 to 1000 ng/mL
Regression Model	1/x weighted quadratic	1/x weighted quadratic	1/x weighted quadratic	1/x ² weighted quadratic	1/x ² weighted quadratic
Precision: (% CV)					
Intra Assay	≤ 4.6%	≤ 0.8%	≤ 3.2%	≤ 6.3%	≤ 7.5%
Inter Assay	ND	ND	≤ 3.2%	ND	ND
Accuracy (%Deviation)	Within ± 13.9%	Within ± 4.7%	Within ± 5.2%	Within ± 14.5	Within ± 11.5
Stability					
RT	24 h	24 h	24 h	ND	ND
-20°C	≥ 14 weeks	≥ 4 weeks	≥ 17 weeks	≥ 18 days	≥ 18 days
Freeze Thaw	3 cycles	ND	3 cycles	ND	ND
DCN	930003278	930010742	930003277	930011547	930011547
Documents Where Methods Were Used	930011508 930003258 930011518	930010604	930003271 930003259 930011520	930011593 930011548	930011593 930011548
ND = not determined					

Table provided by the sponsor.

2.6.4.3 Absorption

Toxicokinetic parameters were evaluated as part of the rat and monkey toxicology studies. Details of the TK evaluations are described under each toxicology study.

Summary of the studies are shown in this section:

Single-dose toxicology in monkeys:

Doses of dasatinib administered: 15, 25, or 45 mg/kg (180, 300, or 540 mg/m²); single doses. Scheduled sacrifices were conducted on Days 4 and 15.

- Systemic exposure following single oral doses of BMS-354825 was dose proportional from LD to MD and was less than dose proportional from MD to HD.
- There were no apparent gender-related differences in the systemic exposure.
- Tmax was 1-2 hrs.

Dose (mg/kg)	Cmax (ng/ml)		Tmax (hr)		AUC* (ng.hr/ml)	
	Male	Female	Male	Female	Male	Female
15	757	1492	1	1	2225	2760
25	1079	828	1.5	1.5	5373	3801
45	1763	2107	1	2	7771	8745
1:1.7:3 Dose Ratio	1:1.4:2.3	1:0.6:1.4			1:2.4:3.5	1:1.4:3.2

* AUC calculated from time zero to the time of last measurable concentration, equal to 24 hr/ ranging between 12 and 24 hr.

Table provided by the sponsor.

One-month toxicology in rats:

Doses administered: 1, 15, and 25 mg/kg (6, 90, and 150 mg/m²) 5 days-on 2-days-off x 4 cycles.

- Systemic exposure to BMS-354825 was generally dose proportional
- Exposures were similar between males and females.
- An overall decrease in exposure occurred upon repeated cycles in both genders at MD and HD.
- Tmax was 2-8 hrs in ♀s and 4-8 hrs in ♂s.

BMS-354825							
Dose [mg/kg/day]	Study Day	C _{max} (ng/mL)		T _{max} (h)		AUCT ^a (ng.h/mL)	
		Male	Female	Male	Female	Male	Female
0.9	1	7.9	9.8	4	4	34	45
	26	6.6	13.4	4	2	32	41
15	1	96.1	88.4	8	8	937	920
	26	57.7	51.8	8	2	827	705
25	1	102.1	184.2	8	4	1315	1737
	26	49	63.8	4	4	951	944
Ratios							
0.9:15:25 Dose Ratio	1	1:12.2:12.9	1:9.0:18.8			1:27.6:38.7	1:20.4:38.6
	26	1:8.7:7.4	1:3.9:4.8			1:25.8:29.7	1:17.2:23

^a Calculated from time zero to the time of last measurable concentration, equal to 24 h, ranging between 8 to 24 h.

Table provided by the sponsor.

Six-month toxicology in rats:

Doses administered: (1.5, 4, and 15/10/8 mg/kg/day) 9, 24, and 90/60/48 mg/m²/day x 6 months (26 weeks).

- Because HD animals were given different doses of dasatinib, a conclusive comparison of LD and MD to HD cannot be made.
- Systemic exposure to BMS-354825, following daily administration of 1.5 mg/kg/day (LD) and 4-mg/kg/day (MD) for 26 weeks was approximately dose proportional.
- At the high-dose, systemic exposure to BMS-354825 was slightly greater than dose proportional.
- There was no clear evidence of drug accumulation over the course of the study. There were instances of ↑ and ↓ in exposure (C_{max} and AUC) after repeated dosing at LD and MD. The AUCs were slightly higher after repeated dosing in LD and MD ♂s and ♀s (Week 13 or 26 compared to Day 1).
- There were no clear gender-dependent effects on exposure.
- T_{max} was mostly 2-4 hrs, with the exception of the HD ♂s, which had t_{max} of 8 hrs on Day 1 and Week 13.

Dose (mg/kg/day)	Study Day (Week)	C _{max} (ng/mL)		T _{max} (hr)		AUC _T ^a (ng/mL•hr)	
		Male	Female	Male	Female	Male	Female
1.5	Day 1	11.9	11.2	4.0	4.0	72.6	62.9
1.5	Week 13	8.3	12.0	4.0	2.0	48.2	114.2
1.5	Week 26	15.8	24.6	2.0	2.0	87.7	104.1
4	Day 1	39.8	34.7	4.0	4.0	250.9	244.3
4	Week 13	46.7	29.7	2.0	4.0	241.0	194.1
4	Week 26	29.8	52.9	2.0	2.0	322.7	416.3
15	Day 1	116.1	92.3	8.0	4.0	1335.2	1077.0
10	Week 13	49.0	82.1	8.0	2.0	564.2	972.6
8	Week 26	120.3	109.6	2.0	2.0	551.4	777.3
Ratios							
1:2.7:10	Day 1	1:3.3:9.8	1:3.1:8.2	-	-	1:3.5:18.4	1:3.9:17.1
1:2.7:6.7	Week 13	1:5.6:5.9	1:2.5:6.8	-	-	1:5.0:11.7	1:1.7:8.5
1:2.7:5.3	Week 26	1:1.9:7.6	1:2.2:4.5	-	-	1:3.7:6.3	1:4.0:7.5

a Calculated from time zero to the time of last measurable concentration, ranging between 8 to 24 hours.

Table provided by the sponsor.

1-month toxicology in monkeys:

Doses administered: 1, 5, and 15 mg/kg (12, 60, and 180 mg/m²); 5-days-on 2-days-off x 4 cycles (4 weeks)

- Systemic exposure to BMS-354825 in monkeys was greater than dose-proportional at HD.
- Exposures were comparable between males and females.
- AUCs were generally similar between Days 1 and 26 indicating BMS-354825 did not accumulate with repeated intermittent dosing in monkey.

BMS-354825							
Dose [mg/kg/day]	Study Day	C _{max} (ng/mL)		T _{max} ^a (h)		AUC _T ^b (ng•h/mL)	
		Male	Female	Male	Female	Male	Female
1	1	20	11	1.5	1	36	17
	26	12	8	2	1.5	34	16
5	1	91	93	1.5	1	206	221
	26	50	64	1.5	2	181	280
15	1	480	399	1	1.5	1162	1053
	26	154	374	1.5	1.5	774	976
Ratios							
1:5:15 Dose Ratio	1	1:4.6:24	1:8.5:36.3			1:5.7:32.3	1:13:61.9
	26	1:4.2:12.8	1:8:46.8			1:5.3:22.8	1:17.5:61

^a Median value

^b Calculated from time zero to the time of last measurable concentration, equal to 24 h. ranging between 8 to 24 h.

Table provided by the sponsor.

9-month toxicology in monkeys:

Doses administered:

- Initial dose: 1, 3, 10 mg/kg/day x 8
No dosing on Days 9-15
- Modified dose (from D16): 1, 3, 6 mg/kg; 5-days on/ 2-days off
- Modified dose (from D83): 1, 3, 4.5 mg/kg; 5-days on/ 2-days off (x 22 weeks for HD)
- Modified dose (from D190): 1, 2.5, no HD animal; 5-days on/ 2-days off (12, 24 mg/m², 5-days on/ 2-days off x 41 weeks for LD and MD)

- Systemic exposure to BMS-354825 on Day 1 increased in a greater-than-dose proportional manner between 1 and 10 mg/kg.
- However, for Weeks 15, 28, and 41, the systemic exposure increased in a generally dose-proportional manner.
- Systemic exposure to BMS-354825 was generally similar between females and males.
- There was no obvious accumulation or reduction in the exposure of BMS-354825 over the 41 weeks of dosing.
- As expected, exposures were generally lower at later times in the HD and MD groups as the dose was reduced.

Dose (mg/kg)	Study Interval	C _{max} (ng/mL)		AUC (ng•hr /mL) ^a	
		Male	Female	Male	Female
1	Day 1	16.4	11.8	38.2	26.3
1	Week 15	13.0	17.5	36.5	38.6
1	Week 28	11.8	19.8	27.1	36.0
1	Week 41	28.7	31.3	56.2	54.1
3	Day 1	50.2	55.6	148.7	130.5
3	Week 15	22.7	47.4	107.3	118.2
2	Week 28	21.0	29.4	85.7	79.1
2	Week 41	55.5	52.4	146.3	93.1
10	Day 1	291.1	244.7	949.0	755.2
4.5	Week 15	115.7	68.9	315.9	206.4
4.5	Week 28	ND	ND	ND	ND
4.5	Week 41	ND	ND	ND	ND

ND = Not determined; due to toxicity, the high-dose group was terminated during Week 26.

a Calculated from time zero to the time of last measurable concentration, ranging between 8 to 24 hours.

Toxicokinetic Ratios

Dose Ratio	Study Interval	C _{max} Ratios		AUC Ratios	
		Male	Female	Male	Female
1:3:10	Day 1	1: 3.1: 17.8	1: 4.7: 20.7	1: 3.9: 24.8	1: 5.0: 28.7
1:3:4.5	Week 15	1: 1.7: 8.9	1: 2.7: 3.9	1: 2.9: 8.7	1: 3.1: 5.3
1:2	Week 28	1: 1.8	1: 1.5	1: 3.2	1: 2.2
1:2	Week 41	1: 1.9	1: 1.7	1: 2.6	1: 1.7

Tables provided by the sponsor.

Additional Studies

Individual single-dose PK studies conducted to obtain the data presented in Table below have not been reviewed; only the summary as submitted by the sponsor is presented.

Pharmacokinetic parameters of dasatinib in mouse, rat, dog, and monkey.

Species	Route	Dose (mg/kg)	C _{max} (µg/mL)	T _{max} (h)	AUC(0-∞) (µg•h/mL)	t _{1/2} (h)	CL (mL/min/kg)	V _{ss} (L/kg)	F (%)
Mouse ^a	IV	10	-	-	2.7	0.9	61.7	4.2	-
	PO	5	0.051	2	0.22	2.5	-	-	17
	PO	15	0.16	2	0.58	2.0	-	-	14
Rat ^a	IA	10	-	-	6.8±2.3	3.3±0.9	26.4±7.8	6.3±2.2	-
	PO	10	0.24±0.09	2.3±3.3	1.9±1.0	3.1±0.3	-	-	27±15
Dog ^b	IV	1.2	-	-	0.82±0.20	4.2±2.0	25±6.3	4.7±0.8	-
	PO	3	0.14±0.04	0.75±0.25	0.68±0.17	5.0±1.8	-	-	34±13
Monkey ^b	IV	2	-	-	0.98±0.11	2.1±0.1	34±4.1	3.5±0.1	-
	PO	5	0.17±0.03	0.6±0.1	0.37±0.02	2.2±0.4	-	-	15±2

^a Dosing vehicle was propylene glycol: water (1:1).

^b Dosing vehicle was 50 mM sodium acetate buffer, pH 4.6.

Table provided by the sponsor.

Rat:

Two pharmacokinetic studies were performed in male Sprague-Dawley rats. One study was conducted to characterize the pharmacokinetics of dasatinib and to assess the absolute oral bioavailability. The second study was conducted to assess the contribution of hepatic extraction on the oral bioavailability of dasatinib.

The mean absolute oral bioavailability of dasatinib was approximately 27% in fasted male Sprague-Dawley rats receiving a single oral gavage dose of 10 mg/kg dasatinib in 50% propylene glycol in water. Dasatinib was also dosed to fasted Sprague-Dawley rats via portal vein infusion over 30 min at a 10-mg/kg dose to assess the contribution of hepatic extraction to the oral bioavailability. The AUC value of dasatinib after portal vein infusion was comparable to that after intra-arterial infusion (10 min) (7.7 ± 2.7 vs 6.8 ± 2.3 µg•h/mL, n = 3), suggesting that dasatinib does not undergo substantial hepatic first-pass extraction in rats.

Monkey:

Two pharmacokinetic studies were performed in male cynomolgus monkeys. One study was conducted to characterize the pharmacokinetics of dasatinib and to assess absolute oral bioavailability. The other study was conducted to assess plasma exposure of dasatinib following a capsule dosage of the free base or the —. The systemic plasma clearance and the steady-state volume of distribution of dasatinib were 34 ± 4.1 mL/min/kg and 3.5 ± 0.1 L/kg, respectively, in fasted male cynomolgus monkeys following a 10-minute intravenous infusion of 2 mg/kg dasatinib.

The mean absolute oral bioavailability of dasatinib was $15 \pm 2\%$, $13 \pm 8\%$, and 10% in fasted male cynomolgus monkeys receiving a single oral dose of dasatinib at 5 mg/kg in

solution in sodium acetate buffer (50 mM, pH 4.6), 4.7 mg/kg as free base in capsule and 4.9 mg/kg as — a capsule, respectively. The systemic exposure in monkeys after solid oral capsule dosage form as either the free base or the — was lower by approximately 19 and 32%, respectively, compared to that from the solution dose group. However, exposure between the free base and the — forms appeared to be comparable.

2.6.4.4 Distribution

Study Title: Tissue distribution of radioactivity in male Long-Evans rats following oral administration of [¹⁴C]BMS-354825

Key study findings:

- Mean concentrations of radioactivity were highest in the GI tract (mainly Hrs 1-12). High levels of radioactivity were also detected in the following organs/tissues from the highest to the lowest levels of radioactivity: liver, adrenal glands, kidneys, lungs, spleen, urinary bladder, thyroid, femoral bone marrow, eyes, heart, skeletal muscle
- Tmax of radioactivity was 4 hrs for most organs/tissues. Tmax for the GI tract was 1 hr. Tmax for the eyes was 12 hrs. Tlast for tissues ranged from 4 hrs (blood, bone, skeletal muscle) to 168 hrs (adrenal glands, eyes, kidneys, and liver).
- Estimation of the exposures to radioactivity indicated the following order from the highest to the lowest exposures (AUC_{last}): GI tract, eyes, liver, adrenals, kidneys, lungs, spleen, thyroid and urinary bladder, bone marrow, heart, skin, plasma, bone, testes, skeletal muscle.
- Elimination half-life estimates for BMS-354825 equivalents ranged from a high of 242 hrs for eyes to a low of 1.6 hrs for plasma. The next highest t_{1/2} estimates were for adrenal glands (40.5 hrs) and liver (40.0 hrs).
- Maximal mean tissue : plasma ratios were highest for tissues of the GI tract followed by: liver (19:1 at Hr 4), adrenal glands (13: 1 at Hr 4), lungs (12:1 at Hr 4), kidneys (11.5:1 at Hr 4), spleen (9:1 at Hr 4), thyroid (6:1 at Hr 4), eyes (5.8:1 at Hr 1), urinary bladder (5.8:1 at Hr 4), femoral bone marrow (5.7:1 at Hr 4), heart (2.8:1 at Hr 4), skeletal muscle (1.8:1 at Hr 4). Only brain, testes, and bone had ratios less than unity at both times.
- The radioactivity was eliminated mainly within the 48 hrs after dosing. Only 0.7% of the radioactivity was detected at Hr 48. Total amount of radioactivity was 0.01% by Day 7.

Study no.: MBA00038

Volume/ Page number: Item 5

Conducting laboratory and location: /

Date of study initiation: April 2004

GLP compliance: yes () no (X)

Drug, lot #, radiolabel, and % purity: [¹⁴C]BMS-354825 — Lot# 001,
radiochemical purity of — 18.7 µCi/mg

<u>Non-radiolabeled Test Article:</u>	BMS-354825-03
Compound:	BMS-354825 monohydrate
Manufacturer/Supplier:	Bristol-Myers Squibb Company
Source Batch Number:	JL71076-017
Physical Description:	Solid
Purity "as is" (free base):	—

Methods:

Species: Long-Evans rats (♂s)

Dose: 10 mg/kg (100µCi/kg); single dose; oral gavage

Study design:

The study consisted of 8 groups of 3 male Long-Evans rats per group.

Group 1: control

Groups 2-8: dosed with test article at 10 mg/kg. Groups 2-8 were sacrificed at different time-points

Group 2: 1 hr post-dose

Group 3: 4 hrs post-dose

Group 4: 12 hrs post-dose

Group 5: 24 hrs post-dose

Group 6: 48 hrs post-dose

Group 7: 96 hrs post-dose

Group 8: 168 hrs post-dose

Aliquots of blood were taken for radio-analysis and the remaining blood was processed for plasma. After euthanasia of the animals, selected tissues and gastrointestinal contents were collected from each animal and the residual carcass was saved. Samples were analyzed for total radioactivity. The concentration of BMS-354825-equivalents of radioactivity in tissue samples was determined and tissue:plasma concentration ratios were calculated. Mean BMS-354825-equivalent concentrations of radioactivity were used to generate a composite concentration-time profile for each tissue. Composite radioactivity concentration-time data for blood, plasma, and tissues were subjected to non-compartmental pharmacokinetic analyses and dosimetry calculations were performed to estimate expected human exposure.

The tissues and gastrointestinal contents listed below were collected from each animal after euthanasia:

Adrenal Glands	Intestinal Contents, Large	Skin, Nonpigmented ^a
Bone (femur) ^a	Intestine, Small	Spleen
Bone Marrow (femur) ^a	Intestinal Contents, Small	Stomach
Brain	Kidneys	Stomach Contents
Cecum	Liver	Testes
Cecum Contents	Lungs	Thyroid
Eyes	Skeletal Muscle (thigh) ^a	Urinary Bladder
Heart	Skeletal Muscle (pectoral) ^a	
Intestine, Large	Skin, Pigmented ^a	Residual Carcass

a: Representative sample: entire tissue was not collected/weighed.

Results:

Concentrations of radioactivity in tissues

See Table below.

- Mean concentrations were highest in the GI tract (mainly Hrs 1-12, up to 46 µg equivalent)
- High levels of radioactivity were also detected in the following organs/tissues (peak levels are listed): liver (8.5 µg equivalent at Hr 4), adrenal glands (5.7 µg equivalent at HR 4), kidneys (5.1 µg equivalent at Hr 4), lungs (5.1 µg equivalent at Hr 4), spleen (4.0 µg equivalent at Hr 4), urinary bladder (2.7 µg equivalent at Hr 4), thyroid (2.7 µg equivalent at Hr 4), femoral bone marrow (2.5 µg equivalent at Hr 4), eyes (1.3 µg equivalent at Hr 12), heart (1.24 µg equivalent at HR 4)
- Based on the maximum levels of radioactivity (see numbers above), the peak distribution of radioactivity was mainly at Hr 4. The highest level of radioactivity in the GI tract was detected at Hr 1 (levels were still high at Hrs 4 and 12) and in the eyes was detected at Hr 12.
- Mean blood:plasma concentration ratios were approximately unity at Hrs 1 and 4, suggesting non-preferential partitioning of [14C]BMS-354825-derived radioactivity.

Pharmacokinetic estimates for concentrations of radioactivity in plasma and tissues:

See Table below.

Estimation of the exposures to radioactivity indicated the following order from the highest to the lowest exposures (AUC_{last}): GI tract, eyes, liver, adrenals, kidneys, lungs, spleen, thyroid and urinary bladder, bone marrow, heart, skin, plasma, bone, testes, skeletal muscle.

Mean percentage of radioactive dose in tissues and GI contents

See Table below.

Mean percent of radioactive dose indicated that:

- The radioactivity was eliminated mainly within the 48 hrs after dosing. Only 0.7% of the radioactivity was detected at Hr 48. The mean total percentage of

the radioactive dose in all matrices at the first sampling time (1 h post-dose) was 100.8%. Mean total percentages decreased to 33.5% at 12 hr post-dose and to 8.2% at 24 hr post-dose.

- At sampling times through 96 hr post-dose, the largest fractions of the radioactive dose were associated with contents of the gastrointestinal tract and the distribution of radioactivity at each time was reflective of movement of the [14C]BMS-354825-derived radioactivity through the gastrointestinal tract.
- High percentage of the radioactive dose was also detected in the liver and the skeletal muscle. High percentage in the liver may reflect liver uptake and metabolism.

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Mean BMS-354825-equivalent Concentrations of Radioactivity in Tissues After a Single Oral (10 mg base/kg, 120 µCi/kg) Administration of [¹⁴C]BMS-354825 to Male Long-Evans Rats

Matrix	Concentration (µg equivalents of BMS-354825/g)																				
	1 h			4 h			12 h			24 h			48 h			96 h			168 h		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD			
Adrenal Glands	0.637	0.172	5.65	1.38	0.808	0.133	0.425	0.0628	0.154	0.0502	0.131	0.0656	0.0215	0.0373	0	0	0	0	0		
Blood	0.0800	0.0295	0.457	0.0762	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Bone (femur)	0	0	0.375	0.0653	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Bone Marrow (femur)	0.223	0.0670	2.48	0.214	0.196	0.055	0	0	0	0	0	0	0	0	0	0	0	0	0		
Brain	0.00340	0.00589	0.0323	0.00471	0.0161	0.00636	0.00691	0.00603	0	0	0	0	0	0	0	0	0	0	0		
Cecum	0.144	0.0344	11.8	4.75	5.00	1.79	0.788	0.580	0.101	0.0906	0.0216	0.0375	0	0	0	0	0	0	0		
Eyes	0.383	0.604	0.535	0.393	1.31	0.712	0.837	0.260	0.566	0.353	0.690	0.253	0.482	0.185	0	0	0	0	0		
Heart	0.136	0.0625	1.24	0.246	0.111	0.0455	0.0295	0.00339	0.00993	0.00905	0.00383	0.00664	0	0	0	0	0	0	0		
Intestine, Large	0.120	0.0458	3.46	1.90	1.69	1.24	0.460	0.267	0.0501	0.0381	0.00680	0.0118	0	0	0	0	0	0	0		
Intestine, Small	45.7	3.55	19.6	11.7	0.537	0.342	0.319	0.219	0.0133	0.0128	0.0197	0.0341	0	0	0	0	0	0	0		
Kidneys	0.789	0.175	5.12	1.32	0.472	0.144	0.172	0.0352	0.0615	0.0168	0.0343	0.0129	0.00570	0.00987	0	0	0	0	0		
Liver	1.25	0.515	8.46	2.13	0.541	0.180	0.188	0.0524	0.0858	0.0322	0.0454	0.0188	0.0110	0.0110	0	0	0	0	0		
Lungs	0.411	0.0962	5.11	0.0557	0.394	0.253	0.0933	0.0160	0.0196	0.00699	0.00497	0.00860	0	0	0	0	0	0	0		
Plasma	0.0790	0.0242	0.438	0.0591	0.0145	0.0251	0	0	0	0	0	0	0	0	0	0	0	0	0		
Skeletal Muscle (pectoral)	0	0	0.790	0.0821	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Skeletal Muscle (thigh)	0	0	0.687	0.0678	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Skin, Nonpigmented	0	0	0.606	0.178	0.111	0.0292	0	0	0	0	0	0	0	0	0	0	0	0	0		
Skin, Pigmented	0	0	0.531	0.0256	0.249	0.0946	0.0829	0.0856	0.0551	0.0480	0	0	0	0	0	0	0	0	0		
Spleen	0.398	0.0875	4.02	0.880	0.439	0.192	0.0891	0.0217	0.0324	0.0163	0.0151	0.0139	0	0	0	0	0	0	0		
Stomach	23.3	8.78	15.3	5.82	0.244	0.142	0.143	0.115	0	0	0	0	0	0	0	0	0	0	0		
Testes	0.00803	0.00696	0.130	0.0229	0.0473	0.0146	0.0179	0.00276	0	0	0.0134	0.0232	0	0	0	0	0	0	0		
Thyroid	0.296	0.0301	2.69	1.16	0.234	0.0220	0	0	0	0	0	0	0	0	0	0	0	0	0		
Urinary Bladder	0.166	0.0943	2.73	2.94	0.0920	0.0176	0.0268	0.00167	0	0	0	0	0	0	0	0	0	0	0		

Table provided by the sponsor.

Pharmacokinetic Parameter Estimates for BMS-354825-equivalent Concentrations of Radioactivity in Blood, Plasma, and Tissues After a Single Oral (10 mg base/kg, 120 µCi/kg) Administration of [¹⁴C]BMS-354825

Matrix	C _{max} (µg equiv/g)	T _{max} (h)	T _{last} (h)	AUC _{last} (µg equiv·h/g)	AUC _{INF} (µg equiv·h/g)	t _{1/2} (h)
Adrenal Glands	5.65	4	168	62.3	63.5	40.5
Blood	0.457	4	4	0.846	NE	NE
Bone (femur)	0.375	4	12 ^a	2.43	2.97	4.0
Bone Marrow (femur)	2.48	4	12	14.9	15.5	2.2
Brain	0.0323	4	24	0.387	0.485	9.8
Cecum	11.8	4	96	134	134	14.6
Eyes	1.31	12	168	111	280	242
Heart	1.24	4	96	9.18	9.32	25.5
Intestine, Large	3.46	4	96	46.4	46.5	12.4
Intestine, Small	45.7	1	96	211	212	21.7
Kidneys	5.12	4	168	42.0	42.3	34.3
Liver	8.46	4	168	64.0	64.7	40.0
Lungs	5.11	4	96	35.4	35.5	17.8
Plasma	0.438	4	12	2.63	2.66	1.6
Skeletal Muscle (pectoral)	0.790	4	4	1.19	NE	NE
Skeletal Muscle (thigh)	0.687	4	4	1.03	NE	NE
Skin, Nonpigmented	0.606	4	12	3.78	4.30	3.3
Skin, Pigmented	0.531	4	48	7.56	9.00	18.1
Spleen	4.02	4	96	30.4	31.1	29.6
Stomach	23.3	1	24	134	137	15.6
Testes	0.130	4	96	1.85	2.01	8.6
Thyroid	2.69	4	12	16.3	17.1	2.3
Urinary Bladder	2.73	4	24	16.4	16.7	6.7

NE: Not estimated; insufficient number of quantifiable data sets.

a: The lower limit of quantitation was used as T_{last} in the pharmacokinetic analysis (see Section 3.8.2. Pharmacokinetics).

Table provided by the sponsor.

Mean Percentages of Radioactive Dose in Tissues and Gastrointestinal Contents After a Single Oral (10 mg base/kg, 120 µCi/kg) Administration of [¹⁴C]BMS-354825

Matrix	Percent of Radioactive Dose													
	1 h		4 h		12 h		24 h		48 h		96 h		168 h	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Adrenal Glands	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Blood	0.06	0.02	0.35	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bone (femur)	0.00	0.00	0.21	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bone Marrow (femur)	0.01	0.00	0.09	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Brain	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cecum	0.01	0.01	0.43	0.19	0.22	0.08	0.04	0.03	0.00	0.01	0.00	0.00	0.00	0.00
Cecum Contents	0.05	0.05	20.31	5.30	13.38	0.59	4.25	3.27	0.28	0.26	0.06	0.11	0.00	0.00
Eyes	0.00	0.01	0.00	0.01	0.01	0.00	0.01	0.00	0.00	0.01	0.01	0.01	0.01	0.01
Heart	0.01	0.01	0.04	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Intestinal Contents, Large	0.00	0.00	9.38	10.30	17.65	10.10	2.55	2.08	0.31	0.40	0.03	0.05	0.00	0.00
Intestinal Contents, Small	31.46	1.22	20.49	13.58	1.22	1.14	0.82	0.98	0.05	0.08	0.06	0.11	0.00	0.00
Intestine, Large	0.01	0.00	0.24	0.17	0.12	0.09	0.03	0.02	0.00	0.01	0.00	0.00	0.00	0.00
Intestine, Small	7.45	0.20	3.00	1.63	0.11	0.07	0.09	0.06	0.00	0.01	0.01	0.01	0.00	0.00
Kidneys	0.06	0.01	0.38	0.07	0.04	0.02	0.01	0.01	0.00	0.01	0.00	0.00	0.00	0.00
Liver	0.41	0.15	2.62	0.44	0.25	0.10	0.09	0.02	0.04	0.02	0.02	0.01	0.01	0.01
Lungs	0.02	0.01	0.20	0.01	0.02	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Skeletal Muscle (thigh)	0.00	0.00	3.31	0.34	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Skin, Nonpigmented	0.00	0.00	0.63	0.19	0.12	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Skin, Pigmented	0.00	0.00	0.27	0.01	0.13	0.05	0.04	0.05	0.03	0.03	0.00	0.00	0.00	0.00
Spleen	0.01	0.00	0.07	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Stomach	1.37	0.58	0.80	0.24	0.02	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Stomach Contents	59.85	13.67	16.51	11.13	0.18	0.24	0.24	0.35	0.01	0.02	0.00	0.00	0.00	0.00
Testes	0.00	0.00	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
Thyroid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Urinary Bladder	0.00	0.00	0.02	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total	100.77	13.86	79.37	9.35	33.49	9.54	8.19	5.49	0.73	0.74	0.19	0.28	0.01	0.01
Skeletal Muscle (pectoral) ^a	0.00	0.00	3.81	0.41	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Carcass	1.37	0.99	6.50	0.76	0.76	0.24	0.41	0.15	0.13	0.11	0.12	0.11	0.00	0.00

a: Presented for comparative purposes. The percentage of the radioactive dose in pectoral muscle was calculated using the same assumption as for thigh muscle (skeletal muscle = 50% of body weight). Therefore, including the values for both thigh and pectoral muscle in the total would result in an over estimation of the percentage of the radioactive dose in tissues.

Table provided by the sponsor.

Mean Tissue: Plasma ratios

- Maximal mean tissue : plasma ratios were highest for tissues of the GI tract followed by: liver (19:1 at Hr 4), adrenal glands (13: 1 at Hr 4), lungs (12:1 at Hr 4), kidneys (11.5:1 at Hr 4), spleen (9:1 at Hr 4), thyroid (6:1 at Hr 4), eyes (5.8:1 at Hr 1), urinary bladder (5.8:1 at Hr 4), femoral bone marrow (5.7:1 at Hr 4), heart (2.8:1 at Hr 4), skeletal muscle (1.8:1 at Hr 4)
- Peak distribution of the radioactivity appeared to be mainly at Hr 4 (Tmax= 4 hr), as was also determined in the “distribution of radioactivity in tissues”

Matrix	Tissue:Plasma Concentration Ratios			
	1 h		4 h	
	Mean	SD	Mean	SD
Adrenal Glands	8.17	1.60	12.8	2.25
Blood	1.00	0.0758	1.04	0.0798
Bone (femur)	0	0	0.852	0.0358
Bone Marrow (femur)	2.88	0.796	5.68	0.353
Brain	0.0530	0.0918	0.0735	0.000850
Cecum	1.84	0.122	27.3	11.6
Eyes	5.78	9.20	1.15	0.763
Heart	1.69	0.548	2.82	0.206
Intestine, Large	1.53	0.471	8.28	5.63
Intestine, Small	613	184	44.5	25.9
Kidneys	10.3	2.61	11.5	1.55
Liver	15.6	2.90	19.2	3.98
Lungs	5.40	1.69	11.8	1.77
Skeletal Muscle (pectoral)	0	0	1.81	0.137
Skeletal Muscle (thigh)	0	0	1.57	0.117
Skin, Nonpigmented	0	0	1.44	0.605
Skin, Pigmented	0	0	1.23	0.192
Spleen	5.13	0.617	9.10	0.811
Stomach	319	168	34.1	9.76
Testes	0.0990	0.0936	0.301	0.0811
Thyroid	3.89	0.775	6.07	2.38
Urinary Bladder	2.12	1.30	5.76	5.67

Table provided by the sponsor.

Summary of the study:

- Mean concentrations of radioactivity were highest in the GI tract (mainly Hrs 1-12, up to 46 µg equivalent)
- High levels of radioactivity were also detected in the following organs/tissues (peak levels are listed): liver (8.5 µg equivalent at Hr 4), adrenal glands (5.7 µg equivalent at HR 4), kidneys (5.1 µg equivalent at Hr 4), lungs (5.1 µg equivalent at Hr 4), spleen (4.0 µg equivalent at Hr 4), urinary bladder (2.7 µg

equivalent at Hr 4), thyroid (2.7 µg equivalent at Hr 4), femoral bone marrow (2.5 µg equivalent at Hr 4), eyes (1.3 µg equivalent at Hr 12), heart (1.24 µg equivalent at HR 4)

- Based on the maximum levels of radioactivity, the peak distribution of radioactivity was mainly at Hr 4 (T_{max}= 4 hrs). T_{max} for the GI tract was 1 hr (levels were still high at Hrs 4 and 12). T_{max} for the eyes was 12 hrs. T_{last} for tissues ranged from 4 hrs (blood, bone, skeletal muscle) to 168 hrs (adrenal glands, eyes, kidneys, and liver).
- Based on the percentage of the dose administered, the highest percentage was detected in the GI tract. High percentage of the radioactive dose was also detected in the liver and the skeletal muscle. The high amount of radioactivity in the liver may reflect liver uptake and metabolism.
- Estimation of the exposures to radioactivity indicated the following order from the highest to the lowest exposures (AUC_{last}): GI tract, eyes, liver, adrenals, kidneys, lungs, spleen, thyroid and urinary bladder, bone marrow, heart, skin, plasma, bone, testes, skeletal muscle.
- Elimination half-life estimates for BMS-354825 equivalents ranged from a high of 242 hrs for eyes to a low of 1.6 hrs for plasma. The next highest half-life estimates were for adrenal glands (40.5 hrs) and liver (40.0 hrs). Half-life estimates for radioactivity in pigmented and non-pigmented skin were 18.1 hrs and 3.3 hrs, respectively.
- Mean blood : plasma concentration ratios were approximately unity at Hrs 1 and 4, suggesting non-preferential partitioning of [14C]BMS-354825-derived radioactivity. Concentration-time profiles for radioactivity in blood and plasma were similar, with concentrations attaining C_{max} at Hr 4 and then decreasing rapidly, with an estimated terminal half-life of 1.6 h for plasma.
- Maximal mean tissue : plasma ratios were highest for tissues of the GI tract followed by: liver (19:1 at Hr 4), adrenal glands (13: 1 at Hr 4), lungs (12:1 at Hr 4), kidneys (11.5:1 at Hr 4), spleen (9:1 at Hr 4), thyroid (6:1 at Hr 4), eyes (5.8:1 at Hr 1), urinary bladder (5.8:1 at Hr 4), femoral bone marrow (5.7:1 at Hr 4), heart (2.8:1 at Hr 4), skeletal muscle (1.8:1 at Hr 4).
- The mean tissue : plasma concentration ratios of radioactivity were greater than unity for majority of tissues at Hrs 1 and 4 post-dose, suggesting a general affinity of radioactivity for tissues. Only brain, testes, and bone had ratios less than unity at both times.
- The radioactivity was eliminated mainly within the 48 hrs after dosing. Only 0.7% of the radioactivity was detected at Hr 48. Total amount of radioactivity was 0.01% by Day 7.

Study Title: In Vitro protein binding determination of BMS-582691 in human serum using equilibrium dialysis

Key study findings: The protein binding of BMS-582691 (metabolite M4) and BMS-354825 was independent of concentrations ranging from 100 to 500 ng/mL. The mean human serum protein binding for both substances was > 93%.

Study No.: 930011593

Volume/ Page number: Item 5

Conducting laboratory and location: BMS

Date of study initiation: not provided

GLP compliance: yes () no (X)

Drug, lot #, radiolabel, and % purity: BMS-582691 (Batch no. DC71117-055 with a purity of ~) and BMS-354825-03 (Batch no. FB59644-076 with a purity of ~)

Study design:

Serum protein binding was determined by equilibrium dialysis, conducted at 37°C using a ~ equilibrium dialysis system ~ Teflon cells (membrane surface area=11.3 cm²) and ~ cellulose high permeability dialysis membranes ~ with a 10 kD molecular weight cut-off.

Sample analysis: Samples were analyzed for BMS-582691 (metabolite M4) and BMS-354825 using a validated LC/MS/MS method at ~

Protein Binding Determination: Following completion of dialysis, bound and free concentrations of BMS-582691 and BMS-354825 were obtained by analyzing samples from the serum and buffer compartments of the dialysis cell. From each dialysis cell, the percentage of free BMS-582691 and BMS-354825 was calculated as follows:

$$\% \text{ free} = 100 \times \left(C_{\text{buffer}} \right) \div \left(C_{\text{protein}} \right),$$

C_{buffer} : concentration of BMS-582691 or BMS-354825 in the buffer compartment of the dialysis cell

C_{protein} : concentration of BMS-582691 or BMS-354825 in the serum compartment of the dialysis cell.

The percentage of BMS-582691 and BMS-354825 bound to human serum proteins was calculated using the equation:

$$\% \text{ bound} = 100 - \% \text{ free}$$

Results:

The protein binding of BMS-582691 (metabolite M4) and BMS-354825 was independent of concentrations ranging from 100 to 500 ng/mL. The mean human serum protein binding for both substances was > 93%.

Concentration (ng/mL)	% Bound	
	BMS-582691	BMS-354825
100	93.7 (0.9)	96.3 (0.2)
500	93.1 (0.3)	96.4 (0.3)

Note: Values shown are the mean of quadruplicate determinations.

Table provided by the sponsor.

2.6.4.5 Metabolism

Study Title: Biotransformation of [¹⁴C]BMS-354825 after Intravenous and Oral Administration to Bile Duct Cannulated Rats

Key study findings:

- The major pathway for the metabolism of BMS-354825 was oxidation. Glucuronidation and sulfate conjugation were also observed.
- M5 was the prominent metabolite detected in plasma, urine, and bile.
- In the plasma, M5 accounted for 15%-18% of the radioactivity in i.v. dosed rats and 13%-14% of the radioactivity in p.o.-dosed rats.
- Approximately 6-17% of the radioactivity recovered in bile and 6-10% of the radioactivity recovered in the urine were attributed to the parent compound, suggesting that BMS-354825 is appreciably cleared through metabolism in rats in vivo.

Study no.: 930010531

Volume/ Page number: Item 5

Conducting laboratory and location: BMS, Princeton, NJ

Date of study initiation: 12/2002

GLP compliance: yes () no (X)

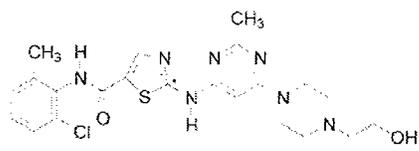
Drug, lot #, radiolabel, and % purity: [¹⁴C]BMS-354825; lot # 52996-102-35;
— pure

BMS-354825; lot# JL71001-001-3; purity not provided

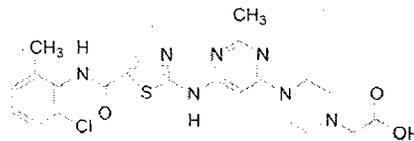
Formulation: sodium acetate buffer (pH 4.0)

Metabolites of BMS-354825 (purities and lot #s not provided)- used as reference standards:

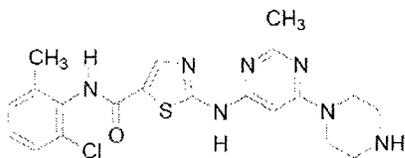
- BMS-573188 (metabolite M6)
- BMS-582691 (metabolite M4)
- BMS-606181 (metabolite M5: major metabolite in rat)



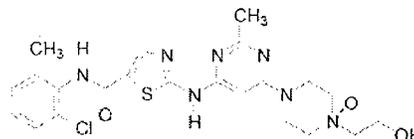
BMS-354825
*denotes the location of the [¹⁴C] label



BMS-573188



BMS-582691



BMS-606181

Structures provided by the sponsor.

Methods:

Doses:

Single i.v.: 10 mg/kg (60 μ Ci/kg)

Single oral: 10 mg/kg (60 μ Ci/kg)

Species: Sprague-Dawley rats (σ 's only)

Bile duct cannulated (BDC) and intact rats

Number of animals/group: 2 σ 's/ group (bile duct cannulated)

6 σ 's/ group (intact)

Biotransformation profiling: HPLC analyses (on a _____ system) were performed for biotransformation profiling in bile, urine, and plasma. LC/MS/MS and LC/MS analyses were performed on pooled bile (0-12 h), urine (0-12 h) and plasma (1, 4, and 8 h) samples.

Objectives:

- To determine route and extent of excretion of total radioactivity in the 12 h interval after a single IV or oral administration of [¹⁴C]BMS- 354825 to BDC rats;
- To determine the biotransformation profiles of [¹⁴C]BMS-354825 in rat bile, urine and plasma, and
- To identify the major metabolites of BMS-354825.

Study Design:

After dosing, bile was collected from the BDC rats at 0-6 and 6-12 h intervals (groups 1 and 3). Recovery of radioactivity in rat bile, urine and GI tract over the study interval (0-12 h) was determined. Of note, the 12-hr GI tract samples from i.v.-dosed rats were not collected.

Summary of bile, urine, plasma, and GI tract samples collected from bile duct cannulated male rats after intravenous or oral administration of [¹⁴C]BMS-354825

Group	Rat	Route ^a	No. of Animals	Dose	Matrix	Sampling time (h)
1	bile duct cannulated	IV	2	10 mg/kg 60 µCi/kg	Bile	0-6, 6-12
					Urine	0-12
					GI tract	^b
2	intact	IV	2	10 mg/kg 60 µCi/kg	Plasma	1
					Plasma	4
					Plasma	8
3	bile duct cannulated	PO	2	10 mg/kg 60 µCi/kg	Bile	0-6, 6-12
					Urine	0-12
					GI tract	12
4	intact	PO	2	10 mg/kg 60 µCi/kg	Plasma	1
					Plasma	4
					Plasma	8

^a IV and PO represent intravenous infusion and oral routes of administration, respectively.

^b The GI tract samples from IV-dosed rats were inadvertently not collected at 12 h.

Table provided by the sponsor.

Results:

Recovery of Radioactivity

- Since the study was conducted for only 12 hrs, and the 12-hr GI tract samples from i.v.-dosed rats were not collected, recovery of radioactivity was not comprehensive.
- For i.v.-dosed rats, the majority of radioactivity was recovered in bile (average of 67%). A smaller percentage (average of 36%) was recovered in the bile of p.o.-dosed rats.
- In urine, the recovery of radioactivity was 12.0% of the radioactivity in i.v.-dosed rats and 3.2% of the radioactivity in p.o.-dosed rats.
- 45% to 61% of the administered oral doses of [¹⁴C]BMS-354825 remained in the GI tract of rats after 12 hrs, suggesting incomplete absorption or secretion into the intestine.

Data for the BDC rats:

Table 2: Recovery of radioactivity in bile, urine and GI tract of male Sprague-Dawley rats following intravenous or oral administration of [¹⁴C]BMS-354825 (10 mg/kg, 60 µCi/kg)

Route ^a	Animal	Recovery of radioactivity (% of dose) ^b			
		Bile (%)	GI tract (%)	Urine (%)	Bile + GI tract + Urine (%)
IV	1	61.2	-	14.9	76.1
IV	2	73.6	-	9.1	82.7
	average	67.4	-	12.0	79.4
PO	3	32.5	61.2	2.7	96.4
PO	4	39.1	44.7	3.6	87.4
	average	35.8	53.0	3.2	92.0

^a IV and PO represent intravenous infusion and oral routes of administration, respectively.

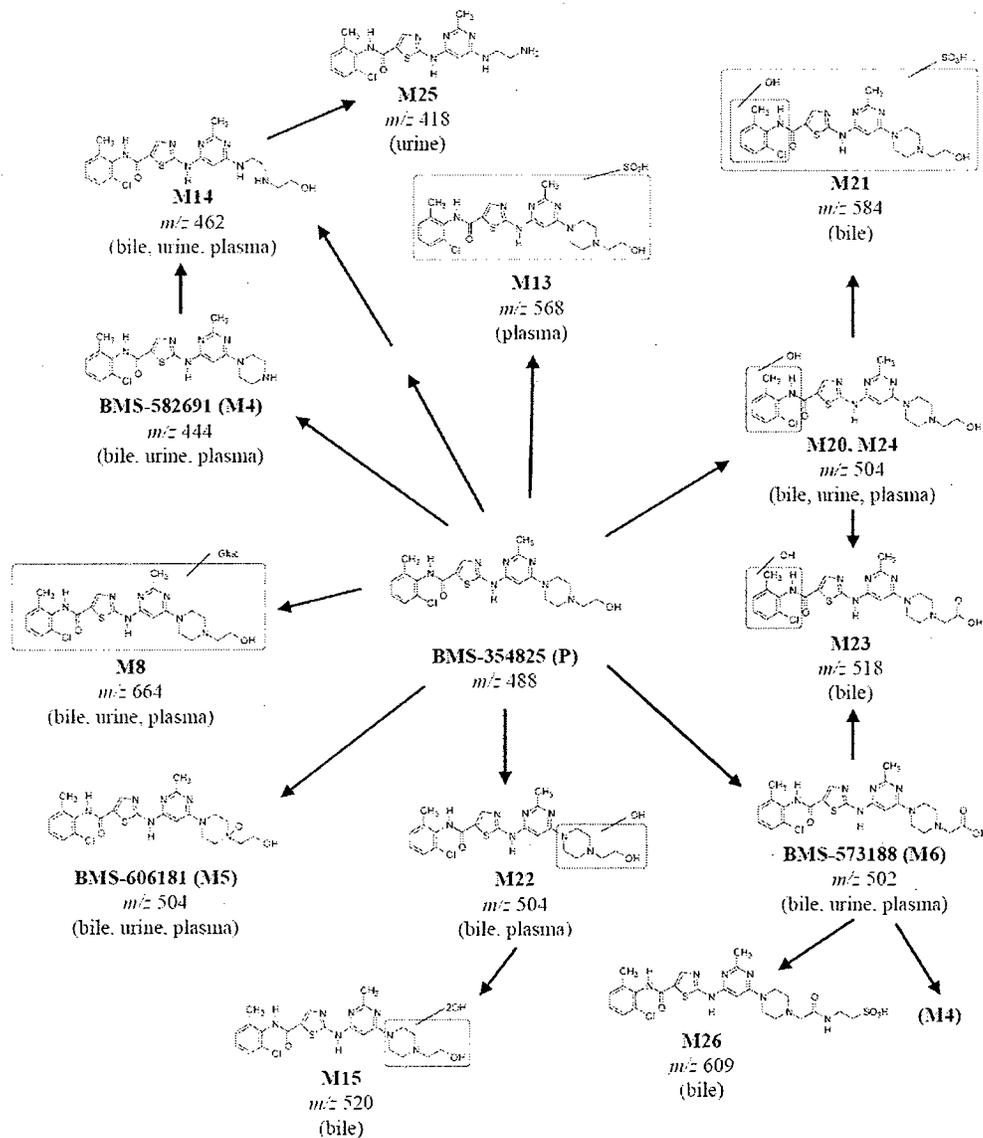
^b Recovery of radioactivity 0-12 h after dosing. Feces were not collected in this study. Data are the average of duplicate sample analyses.

Table provided by the sponsor.

Metabolic pathway

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ON ORIGINAL**

Proposed pathways for biotransformation of BMS-354825 in bile duct cannulated rats:



Schema provided by the sponsor.

The metabolism of BMS-354825 in the bile-duct cannulated and intact rats includes the following pathways:

- N-oxidation of the piperazine (M5)
- Monooxygenation of the chloromethylphenyl group (M20, M24)
- Sulfate conjugation of a mono-oxygenated metabolite (M21)
- Oxidation of the N-hydroxyethyl group to a carboxylic acid (M6) followed by taurine conjugation (M26)
- A combination of monooxygenation of the chloromethylphenyl group and oxidation of the N-hydroxyethyl group to a carboxylic acid (M23)

- Monooxygenation of the N-hydroxyethylpiperazine (M22)
- Sulfate conjugation of the N-hydroxyethyl group (M13)
- N-dealkylation of the piperazine leading to ring opening (M14, M25)
- N-dealkylation of the N-hydroxyethyl group (M4)
- Glucuronidation of the intact parent compound (M8)
- Bis-oxygenation of N-hydroxyethylpiperazine group (M15)

Metabolite profile in bile and urine:

Metabolite	Pooled Bile (0-12 hrs) % distribution (% of dose)		Pooled Urine (0-12 hrs) % distribution (% of dose)	
	i.v.	p.o.	i.v.	p.o.
M20	—	—	0.9 (0.11)	2.6 (0.083)
M21	9.1 (6.13)	9.2 (3.29)	—	—
M23	2.0 (1.35)	2.9 (1.04)	—	—
M24	2.3 (1.55)	3.2 (1.15)	2.4 (0.29)	3.7 (0.12)
M15	1.2 (0.81)	1.3 (0.47)	—	—
M14	1.4 (0.94)	0.2 (0.07)	1.1 (0.13)	0.8 (0.026)
M26	1.9 (1.28)	4.0 (1.43)	—	—
M22	1.4 (0.94)	1.1 (0.39)	—	—
M8	5.6 (3.77)	10.6 (3.79)	1.3 (0.16)	2.2 (0.07)
M4 (BMS-582691)	20.9 (14.09)	18.6 (6.66)	8.1 (0.97)	8.6 (0.28)
M6 BMS-573188				
P (BMS-354825)	16.6 (11.19)	15.9 (5.69)	5.6 (0.67)	10.4 (0.33)
M5 (BMS-606181)	24.7 (16.65)	17.8 (6.37)	66.5 (7.98)	51.3 (1.64)
I (impurity)	—	—	—	—
Unidentified peaks	6.5 (4.38)	7.1 (2.54)	5.4 (0.65)	11.3 (0.36)
Total	—	—	92.4 (11.1)	92.5 (2.96)

- The relative % distribution of radioactivity represents the percent of radioactivity in each peak compared to the total radioactivity recovered from the HPLC column eluate after background subtraction. The percentage of dose for metabolites was calculated from the relative % distribution of radioactivity and the average percent of the radioactive dose excreted in the bile (67.4% for IV-dosed rats and 35.8% for PO-dosed rats) or urine (12.0% for i.v.-dosed rats and 3.2% for p.o.-dosed rats).
- M4 and M6 co-eluted in the HPLC.
- Unidentified peaks includes those individually representing <3.0% of the total bile radioactivity, <2.2% of the total urinary radioactivity.
- Total radioactivity less than 100% appear to be due to small amounts of radioactivity distributed throughout the radiochromatogram, not observed as distinct peaks.

Metabolite profile in the plasma:

Metabolite ^a	[M+H] ⁺	Retention Time range ^b	Pooled Plasma Samples % Distribution ^c					
			IV Route			PO Route		
			1 h	4 h	8 h	1 h	4 h	8 h
M24	504	25.5-27 min	0.6	1.0	0.8	1.4	0.7	2.2
M13	568	31-32.5 min	3.3	6.5	6.3	5.9	7.7	7.6
M14	462	32.5-33.5 min	0.5	0.8	0.5	-	0.7	0.6
M22 ^d	504	34-36 min	1.9	4.4	4.4	5.6	4.2	3.8
M8 ^d	664							
M4 BMS-582691 ^e	444	36.5-37.8 min	1.2	1.7	1.2	1.7	1.2	0.6
M6 BMS-573188 ^e	502							
P BMS-354825	488							
M5 BMS-606181	504	42.5-45 min	14.6	17.8	15.1	12.9	12.8	13.6
I impurity ^f		58-60 min						
Unidentified peaks ^g			16.1	22.0	24.8	20.8	14.8	22.5
		Total ^h						

^a Structures of metabolites are shown in Table 6.

^b Approximate retention times of peaks from HPLC biotransformation profiles; minor variations in retention time were observed between injections.

^c The relative % distribution of radioactivity represents the radioactivity in each metabolite peak(s) compared to the total radioactivity recovered from the HPLC column after background subtraction.

^d Metabolites M22 and M8 co-eluted in the HPLC.

^e BMS-582691 (M4) and BMS-573188 (M6) co-eluted in the HPLC.

^f The identity of this peak could not be confirmed by LC/MS analysis; however, its retention time matched that of a — impurity in the dosing solution.

^g Radioactivity from peaks that have not been identified (each individual peak represents <7.9% of the total plasma radioactivity).

^h Total radioactivity is less than 100% due to small amounts of radioactivity distributed through out the radiochromatogram, not observed as distinct peaks.

Table provided by the sponsor.

Metabolite identification by LC/MS/MS analysis:

The identities of all metabolites were confirmed by LC/MS/MS or LC/MS analysis.

Summary of the study:

Biotransformation of BMS-354825 in bile and urine was evaluated after administration of a single i.v. or oral dose of [¹⁴C]BMS-354825 (10 mg/kg) to bile duct cannulated rats. In addition, plasma metabolite profiles in intact rats were obtained after i.v. and oral administration of [¹⁴C]BMS-354825 at the same dose.

Since the study was conducted for only 12 hrs, and the 12-hr GI tract samples from i.v.-dosed rats were not collected, recovery of radioactivity was not comprehensive. The following results were obtained:

- For i.v.-dosed rats, the majority of radioactivity was recovered in bile (average of 67%). A smaller percentage (average of 36%) was recovered in the bile of p.o.-dosed rats.
- In urine, the recovery of radioactivity was 12.0% in i.v.-dosed rats and 3.2% in p.o.-dosed rats.
- 45%-61% of the administered oral doses of [¹⁴C]BMS-354825 remained in the GI tract of rats after 12 hrs. This information, together with the ↓bile duct and urinary excretion in these animals suggest incomplete absorption. Secretion into the intestine may also contribute to this finding.

Metabolites in bile, urine and plasma were identified by comparison of HPLC retention times with those of available synthetic standards and characterization by LC/MS/MS analysis. The following is a summary of metabolite profiling in the bile, urine, and plasma samples.

Bile:

- For the pooled 0-12 hr bile samples, biotransformation profiles were qualitatively similar for i.v. and oral routes of administration.
- The parent compound, BMS-354825, accounted for 16-17% of the total radioactivity in rat bile samples.
- The prominent metabolites identified in 0-12 h rat bile were an *N*-oxide formed on the piperazine ring (18-25%, BMS-606181, **M5**); a monooxygenated sulfate conjugate of BMS-354825 (9%, M21); a glucuronide conjugate (6-11%, M8); a carboxylic acid of BMS-354825 (BMS-573188, M6); and an *N*-dealkylation product of BMS-354825 (BMS-582691, M4). Because M4 and M6 co-eluted on the HPLC, the individual contributions of these metabolites to the overall profile could not be calculated; the total radioactivity associated M4 and M6 peak was 19-21%.
- Minor metabolites, which individually accounted for 1% to 4% of the radioactivity, were identified as mono-oxidation products (M23, M22, and M24); a bis-oxidation product (M15); a piperazine ring fragmentation product of BMS-354825 (M14); and a taurine conjugate (M26) of BMS-573188.

Urine:

- The biotransformation profiles of pooled 0-12 h urine samples from rats that were administered i.v. and oral doses of [¹⁴C]BMS-354825 were qualitatively similar.
- Parent compound accounted for 6-10% of the total radioactivity in rat urine. The predominant metabolite identified in 0-12 h rat urine for both the i.v. and oral route of administration was the *N*-oxide (BMS-606181, **M5**), which accounted for 67% of the radioactivity in i.v.-dosed rats and 51% of the radioactivity in p.o.-dosed rats.

- Minor metabolites, which individually accounted for 1% to 9% of the radioactivity, were mono-oxidation products (M20 and M24); piperazine ring fragmentation products of BMS-582691 and BMS-354825 (M25 and M14, respectively); a glucuronide conjugate (M8); BMS-582691 (M4); and BMS-573188 (M6).

Plasma:

- The biotransformation profiles for 1, 4, and 8 hr plasma samples from rats that were administered i.v. and oral doses of [¹⁴C]BMS-354825 were also qualitatively similar.
- Parent compound accounted for the largest percentage of radioactivity in rat plasma, 36-56% for i.v.-dosed rats and 34-48% for p.o.-dosed rats.
- The major circulating metabolite was identified as the N-oxide, BMS-606181 (M5), which accounted for 13 to 18% of the total radioactivity in rat plasma samples.
- A sulfate conjugate (M13) accounted for 3-8% of the plasma radioactivity.
- Minor metabolites which individually accounted for less than 1 to 6% of the radioactivity were M4, M6, M8, M14, M22 and M24.

In conclusion, the major pathway for the metabolism of BMS-354825 was oxidation. Glucuronidation and sulfate conjugation were also observed. Approximately 6-17% of the radioactivity recovered in bile and 6-10% of the radioactivity recovered in the urine were attributed to the parent compound, suggesting that BMS-354825 is appreciably cleared through metabolism in rats in vivo. M5 was the prominent metabolite detected in plasma, urine, and bile.

Study Title: Biotransformation of [¹⁴C]dasatinib (BMS-354825) in rats, monkeys, and humans

Key study findings:

- Dasatinib was highly metabolized in human (parent compound accounted for 26% of the plasma radioactivity) and monkey (parent compound accounted for 32% of the plasma radioactivity). In rat plasma, the parent drug was 53% of the radioactivity.
- Major metabolic pathways of dasatinib in human involved hydroxylation on the chloromethylphenyl ring followed by sulfation, oxidation of the hydroxyethyl moiety to the carboxylic acid, N-oxidation of the piperazine ring, and products formed by a combination of the above pathways
- In human, metabolite M20 (4-OH-chloromethylphenyl dasatinib) and its sulfate conjugate (M21) were detected in significant amount (13% and 10%, respectively) in the plasma. Rats had no detectable amounts of M20. In monkeys, the amount of M20 in plasma was only 2.8% of the radioactivity. Therefore, neither species could- adequately- assess toxicities of M20 in humans.

- In the bile duct cannulated monkeys (i.v. dosing), the unchanged parent drug represented only 3% and 0.1% of the dose in bile and urine, respectively, suggesting that dasatinib undergoes extensive metabolism in monkeys.
- Biliary excretion appears to be the major excretion route in i.v.-dosed monkeys as indicated by the amount of total biliary radioactivity (approximately 61% of the dose was detected in the bile). 9% of the dose was detected in the urine

Study no.: 930011321

Volume/ Page number: Item 5

Conducting laboratory and location: BMS

Date of study initiation: 1/2005

GLP/GCP compliance: yes () no (X)

Drug, lot #, radiolabel, and % purity: [14C]dasatinib, lot# 002, 17.0 µCi/mg, radiochemical purity of —

Reference standards:

- Dasatinib (lot JL71001-001-3)
- BMS-573188-01-001

Methods:

Species: Sprague-Dawley rats (♂s); Cynomolgus Monkeys (♂s); human (healthy subjects)

Doses: single doses

Rat: 15 mg/kg oral; 6.7 µCi/mg

Monkey: 10 mg/kg oral; 3.0 µCi
2 mg/kg i.v.; 15 µCi/mg

Human: 100 mg p.o.; (120 µCi/mg)

Species	Dose	Route	Samples Collected	N
Rat (Group 1)	15 mg/kg	p.o.	• Plasma: 1, 4, 8, and 24 hrs	3 rats/collection
Rat (Group 2)	15 mg/kg	p.o.	• Urine: 0-6, 6-12, 12-24, and at 24 hr intervals up to 168 hrs • Feces: 0-168 hrs at 24-hr intervals	N=3
Monkey	10 mg/kg	p.o.	• Plasma: 1, 4, 8, 24, and 96 hrs • Urine: 0-6, 6-12, 12-24, and at 24 hr intervals up to 168 hrs • Feces: 0-168 hr at 24 intervals	N=3
Monkey (BDC)	2 mg/kg	i.v.	• Bile: 0-4, 4-8, 8-24, 24-48, and 48-72 hrs • Urine: 0-8, 8-24, 24-48, and 48-72 hrs	Not provided
Human Healthy subjects	100 mg	p.o.	• Plasma: 1, 2, 4, 8, 12, and 24 hrs • Urine: 0-12, 12-24, and at 24 hr intervals up to 312 hrs • Feces: 0-312 hrs at 24-hr intervals	N=8

Formulation: citrate buffer, pH 3.

BDC: Bile duct cannulated.

Biotransformation profiling:

HPLC was performed on an

Identification of Metabolites by LC/MS:

The LC/MS system used for analysis of plasma, urine, bile, and fecal samples consisted of an HPLC system connected to a mass spectrometer. Samples were analyzed using positive electrospray ionization.

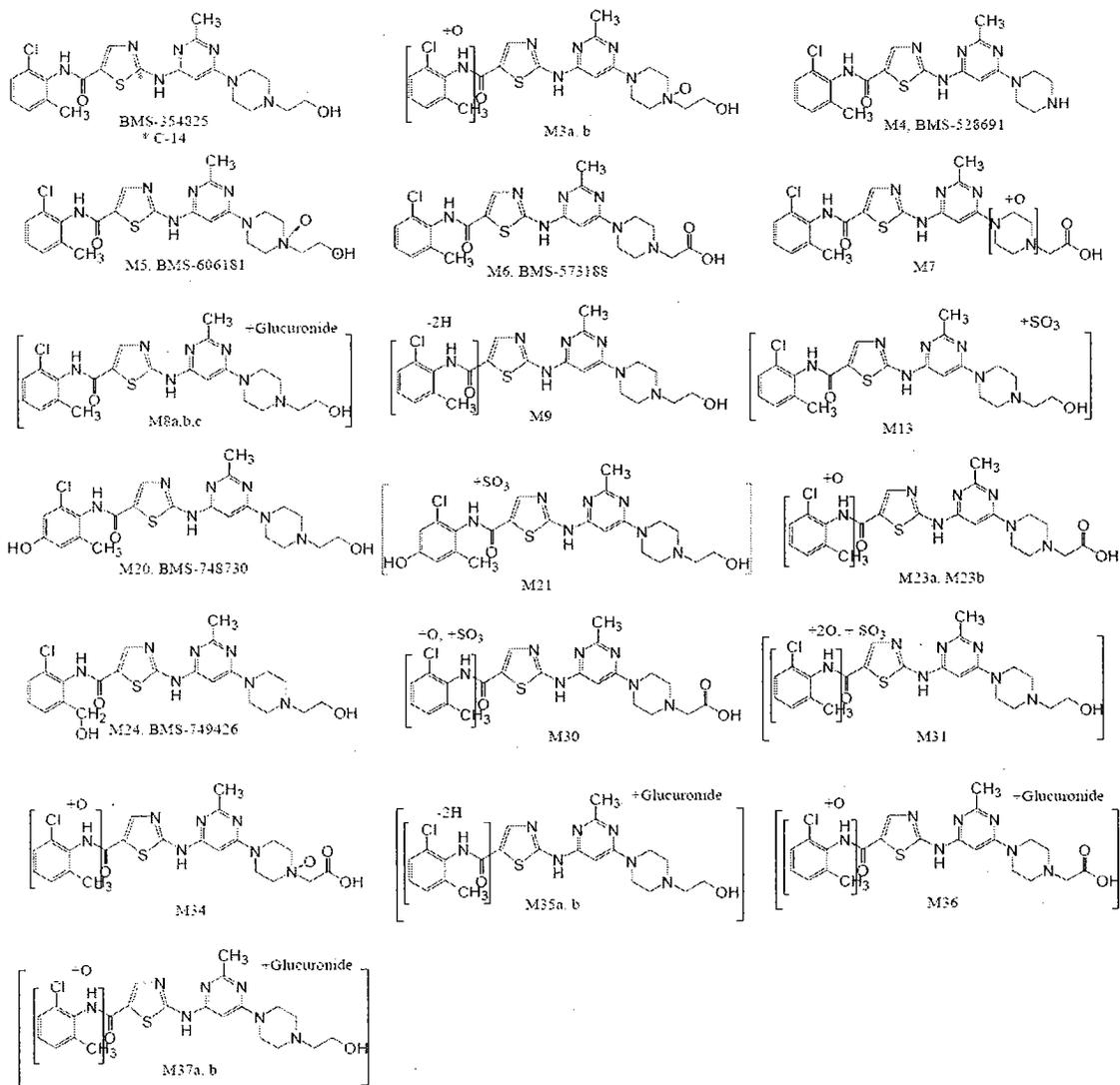
Objectives:

- To compare biotransformation profiles in plasma, urine and feces in rats, monkeys, and humans administered [¹⁴C]dasatinib,
- To determine metabolite profiles of dasatinib in urine and bile from BDC monkeys
- To characterize and identify the major metabolites of dasatinib

Results:

Proposed structures of metabolites:

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Structures provided by the sponsor.

Distribution of radioactive metabolites in pooled plasma samples after oral administration of [¹⁴C]dasatinib to rats, monkeys, and humans

Metabolite ID ^a	% Distribution		
	Rat (4 h)	Monkey (4 h)	Human (2 h)
M3a, b ^b	nd	1.4	3.3
M4	2.6	3.0	MS ^e
M5	18.7	2.6	4.5
M6	2.2	2.7	3.6
M7	nd	2.1	3.3
M8a	nd	12.8	3.4
M8b, M23a, b ^{b,c}	nd	4.5 ^c	1.4 ^c
M8c, M13 ^d	17.6 ^d	nd	nd
M20	nd	2.8	12.5
M21	nd	4.7	9.5
M24	nd	0.7	3.1
M30	nd	9.7	6.9
M31	nd	1.7	3.6
M34	nd	1.4	1.1
M35a	nd	MS ^e	3.6
M37a, b ^b	nd	3.7	4.1
Parent	52.8	32.1	25.5
Total	93.9	85.9	89.4

^aStructures of metabolites are shown in Table 5 and Figure 2.

^bMetabolites M3a, b, M23a, b and M37a, b were positional isomers and were not well resolved on HPLC.

^cMetabolites M8b and M23a, b were not well resolved on HPLC. The % of distribution is the total percentage of all three metabolites.

^dMetabolites M8c and M13 were not well resolved on HPLC. The % of distribution is the total percentage of the two metabolites.

^eMetabolite M35a was only detected by mass spectrometry in monkey plasma.
nd, Not detected.

MS: detected by mass spectrometry.

Table provided by the sponsor.

Due to the low amount of radioactivity in plasma, only samples from single time point (2 or 4 h) were analyzed for each species.

Distribution of radioactive metabolites in pooled urine samples after oral administration of [14C]dasatinib to rats, monkeys, and humans

Metabolite ID ^a	Metabolites in Urine (0-168 hr)					
	Rat		Monkey		Human	
	% Rad ^b	% Dose	% Rad	% Dose	% Rad	% Dose
M3a, b ^c	2.3	0.1	4.1	0.1	6.8	0.2
M4	4.4	0.3	1.7	0.05	1.3	0.05
M5	52.4	3.4	35.8	1.1	39.8	1.4
M6	6.5	0.4	2.2	0.06	1.3	0.05
M7	0.3	0.02	10.3	0.3	2.1	0.08
M8a	0.9	0.06	1.2	0.04	5.5	0.2
M8b	0.8	0.05	2.3	0.07	nd	nd
M20	9.9	0.6	0.6	0.02	4.1	0.2
M21	1.5	0.1	2.7	0.08	7.8	0.3
M24	nd	nd	1.9	0.06	6.0	0.2
M34	nd	nd	3.9	0.1	2.3	0.08
M35a	nd	nd	nd	nd	4.2	0.2
M36	nd	nd	nd	nd	4.4	0.2
M37a, b ^c	nd	nd	nd	nd	2.5	0.1
Parent	11.6	0.7	21.2	0.6	3.6	0.1
Total	90.6	5.7	87.9	2.6	91.7	3.4

^a Structures of metabolites are shown in Table 5 and Figure 2.

^b % of total radioactivity in sample.

^c These metabolites are positional isomers that were not well resolved on HPLC.

nd. Not detected.

Table provided by the sponsor.

Distribution of radioactive metabolites in pooled fecal extracts after oral administration of [¹⁴C]dasatinib to rats, monkeys, and humans

Metabolite ID ^a	Metabolites in Feces (0-168 hr)					
	Rat		Monkey		Human	
	% Rad ^b	% Dose	% Rad	% Dose	% Rad	% Dose
M4	2.5	1.9	3.8	2.9	3.1	2.6
M6	17.3	13.2	18.2	14.0	10.4	8.9
M7	1.0	0.8	0.5	0.4	nd	nd
M9	nd	nd	1.7	1.3	1.8	1.5
M20	9.9	7.6	15.2	11.7	36.6	31.2
M23a, b ^c	3.8	2.9	12.6	9.7	14.7	12.5
M24	4.0	3.1	8.6	6.6	4.7	4.0
Parent	54.5	41.6	32.2	24.7	22.4	19.1
Total	93.0	71.1	92.8	71.3	93.7	79.8

^aStructures of metabolites are shown in Table 5 and Figure 2.

^b% of total radioactivity in sample.

^cThese metabolites positional isomers that were not well resolved on HPLC.

nd. Not detected.

Table provided by the sponsor.

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Relative percent distribution of radioactive metabolites in urine and bile after IV administration of [14C]dasatinib to bile-duct cannulated monkeys

Metabolite ID ^a	Metabolites in BDC-Monkeys (0-72 hr)			
	Urine		Bile	
	% Rad ^b	% Dose	% Rad	% Dose
M3a, b ^c	4.3	0.4	3.0	2.0
M4	nd	nd	0.7	0.5
M5	70.7	7.0	7.4	5.0
M6	2.8	0.3	9.4	6.3
M7	9.5	0.9	12.4	8.3
M8a	nd	nd	1.4	0.9
M8b, M23a, b ^{c, d}	nd	nd	4.6 ^d	3.1 ^d
M20	2.7	0.3	3.5	2.4
M21	nd	nd	13.3	8.9
M24	1.4	0.1	4.4	3.0
M30	nd	nd	9.1	6.1
M31	nd	nd	4.8	3.2
M34	2.6	0.3	5.1	3.4
M35a	nd	nd	MS ^e	MS ^e
M35b	nd	nd	3.2	2.2
M36	nd	nd	1.3	0.9
M37a, b ^c	nd	nd	1.7	1.1
Parent	1.4	0.1	4.7	3.2
Total	95.4	9.4	90.0	60.5

^aStructures of metabolites are shown in Table 5 and Figure 2.

^b% of total radioactivity in sample.

^cThese metabolites are positional isomers that were not well resolved on HPLC.

^dMetabolites M8b and M23a,b were not well resolved on HPLC. The % of distribution is the total percentage of all three metabolites.

^eMetabolite was only detected by mass spectrometry

nd. Not detected.

Table provided by the sponsor.

Summary of the study:

Metabolite profiling were evaluated after single doses of [14C]dasatinib to rats, monkeys, and healthy subjects. Below is the summary of the findings.

Plasma (p.o.):

- Dasatinib was highly metabolized in human (parent compound accounted for 26% of the radioactivity) and monkeys (parent compound accounted for 32% of the radioactivity). In rats' plasma, the parent drug was 53% of the radioactivity.
- Dasatinib was the major circulating compound and represented 53, 32, and 26% of total radioactivity in plasma from rats, monkeys, and humans, respectively.
- Plasma metabolite profiles were qualitatively similar for monkey and human, while rat profile was somewhat different.
- In addition to dasatinib, multiple (18-19) circulating metabolites were detected by radioactivity in plasma from monkeys and humans, whereas, only five circulating metabolites were detected in rat plasma.
- **In human**, metabolite **M20** (4-hydroxy-chloromethylphenyl dasatinib) and its sulfate conjugate (**M21**) were also detected in significant amount (13% and 10%, respectively). In monkey plasma, the glucuronide of dasatinib (**M8a**) was a prominent circulating metabolite. In rat plasma, the piperazine N-oxide (**M5**) and sulfate and glucuronide of dasatinib (**M13** and **M8c**) were the prominent species (as was detected in the study in BDC rats).
- It should be noted that rats had no detectable amounts of **M20**. In monkeys, the amount of **M20** in plasma was only 2.8% of the radioactivity. Therefore, neither species could- adequately- assess toxicities of **M20** in humans.

Urine and feces (p.o.):

- In urine and feces, metabolite profiles of dasatinib were qualitatively similar for rats, monkeys and humans.
- Unchanged parent drug was the major component in fecal extracts and accounted for 42%, 25%, and 19% of the dose for rats, monkeys, and humans, respectively.
- Prominent fecal metabolites in all three species were products of chloromethylphenyl ring hydroxylation (**M20** and **M24**), the carboxylic acid (**M6**), and the combined products of the two pathways.
- No conjugated or N-oxide derived metabolites were observed in feces from all three species. This might be suggestive of hydrolysis and reduction of these metabolites in the GI tract.
- Multiple metabolites were observed in urine samples from the three species and dasatinib N-oxide (**M5**) was the major metabolite. Other urinary metabolites in all three species included both oxidative and conjugated products.

Bile duct cannulated monkeys (i.v.):

- The unchanged parent drug represented only 3% and 0.1% of the dose in bile and urine, respectively, suggesting dasatinib undergoes extensive metabolism in monkeys.
- Approximately 61% of the dose was detected in the bile (parent compound and all metabolites combined) and 9% of the dose was detected in the urine. This suggests that biliary excretion is the major excretion route in monkeys, when dosed i.v.
- At least 21 metabolites formed through both oxidative and conjugated pathways were detected in monkey bile.

- Prominent biliary metabolites included the sulfate of phenyl ring-hydroxylated dasatinib (M21), the N-oxide of the carboxylic acid metabolite (M7), and the carboxylic acid metabolite (M6).

2.6.4.6 Excretion

Study Title: Biotransformation of [¹⁴C]dasatinib (BMS-354825) in rats, monkeys, and humans

Key study findings:

- Fecal excretion was the main route of excretion for dasatinib and its metabolites, when dosed orally to monkeys, as shown by the 77% recovery of radioactivity in feces.
- Radioactivity was excreted at a moderately rapid rate, with >80% of the dose recovered within 48 h of dosing.
- Urinary excretion was low (only 3% of radioactivity was recovered in the urine)
- Total recovery of radioactivity was 89%, suggesting that some of the radioactivity may be remaining in the animal after 168 days (7 days).
- Mean plasma concentrations of BMS-354825 equivalents increased to a maximum of 440 ng equivalents/g at 2 h post-dose and then declined in a multi-phasic manner. Mean concentrations were measurable through 8 hrs post-dose.

Study no.: MBA00097

Report Number: MBA00097-05-221

Volume/ Page number: Item 5

Conducting laboratory and location:

Date of study initiation: 1/2005

GLP compliance: yes () no (X)

Drug, lot #, radiolabel, and % purity: [¹⁴C]dasatinib, lot# 002, 17.0 µCi/mg, radiochemical purity of —

<u>Non-radiolabeled Test Article:</u>	BMS-354825-03
Compound:	BMS-354825 monohydrate
Manufacturer/Supplier:	Bristol-Myers Squibb Company
Batch Number:	4H74122
Physical Description:	Solid
As-is Purity:	—

Methods:

Species: Cynomolgus Monkeys (♂s)

Dose: single dose, oral gavage
10 mg/kg (30 µCi/kg)

Study Design:

The study was conducted in 3 ♂ cynomolgus monkeys. After an overnight fast, each animal received a single oral gavage dose of [¹⁴C]BMS-354825 dissolved in 80 mM citrate buffer (pH 3.1), at a target dose level of 10 mg/kg (30 µCi/kg).

Blood samples for pharmacokinetic analysis were collected from each animal before dosing and at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144, and 168 h after dosing. Blood samples for biotransformation analysis were collected from each animal before dosing and at 1, 4, 8, 24, and 96 h after dosing. However, biotransformation was not evaluated as part of this study. Urine and feces were collected separately for at least 13 h before dosing. After dosing, urine was collected from 0-6, 6-12, and 12-24 h, feces were collected from 0-24 h, and then urine and feces were collected separately over 24-h intervals through 168 h after dosing. Cage debris was collected from each cage after each post-dose excreta collection through 144 h after dosing.

Results:

Recoveries of radioactivity for each interval after a single oral administration of [14C]BMS-354825 (10 mg free base/kg, 30 µCi/kg) to male Cynomolgus monkeys:

Matrix	Time (h)	Percent of Radioactive Dose			Mean	SD
		Animal Number				
		1001	1002	1003		
Urine	Predose				0.00	0.00
	0-6				1.31	0.47
	6-12				0.31	0.27
	12-24				1.11	1.50
	24-48				0.17	0.08
	48-72				0.05	0.02
	72-96				0.02	0.03
	96-120				0.00	0.01
	120-144				0.01	0.02
	144-168				0.00	0.00
	Subtotal	2.72	1.59	4.65	2.99	1.55
Feces	Predose				0.00	0.00
	0-24				32.11	19.47
	24-48				40.67	20.60
	48-72				3.42	2.19
	72-96				0.36	0.31
	96-120				0.16	0.12
	120-144				0.06	0.03
	144-168				0.04	0.01
	Subtotal	82.18	65.56	82.71	76.82	9.75
Cage Debris	0-24				6.83	8.71
	24-48				0.96	0.79
	48-72				0.27	0.24
	72-96				0.19	0.09
	96-120				0.09	0.07
	120-144				0.07	0.03
	Subtotal	4.16	18.13	2.94	8.41	8.44

SC Sample combined with sample for next collection interval due to insufficient quantity for analysis.

a Value includes radioactivity recovered in the cage debris spill / and in gauze used to recover spill /

Table provided by the sponsor.

Total recoveries of radioactivity after a single oral administration of [14C]BMS-354825 (10 mg free base/kg, 30 µCi/kg) to male Cynomolgus monkeys

Matrix	Time (h)	Percent of Radioactive Dose				
		Animal Number			Mean	SD
		1001	1002	1003		
Urine	0-168				2.99	1.55
Feces	0-168				76.82	9.75
Cage Debris	0-144				3.41	8.44
Cage Wash	168				0.28	0.15
Cage Wipe	168				0.15	0.10
Total	168				88.65	2.42

Table provided by the sponsor.

Concentrations of Radioactivity in Plasma

Mean plasma concentrations of BMS-354825 equivalents increased to a maximum of 440 ng equivalents/g at 2 hr post-dose and then declined in a multi-phasic manner. Mean concentrations were measurable through 8 h post-dose.

Time (h)	Concentration (ng equivalents of BMS-354825/g)				
	Animal Number			Mean	SD
	1001	1002	1003		
Predose				BQL	NA
0.25				BQL	NA
0.5				BQL	NA
1				290	222
2				440	109
3				350	48.7
4				346	79.8
6				190	33.9
8				97.3	21.1
12				BQL	NA
24				BQL	NA
48				BQL	NA
72				BQL	NA
96				BQL	NA
120				BQL	NA
144				BQL	NA
168				BQL	NA

BQL Below the quantifiable limit (52.8 ng equiv/g).
 NA Not applicable.

Table provided by the sponsor.

Summary of the study:

Male Cynomolgus monkeys were administered single doses of radiolabeled dasatinib. Blood samples were collected for plasma PK evaluations. Urine and fecal excretions were determined by sampling up to 168 hrs (7 days).

- Fecal excretion was the main route of excretion for dasatinib and its metabolites, when dosed orally to monkeys, as shown by the 77% recovery of radioactivity in feces.
- Radioactivity was excreted at a moderately rapid rate, with >80% of the dose recovered within 48 h of dosing.
- Urinary excretion was low (only 3% of radioactivity was recovered in the urine)
- Total recovery of radioactivity was 89%, suggesting that some of the radioactivity may be remaining in the animal after 168 days (7 days).
- Mean plasma concentrations of BMS-354825 equivalents increased to a maximum of 440 ng equivalents/g at 2 h post-dose and then declined in a multi-phasic manner. Mean concentrations were measurable through 8 hrs post-dose.

Study title: Biliary excretion of radioactivity after intravenous administration of [14C]BMS-354825

Key study findings:

- Biliary excretion was the main route of elimination of dasatinib and its metabolites, as indicated by approximately 67% recovery of the radioactivity in the bile.
- 14% of the radioactivity was recovered in the feces and 10% in the urine.
- Elimination of the radioactivity occurred mainly during the first 48 hrs.
- The mean total recovery in all samples collected through 72 h after dosing was 90.80%.
- The mean plasma concentration of BMS-354825 equivalents was 337 ng equivalents/g at 1 h post-dose and then declined in a mono-phasic manner. Mean concentrations were measurable through 8 h post-dose.

Study number: MBA00127

Volume/ Page number: Item 5

Conducting laboratory and location: /

Date of study initiation: 2/2005

GLP compliance: yes () no (X)

Drug, lot #, radiolabel, and % purity: [14C]dasatinib, lot# 002, 17.0 µCi/mg, radiochemical purity of —

<u>Non-radiolabeled Test Article:</u>	BMS-354825-03
Compound:	BMS-354825 monohydrate
Manufacturer/Supplier:	Bristol-Myers Squibb Company
Batch Number:	4H74122
Physical Description:	Solid
As-is Purity:	—

Species: Cynomolgus monkeys (♂s)

Dose: 2 mg/kg, 10-min i.v. infusion (30 µCi/kg)

Study Design

The study was conducted in 3 ♂ bile duct cannulated (BDC) cynomolgus monkeys. After an overnight fast, each animal received a single i.v. infusion of [14C]BMS-354825 dissolved in 80 mM citrate buffer (pH 3.1), at a target dose level of 2 mg/kg (30 µCi/kg).

Blood samples for biotransformation analysis were collected from each animal before dosing and at 1, 4, 8, 24, and 72 hrs after dosing (biotransformation was not reported as part of this study). Plasma was prepared from all blood samples. Bile was collected for at least 19 hrs before dosing and from 0-4, 4-8, 8-24, 24-48, and 48-72 hrs after dosing. Urine and feces were collected separately for at least 19 hrs before dosing. After dosing, urine and feces samples were collected from 0-8 and 8-24 hrs, and then over 24-hrs intervals through 72 hrs after dosing.

Results:

Recoveries of radioactivity during each interval after a single i.v. infusion (2 mg/kg, 30 µCi/kg) of [14C]BMS-354825 to male Cynomolgus monkeys

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ON ORIGINAL**

Matrix	Time (h)	Percent of Radioactive Dose				
		Animal Number			Mean	SD
		1001	1002	1003		
Urine	Predose				0.00	0.00
	0-8				8.50	3.00
	8-24				0.72	0.38
	24-48				0.43	0.29
	48-72				0.24	0.04
	Subtotal	11.57	11.54	6.56	9.89	2.88
Bile	Predose				0.00	0.00
	0-4				56.19	11.23
	4-8				8.14	2.87
	8-24				2.59	2.24
	24-48				0.28	0.08
	48-72				0.04	0.02
	Subtotal	48.31	77.08	76.34	67.24	16.40
Feces	Predose				0.00	0.00
	0-8				0.05	0.07
	8-24				3.62	2.09
	24-48				6.69	6.33
	48-72				3.31	4.77
	Subtotal	28.04	4.18	8.79	13.67	12.66

a The percent of dose was calculated based on a combination of the sample weights of the primary sample and the overflow sample.

NF No feces produced.

Table provided by the sponsor.

Total recoveries of radioactivity after a single i.v. infusion (2 mg/kg, 30 µCi/kg) of [14C]BMS-354825 to male Cynomolgus monkeys

Matrix	Time (h)	Percent of Radioactive Dose				
		Animal Number			Mean	SD
		1001	1002	1003		
Urine	0-72				9.89	2.88
Bile	0-72				67.24	16.40
Feces	0-72				13.67	12.66
Total	0-72				90.80	2.56

Table provided by the sponsor.

Concentrations of Radioactivity in Plasma

The mean plasma concentration of BMS-354825 equivalents was 337 ng equivalents/g at 1 h post-dose and then declined in a mono-phasic manner. Mean concentrations were measurable through 8 h post-dose.

Time (h)	Concentration (ng equivalents of BMS-354825/g)			Mean	SD
	Animal Number				
	1001	1002	1003		
Predose				BQL	NA
1				337	45.6
4				135	37.5
8				34.6	7.12
24				BQL	NA
72				BQL	NA

BQL Below the quantifiable limit (12.3 ng equiv/g).

NA Not applicable.

Table provided by the sponsor.

Summary of the study:

Male bile duct cannulated Cynomolgus monkeys were administered single dose of radiolabeled dasatinib by 10-min i.v. infusion. Blood samples were collected for PK evaluation. Bile, urine, and feces were collected up to 72 hrs for determination of elimination of radioactivity.

- Biliary excretion was the main route of elimination of dasatinib and its metabolites, as indicated by approximately 67% recovery of the radioactivity in the bile.
- 14% of the radioactivity was recovered in the feces and 10% in the urine.
- Elimination of the radioactivity occurred mainly during the first 48 hrs.
- The mean total recovery in all samples collected through 72 h after dosing was 90.80%.
- The mean plasma concentration of BMS-354825 equivalents was 337 ng equivalents/g at 1 h post-dose and then declined in a mono-phasic manner. Mean concentrations were measurable through 8 h post-dose.

2.6.4.7 Pharmacokinetic drug interactions

For CYP induction/inhibition and p-glycoprotein studies see the clinical pharmacology review of the NDA.

2.6.4.8 Other Pharmacokinetic Studies

For Caco-2 cell permeability studies see the clinical pharmacology review of the NDA.

2.6.4.9 Discussion and Conclusions

See section 2.6.4.1.

2.6.4.10 Tables and figures to include comparative TK summary

Dasatinib exposures in rats vs humans

Species	Study	Dose (mg/kg)	Mean AUC (ng-hr/mL, 0-24 hour ^a)		Multiple of Human Exposure Based on AUC 70 mg BID	
			Males	Females	Males	Females
Human	Continuous daily dose	70 mg BID	308 ^b (Daily AUC, ng-hr/mL)		-	-
Rat	2 week, daily oral	1	70	51	0.2	0.2
		15	1,449	1,567	4.7	5.1
		30 ^c	2,905	5,111	9.4	16.6
Rat	1 month, 5-days on/ 2-days off	0.9	34	45	0.1	0.1
		15	937	920	3.0	3.0
		25 ^c	1,315	1,737	4.3	5.6
Rat	6 months, daily oral	1.5	88	114	0.3	0.4
		4	323	416	1.0	1.4
		15 ^c	1,355	1,077	4.4	3.5
		10 ^{c,d}	564	973	1.8	3.2
		8 ^{c,d}	551	777	1.8	2.5

a For the 6-month rat study, AUC values were calculated from time zero to the time of last measurable concentration, ranging between 8 to 24 hours.

b Daily AUC based on twice the geometric mean AUC(0-12hr) of 154 ng-hr/mL.

c Doses associated with severe toxicity and lethality.

d In surviving animals, the high dose was lowered from 15 to 10 mg/kg/day in Week 8 and then to 8 mg/kg/day in Week 17.

Table provided by the sponsor.

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Dasatinib exposures in monkeys vs humans

Species	Study	Dose (mg/kg)	Mean AUC (ng·hr/mL, 0-24 hour ^a)		Multiple of Human Exposure Based on AUC 70 mg BID	
			Males	Females	Males	Females
Human	Continuous daily dose	70 mg BID	308 ^b (Daily AUC, ng·hr/mL)			
Monkey	10 day, 5-days on/ 2-days off	1	83	29	0.3	0.1
		10	1,322	825	4.3	2.7
		15	1,654	1,308	5.4	4.2
		25 ^c	7,091	4,719	23.0	15.3
		62.5 ^c	8,467	10,624	27.5	34.5
Monkey	1 month, 5-days on/ 2-days off	1	36	17	0.1	0.06
		5	206	280	0.7	0.9
		15	1,162	1,053	3.8	3.4
Monkey	9 months, 5-days on/ 2-days off	1	56	54	0.2	0.2
		3 ^c	149	131	0.5	0.4
		2 ^d	146	93	0.5	0.3
		10 ^c	949	755	3.1	2.5
		4.5 ^{c,d}	316	206	1.0	0.7

a For the 9-month monkey study, AUC values were calculated from time zero to the time of last measurable concentration, ranging between 8 to 24 hours.

b Daily AUC based on twice the geometric mean AUC(0-12hr) of 154 ng·hr/mL.

c Doses associated with severe toxicity and lethality.

d The high dose was lowered from 10 to 6 mg/kg/day in Week 3 and then to 4.5 mg/kg/day in Week 12; toxicokinetics were not assessed at 6 mg/kg/day. The intermediate dose was lowered from 3 to 2 mg/kg/day in Week 28.

Table provided by the sponsor.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Tabulated summary of selected studies are presented below. Tables have been extracted from the sponsor's submission.

Study Description or Title: Single dose pharmacokinetic study of dasatinib in rats

Test Article: Dasatinib
Study Type: non-GLP

Species/Strain: Rat/Sprague-Dawley
Study No./Document Control Number: MAP005/554825/930003190
Location in Dossier: map005.pdf

Gender (M/F) / Number of Animals: M/3 animals M/3 animals M/3 animals
Feeding condition: Fasted overnight/ fed 4 h postdose Fasted overnight/ fed 4 h postdose Fasted overnight/ fed 4 h postdose
Vehicle/Formulation: Propylene glycol: Water (1:1) Propylene glycol: Water (1:1) 50 mM Sodium acetate buffer (pH 4.6)

Method of Administration: Intra-arterial Oral Gavage 30 min infusion intra-portal
Dose (mg/kg): 10 10 10
Sample (Whole blood, plasma, serum etc.): Plasma Plasma Plasma
Analyte: Dasatinib Dasatinib Dasatinib
Assay: LC:MS/MS LC:MS/MS LC:MS/MS

PK parameters:

Dose (mg/kg)	10	10	10
Parameter / Route	Intra-arterial	PO	Portal
C _{max} (µg/mL)	6.8 ± 2.3	0.24 ± 0.09	3.7 ± 0.6
AUC ₀₋₂₄ (µg•h/mL)	26.4 ± 7.8	1.9 ± 1.0	7.7 ± 2.7
T _{max} (h)		2.3 ± 3.3	0.5 ± 0.0
Cl (mL/min/kg)			

V _{ss} (L/kg)	6.3 ± 2.2	-	-
t _{1/2} (h)	3.3 ± 0.9	3.1 ± 0.3	6.7 ± 3.0
Bioavailability (%)	-	27 ± 15	114 ± 39
% of Dose excreted in urine as parent (0-10 h)	1.6 ± 0.5	0.2 ± 0.04	0.5 ± 0.2

Additional Information: Dasatinib shows a moderate systemic clearance in rat. The systemic exposure was not decreased when dasatinib was administered via portal vein infusion compared to that via intra-arterial administration, suggesting minimum first pass effect in rat.

**APPEARS THIS WAY
ON ORIGINAL**

Study Description or Title: Single dose pharmacokinetic study of dasatinib in monkeys

Test Article: Dasatinib
Study Type: non-GLP

Species/Strain Monkey/Cynomolgus

Study No./Document Control Number MAP005/354825/930003190

Gender (M/F) / Number of Animals M/3 animals M/3 animals M/2 animals
Feeding condition Fasted overnight/fed 4 h postdose Fasted overnight/fed 4 h postdose Fasted overnight/fed 4 h postdose

Vehicle/Formulation 50 mM Sodium acetate buffer (pH 4.6) 50 mM Sodium acetate buffer (pH 4.6) Oral Capsule/Free base Oral Capsule/HCl salt

Method of Administration IV PO PO PO

Sample (Whole blood, plasma, serum etc.) Plasma Plasma Plasma

Analyte Dasatinib Dasatinib Dasatinib

Assay LC/MS/MS LC/MS/MS LC/MS/MS LC/MS/MS

PK parameters:

Parameter / Route	Dose (mg/kg)	2 (free base solution)	5 (free base solution)	4.7 (free base capsule)	4.9 (capsule)
C _{max} (µg/mL)	IV	-	0.17 ± 0.03	PO	PO
AUC ₀₋₂₄ (µg•h/mL)		0.98 ± 0.11	0.37 ± 0.02	0.09 ± 0.02	0.08
T _{max} (h)		-	0.6 ± 0.1	0.30 ± 0.18	0.25
Cl (mL/min/kg)		34 ± 4.1	-	2.3 ± 1.6	3.4
V _{ss} (L/kg)		3.5 ± 0.1	-	-	-

$t_{1/2}$ (h)	2.1 ± 0.1	2.2 ± 0.4	-	-
Bioavailability (%)	-	15.2 ± 2.1	13 ± 8	10.3
% of Dose excreted in urine as parent (0-24h)	0.7 ± 0.3	0.1 ± 0.1	0.04 ± 0.01	0.03

Additional Information: Dasatinib shows a moderate systemic clearance in monkey. The oral bioavailability of dasatinib from the solid dosage forms was slightly lower than that from the oral solution. The plasma exposure from the free base and the ~~---~~ appeared to be comparable.

**APPEARS THIS WAY
ON ORIGINAL**

Study Description or Title: Single dose toxicokinetic study of dasatinib in monkeys

Test Article: Dasatinib
Study Type: GLP

Species/Strain: Monkey/Cynomolgus

Study No./Document Control Number: DS02147/930003271
 ds02147.pdf

Location in Dossier

Gender (M/F) / Number of Animals M/F, 2/sex

M/F, 2/sex

M/F, 2/sex

Feeding condition

Fasted overnight

Fasted overnight

Fasted overnight

Vehicle/Formulation

80 mM Sodium acetate buffer

80 mM Sodium acetate buffer

80 mM Sodium acetate buffer

Method of Administration

Oral gavage

Oral gavage

Oral gavage

Dose (mg/kg)

15

25

45

Sample (Whole blood, plasma, serum etc.)

Plasma

Plasma

Plasma

Analyte

Dasatinib

Dasatinib

Dasatinib

Assay

LC/MS/MS

LC/MS/MS

LC/MS/MS

PK parameters:

Dose (mg/kg)	15		25		45	
Sex	M	F	M	F	M	F
C _{max} (µg/mL)	0.757	1.492	1.079	0.828	1.763	2.107
AUC ₀₋₂₄ (µg•h/mL)	2.225	2.760	5.373	3.801	7.771	8.745
T _{max} (h)	1	1	1.5	1.5	1	2

Additional Information: The exposure of the monkey to dasatinib after single oral doses was dose-related with no apparent sex-related differences in the systemic exposure.

Study Description or Title: Toxicokinetic evaluation of dasatinib in one month intermittent dose oral toxicity study in rats

Test Article: Dasatinib

Study Type: GLP

Species/Strain

Rat/Sprague-Dawley

Study No./Document Control Number

DS02158-354825/930003258

Location in Dossier

ds02158.pdf

Gender (M/F) / Number of Animals

M/F, 15/sex

M/F, 15/sex

M/F, 15/sex

Feeding condition

Ad lib

Ad lib

Ad lib

Vehicle/Formulation

80 mM Sodium citrate

80 mM Sodium citrate

80 mM Sodium citrate

Method of Administration

Oral gavage

Oral gavage

Oral gavage

Dose

1 mg/kg/day for 5 days on then 2 days off for 4 cycles

15 mg/kg for 5 days on then 2 days off for 4 cycles

25 mg/kg/day for 5 days on then 2 days off for 4 cycles

Sample (Whole blood, plasma, serum etc.)

Plasma

Plasma

Plasma

Analyte

Dasatinib

Dasatinib

Dasatinib

Assay

LC/MS/MS

LC/MS/MS

LC/MS/MS

PK parameters:

Parameter / Sex	Dose (mg/kg/day)		15		25		
	M	F	M	F	M	F	
C _{max} (ng/mL)	Day 1	7.9	9.8	96.1	88.4	102.1	184.2
	Day 26	6.6	13.4	57.7	51.8	49	63.8
AUC ₀₋₂₄ (ng•h/mL)	Day 1	34	45	937	920	1315	1737
	Day 26	32	41	827	705	951	944
T _{max} (h)	Day 1	4	4	8	8	8	4
	Day 26	4	2	8	2	4	4

Additional Information: The systemic exposure was similar in females compared to males. The C_{max} values increased in a less than dose proportional manner, while the AUC values appeared to increase in roughly a dose proportional manner on both day 1 and day 26. The AUC values on day 26 appeared less than those on day 1 at the 15 and 25 mg/kg/day dose levels for both male and female rats.

Study Description or Title: Toxicokinetic analysis of dasatinib in a 6-month oral toxicology study in rats
Test Article: Dasatinib
Study Type: GLP

Species/Strain: Rat/Sprague-Dawley
Study No./Document Control Number: DS03072/930011518
Location in Dossier: ds03072.pdf
Gender (M/F) / Number of Animals: M/F, 9/sex/dose level
Feeding condition: Ad lib
Vehicle/Formulation: 80 mM sodium citrate buffer
Method of Administration: Oral
Dose (mg/kg/day): The dose for the low- and intermediate-dose groups were 1.5, 4 mg/kg/day. The dose for the high-dose group was 15 mg/kg/day during weeks 1 through 7. 10 mg/kg/day during weeks 8 through 16, and 8 mg/kg/day beginning on week 17.

Sample (Whole blood, plasma, serum etc.): Plasma

Analyte: Dasatinib
Assay: LC/MS/MS

PK parameters:

Parameter / Sex	Dose (mg/kg)			15/10:8 ^a		
	M	F	Study Day	M	F	Study Day
C _{max} (ng/mL)	11.9	11.2	Day 1	39.8	34.7	Day 1
	8.3	12.0	Week 13	46.7	29.7	Week 13
	15.8	24.6	Week 26	29.8	52.9	Week 26
AUC(0-T) ^b (ng/mL•h)	72.6	62.9	Day 1	250.9	244.3	Day 1
	48.2	114.2	Week 13	241.0	194.1	Week 13
	87.7	104.1	Week 26	322.7	416.3	Week 26

Study Description or Title: Toxicokinetic analysis of dasatinib in a 6-month oral toxicology study in rats
 Test Article: Dasatinib
 Study Type: GLP

Parameter / Sex	1.5			4			15/10/8 ^a	
	Study Day	M	F	M	F	M	F	
T _{max} (h)	Day 1	4.0	4.0	4.0	4.0	8.0	4.0	
	Week 13	4.0	2.0	2.0	4.0	8.0	2.0	
	Week 26	2.0	2.0	2.0	2.0	2.0	2.0	

Additional information: Systemic exposure of the rats to dasatinib following daily administration of 1.5 to 4 mg/kg/day doses for 26 weeks was roughly dose proportional. At the high doses the AUC values to dasatinib appeared to increase in a slightly greater than dose proportional manner. The C_{max} values appeared to increase in a dose proportional manner over the entire dose range. The systemic exposure appeared to be similar between male and female rats. There was no consistent accumulation or reduction in systemic exposure of rats to dasatinib after 26 weeks of dosing.

^a The dose for the high-dose group was 15 mg/kg/day during weeks 1 through 7, 10 mg/kg/day during weeks 8 through 16, and 8 mg/kg/day beginning on week 17.
^b Calculated from time zero to the time of the last measurable concentration ranging between 8 to 24 h.

**APPEARS THIS WAY
ON ORIGINAL**

Study Description or Title: Toxicokinetic Analysis of Dasatinib: Oral Study of Embryo-Fetal Development in Rats.

Test Article: Dasatinib

Study Type: GLP

Species/Strain: Rat CD® (SD) IGS BR
Study No./Document Control Number: DN04078/930011508
Location in Dossier: dn04078.pdf
Gender (M/F) / Number of Animals: F, 10/dose level
Feeding condition: Ad lib
Vehicle/Formulation: 80 mM sodium citrate buffer
Method of Administration: Oral gavage
Dose (mg/kg/day): 2.5, 5, 10, or 20 mg/kg/day once daily on days 6 to 15 of presumed gestation.
Sample (Whole blood, plasma, serum etc.): Plasma

Analyte

Assay

PK parameters: Dasatinib LC/MS/MS

Dose (mg/kg/day)	2.5	5	10	20
Parameter / Sex	F	F	F	F
C _{max} (ng/mL)	21.1	43.7	128.2	107.2
AUC(0-T) ^a (ng/mL•h)	105 ^b	239 ^b	1490	1270

Additional information: The systemic exposure of pregnant rats to dasatinib was dose-related. Between 2.5 and 5 mg/kg/day, the AUC values increased in a roughly dose-proportional manner, while at the dose levels between 5 and 10 mg/kg/day, the increase in AUC appeared to be greater than dose-proportional. Between 10 and 20 mg/kg/day, there was no increase in systemic exposure.

^a Calculated from time zero to 24 h unless otherwise noted.

^b Calculated from time zero to 8 h.

Study Description or Title: Toxicokinetic Analysis of Dasatinib: Oral Study of Embryo-Fetal Development in Rabbits

Test Article: Dasatinib

Study Type: GLP

Species/Strain: Rabbit/New Zealand White Hra:(NZW) SPF

Study No./Document Control Number: DN04080/930010604

Location in Dossier: dn04080.pdf

Gender (M/F) / Number of Animals:

Feeding condition: Ad lib

Vehicle/Formulation: 80 mM sodium citrate buffer

Method of Administration: Oral gavage

Dose (mg/kg/day): 0.5, 2 or 6 mg/kg/day once daily on days 7 to 19 of presumed gestation

Sample (Whole blood, plasma, serum etc.): Plasma

Analyte: Dasatinib

Assay: LC/MS/MS

PK parameters:

Dose (mg/kg)		0.5	2	6
Parameter / Sex	Study Day	F	F	F
C _{max} (ng/mL)	Day 19	14 ± 11	63 ± 16	227 ± 111
AUC(0-T) (ng/mL* ^a h)	Day 19	44 ± 35 ^b	248 ± 94	834 ± 213

Additional Information: The systemic exposure of pregnant rabbits to dasatinib was dose-related. Between 0.5 to 2 mg/kg/day and 2 to 6 mg/kg/day, the AUC values increased in a roughly dose-proportional manner.

^a Calculated from time zero to 24 h otherwise noted.

^b Calculated from time zero to 8 h.

Study Description or Title: Toxicokinetic evaluation of dasatinib in one-month intermittent dose oral toxicity study in monkeys

Test Article: Dasatinib
Study Type: GLP

Species/Strain: Monkey/Cynomolgus
Study No./Document Control Number: DS02159/930003259
Location in Dossier: ds02159.pdf

Gender (M/F) / Number of Animals: M/F, 4/sex Ad lib M/F, 4/sex Ad lib M/F, 4/sex Ad lib

Feeding condition: Ad lib

Vehicle/Formulation: 87.5 mM Sodium citrate

Method of Administration: Nasal intubation

Dose: 1 mg/kg/day for 5 days on then 2 days off for 4 cycles
 5 mg/kg/day for 5 days on then 2 days off for 4 cycles
 15 mg/kg/day for 5 days on then 2 days off for 4 cycles

Sample (Whole blood, plasma, serum etc.): Plasma

Analyte Assay: Dasatinib LC/MS/MS

PK parameters: Dasatinib LC/MS/MS

Parameter / Sex	Dose (mg/kg/day)		1		5		15	
	M	F	M	F	M	F	M	F
C _{max} (µg/mL)	0.020	0.011	0.091	0.093	0.480	0.399		
	0.012	0.008	0.050	0.064	0.154	0.374		
AUC ₀₋₂₄ (µg•h/mL)	0.036	0.017	0.206	0.221	1.162	1.053		
	0.034	0.016	0.181	0.280	0.774	0.976		
T _{max} (h)	1.5	1	1.5	1	1	1.5		
	2	1.5	1.5	2	1.5	1.5		

Additional Information: Between the 1 and 15 mg/kg/day dose levels, the C_{max} and AUC values increased in a greater than dose proportional manner. The systemic exposure to dasatinib was similar between female and male monkeys. The AUC values on day 26 were comparable to those on day 1 for both sexes, suggesting that dasatinib did not accumulate over the 26 days of intermittent dosing.

Study Description or Title: Toxicokinetic evaluation of dasatinib in nine-month oral toxicity study in *Cynomolgus* monkeys

Test Article: Dasatinib

Study Type: GLP

Species/Strain: Monkey: *Cynomolgus*
 Study No./Document Control Number: DS03073-930011520
 Location in Dossier: ds03073.pdf
 Gender (M/F) / Number of Animals: M/F, 6/sex/dose level
 Feeding condition: Ad lib
 Vehicle/Formulation: 80 mM sodium citrate buffer
 Method of Administration: Oral
 Sample (Whole blood, plasma, serum etc.): Plasma
 Analyte: Dasatinib
 Assay: LCMS/MS

PK parameters:

Parameter / Sex	Dose (mg/kg/day) ^a		1		3, 2 ^b		10, 6, 4, 5 ^c	
	Study Day	M	F	M	F	M	F	
C _{max} (ng/mL)	Day 1	16.4 ± 14.1	11.8 ± 9.6	50.2 ± 41.7	55.6 ± 23.7	291.1 ± 219.7	244.7 ± 97.6	
	Day 100 (Week 15)	13.0 ± 9.1	17.5 ± 10.9	22.7 ± 5.7	47.4 ± 49.5	115.7 ± 85.4	68.9 ± 41.4	
	Day 195 (Week 28)	11.8 ± 6.8	19.8 ± 14.1	21.0 ± 15.5	29.4 ± 5.0	ND	ND	
AUC _T (ng/mL·h) ^d	Day 282 (Week 41)	28.7 ± 9.9	31.3 ± 9.2	35.5 ± 14.6	52.4 ± 4.3	ND	ND	
	Day 1	38.2 ± 27.9	36.3 ± 18.0	148.7 ± 59.6	130.5 ± 37.9	949.0 ± 431.1	755.2 ± 227.9	
	Day 100 (Week 15)	36.5 ± 21.3	38.6 ± 18.1	107.3 ± 18.3	118.2 ± 75.6	315.9 ± 179.4	206.4 ± 87.3	
T _{max} (h) ^e	Day 195 (Week 28)	27.1 ± 13.7	36.0 ± 21.0	85.7 ± 57.2	79.1 ± 15.2	ND	ND	
	Day 282 (Week 41)	56.2 ± 17.7	54.1 ± 14.6	146.3 ± 28.3	93.1 ± 16.9	ND	ND	
	Day 1	1.0 (1.0, 2.0)	2.0 (1.0, 4.0)	1.5 (1.0, 2.0)	1.0 (1.0, 1.0)	1.0 (1.0, 2.0)	1.0 (1.0, 2.0)	
T _{max} (h) ^e	Day 100 (Week 15)	1.0 (1.0, 2.0)	1.0 (1.0, 2.0)	2.0 (1.0, 2.0)	1.0 (1.0, 2.0)	1.0 (1.0, 2.0)	1.0 (1.0, 2.0)	
	Day 195 (Week 28)	1.0 (1.0, 2.0)	1.0 (1.0, 1.0)	1.0 (1.0, 2.0)	1.0 (1.0, 2.0)	ND	ND	
	Day 282 (Week 41)	1.0 (1.0, 1.0)	1.0 (1.0, 1.0)	1.0 (1.0, 1.0)	1.0 (1.0, 1.0)	ND	ND	

Additional information: Systemic exposure of the monkeys to dasatinib was dose-related. The AUC values appeared to increase in a greater than dose proportional manner between 1 and 10 mg/kg on day 1, however, they increased in nearly a dose proportional manner for Days 100, 195 and 282. The systemic exposure to dasatinib appeared to be similar between males and females. There was no obvious accumulation or reduction in the exposure to dasatinib over the 41 weeks of dosing.

- a Beginning early in week 2, the monkeys were not dosed for about a week, after which time the dosing regime was changed from daily dosing to a 5-days-on and 2-days-off dosing schedule
- b Due to toxicity observed, the mid-dose level was modified from 3 mg/kg to 2 mg/kg (beginning on Day 190; Week 27)
- c Due to toxicity observed, the high-dose level was modified from 10 mg/kg to 6 mg/kg (beginning on Day 15, Week 3) and from 6 mg/kg to 4.5 mg/kg beginning on Day 83 (Week 12)
- d Calculated from time zero to the time of last measurable concentration
- e Median (minimum, maximum)

ND = Not Determined. Due to toxicity observed, the high-dose group was terminated during week 26

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Study Description or Title: Serum Protein Binding of Dasatinib and its N-dealkylated metabolite BMS-582691

Test Article: Dasatinib and BMS-582691

Study Type: Non-GLP

Study system: Pooled fresh serum from mouse, rat, dog, monkey or human

Target entity, Test system and method: Equilibrium dialysis was carried out at 37°C for 4 hours. Dasatinib and BMS-582691 were analyzed by LC/MS/MS

Species	Conc. tested	% Bound (mean ± SD, n = 3 or 4)	Study No./ Document Control No.	Location in Dossier
Mouse	10 µM, dasatinib	91.8 ± 4.6	MAP005/354825/930003190	
Rat	10 µM, dasatinib	97.4 ± 0.6	MAP005/354825/930003190	
Dog	10 µM, dasatinib	95.8 ± 1.1	MAP005/354825/930003190	
Monkey	10 µM, dasatinib	96.9 ± 0.6	MAP005/354825/930003190	
Human	10 µM, dasatinib	93.9 ± 5.4	MAP005/354825/930003190	
Human	100 ng/mL (0.20 µM), dasatinib	96.3 ± 0.2	NA/930011593	
			NA/930011548	
	500 ng/mL (1.02 µM), dasatinib	96.4 ± 0.3	NA/930011593	
			NA/930011548	
	100 ng/mL (0.23 µM), BMS-582691	93.7 ± 0.9	NA/930011593	
			NA/930011548	
	500 ng/mL (1.13 µM), BMS-582691	93.1 ± 0.3	NA/930011593	
			NA/930011548	

Additional Information: Relatively high protein bindings of dasatinib and its dealkylated metabolite BMS-582691 were observed across species. The protein binding of dasatinib and BMS-582691 did not appear to be concentration-dependent from 100 ng/mL to 500 ng/mL. The slight difference in human serum protein binding of dasatinib between the two experiments might be due to experimental variations.

Study Description or Title: Biotransformation of [¹⁴C]dasatinib in Sprague Dawley Rat, Cynomolgus Monkey and Human

Test Article: [¹⁴C]Dasatinib

Study Type: Non-GLP

Study No./Document Control No.: NA/930011321

Location in Dossier: 930011321.pdf

Species/Strain Gender (M/F)/Number of animals: Feeding condition: Vehicle/Formulation: Method of Administration: Dose: Radionuclide: Specific Activity:	Rat/Sprague Dawley M/3 Fasted overnight/fed 4 h postdose 80 mM citrate buffer, pH 3.1 Oral gavage 15 mg/kg (80 µCi/kg) ¹⁴ C 5.26 µCi/mg		Monkey/Cynomolgus M/3 Fasted overnight/fed 4 h postdose 80 mM citrate buffer, pH 3.1 Oral gavage 10 mg/kg (30 µCi/kg) ¹⁴ C 3.17 µCi/mg		Human M/8 Fasted for ≥10 h/ fed 4 h postdose 25 mM citrate Oral 100 mg (120 µCi) ¹⁴ C 1.2 µCi/mg				
	% Distribution of Radioactivity ^b								
Metabolite ^a	Feces 0-168 h (% of Dose)			Urine 0-168 h (% of Dose)		Plasma ^d (% of Sample)			
	Rat	Monkey	Human ^c	Rat	Monkey	Human	Rat 4 h	Monkey 4 h	Human 2 h
Parent	41.6	24.7	19.1	0.7	0.6	0.1	52.8	32.1	25.5
M3a	-	-	-	0.1	0.1	0.2	-	1.4	3.3
M3b	-	-	-	-	-	-	-	-	-
M4	1.9	2.9	2.6	0.3	0.05	0.05	2.6	3.0	MS ^f
M5	-	-	-	3.4	1.1	1.4	18.7	2.6	4.5
M6	13.2	14.0	8.9	0.4	0.06	0.05	2.2	2.7	3.6
M7	0.8	0.4	-	0.02	0.3	0.08	-	2.1	3.3
M8a	-	-	-	0.06	0.04	0.2	-	12.8	3.4
M9	-	1.5	1.5	-	-	-	-	-	-
M8h	-	-	-	0.05	0.07	-	-	-	-
M23a	-	-	-	-	-	-	-	-	-
M23b	2.9	9.7	12.5	-	-	-	-	4.5	1.4

Study Description or Title: Biotransformation of [¹⁴C]Dasatinib in Bile-Duct Cannulated and Intact Sprague Dawley Rat and Bile-Duct Cannulated Cynomolgus Monkey

Test Article: [¹⁴C]Dasatinib
Study Type: Non-GLP
Study No./Document Control No.: NA/930010531
 NA/930011321
Location in Dossier: 930010531.pdf 930011321.pdf

Species/Strain Gender (M/F)/Number of animals; Feeding condition; Vehicle/Formulation; Method of Administration; Dose; Radionuclide; Specific Activity;	Rat/Sprague Dawley		Monkey/Cynomolgus											
	M/2 per treatment/sample time or time period Fasted overnight and for duration of study 50 mM sodium acetate buffer, pH 4.0 Single dose PO, and IV 10 mg/kg (60 µCi/kg) ¹⁴ C 6 µCi/mg	M/3 per sample time or time period Fasted overnight and fed 4 h post-dose 80 mM citrate buffer, pH 3.1 Single dose IV 2 mg/kg (30 µCi/kg) ¹⁴ C 13.3 µCi/mg												
Metabolite ^{a,b}	Bile (% of Dose)				Urine (% of Dose)				Plasma ^d (% of Sample)					
	Rat-PO (0-12 h)	Rat-IV (0-12 h)	Monkey-IV (0-168 h)	Rat-PO (0-12 h)	Rat-IV (0-12 h)	Monkey-IV (0-168 h)	Rat-PO (0-12 h)	Rat-IV (0-12 h)	Rat-PO (0-12 h)	Rat-IV (0-12 h)	Rat-PO (0-12 h)	Rat-IV (0-12 h)	Rat-PO (0-12 h)	Rat-IV (0-12 h)
Parent	5.7	11.2	3.2	0.3	0.7	0.1	41.6	48.0	34.4	55.5	37.6	35.7	-	-
M3a	-	-	2.0	-	-	0.4	-	-	-	-	-	-	-	-
M3b	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M4	6.7	14.1	0.5	0.3	1.0	-	1.7	1.2	0.6	1.2	1.7	1.2	-	-
M6	-	-	6.3	-	-	0.3	-	-	-	-	-	-	-	-
M5	6.4	16.7	5.0	1.6	8.0	7.0	12.9	12.8	13.6	14.6	17.8	15.1	-	-
M7	-	-	8.3	-	-	0.9	-	-	-	-	-	-	-	-
M8	3.8	3.8	-	0.1	0.2	-	5.6	4.2	3.8	1.9	4.4	4.4	-	-
M22	0.4	0.9	-	-	-	-	-	-	-	-	-	-	-	-
M8a	-	-	0.9	-	-	-	-	-	-	-	-	-	-	-

^c % Distribution of Radioactivity

- a The following metabolites co-eluted on the HPLC system used for separation of samples from rats: (M4 and M6) and (M8 and M22, for the plasma analysis). The radioactivity reported represents the combined contribution of the co-eluting metabolites, as indicated in the table.
- b The following metabolites co-eluted on the HPLC system used for separation of monkey samples (M3a with M3b), (M8b with M23a, and M23b), and (M37a with M37b). The radioactivity reported represents the combined contribution of the co-eluting metabolites, as indicated in the table.
- c The relative distribution of dactinib and its metabolites in bile and urine is expressed as the % of the dose that each radioactive component represents in a particular matrix. The relative distribution in plasma is expressed as the % distribution of radioactivity of each radioactive component in the sample extract.
- d Blood samples were collected from intact rats (n=2 per time point). Plasma was prepared from blood collected with EDTA as the anti-coagulant. Plasma samples from bile-duct cannulated monkeys were not analyzed.
- e A dash (-) means that the metabolite was not detected in the sample.
- f MS = Metabolite was detected by mass-spectrometry, but not by radioactivity.
- g Other = radioactive components that were not identified or analyzed.
- h An additional 53.0% of the radioactive dose was found in the GI tract of PO-dosed rats at 12 h.

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Study Description or Title: Biotransformation of [¹⁴C]Dasatinib in Liver, Microsomal and Hepatocyte Preparations from Sprague-Dawley Rat, Cynomolgus Monkey and Human

Test Article: [¹⁴C]Dasatinib
 Study Type: Non-GLP
 Location in Dossier: 930011324.pdf
 Study No./Document Control No.: NA/930011324

Study system: Microsomes, 20 µM [¹⁴C]Dasatinib
 Hepatocytes, 20 µM [¹⁴C]Dasatinib
 Time (h): Microsome Incubation time = 0.25 h
 Hepatocyte Incubation time = 3 h

Metabolites	% Distribution of Radioactivity (% of sample)						
	Microsomes			Hepatocytes			
	Sprague-Dawley Rat	Cynomolgus Monkey	Human	Sprague-Dawley Rat	Cynomolgus Monkey	Human	Human
M3a, M3b ^a	1.4	6.0	2.9	ND	ND	ND	ND
M4	1.8	5.8	4.0	1.6	3.7	1.0	1.0
M5	37.3	8.6	4.0	2.7	3.2	2.3	2.3
M6	0.7	2.1	1.6	4.9	6.5	9.2	9.2
M7	ND	0.8	ND	ND	2.1	1.6	1.6
M9	ND	3.2	1.1	ND	1.4	ND	ND
M20, M24 ^a	4.5	30.0	39.2	1.1	4.5	2.6	2.6
M21	ND	ND	ND	0.8	6.9	2.2	2.2
M23a, M23b ^a	ND	1.2	ND	ND	1.1	0.6	0.6

Metabolites	% Distribution of Radioactivity (% of sample)					
	Microsomes			Hepatocytes		
	Sprague-Dawley Rat	Cynomolgus Monkey	Human	Sprague-Dawley Rat	Cynomolgus Monkey	Human
M28a, M28b ^a	ND	2.8	1.7	ND	ND	ND
M29a, M29b ^a	ND	4.9	4.8	ND	ND	ND
M29c	ND	2.5	3.8	ND	ND	ND
M30	ND	ND	ND	ND	4.9	1.5
M31	ND	ND	ND	ND	0.8	ND
P. Dasatinib (BMS-354835)	54.2	27.7	35.5	88.8	61.2	77.7
Total	99.9	95.6	98.6	99.9	96.3	98.7

Additional Information: Exploratory studies in microsomes and hepatocytes were conducted with non-radiolabeled dasatinib order to identify metabolites of dasatinib (M4A9005-354825-930003190). The results from those studies are not presented in a tabulated summary of the CTD because a quantitative estimate of metabolite distribution could not be made.

^a Chromatographic peaks were not well-resolved. Radioactivity reported represents the combined radioactivity for the co-eluting peaks.

ND = Not detected by radioactivity.

Study Description or Title: Identification of the Major Enzymes Involved in the Oxidative Metabolism of Dasatinib

Test Article: Dasatinib
 Study Type: Non-CLP
 Location in Dossier: map005.pdf

Study system: Studies performed with human recombinant singly expressed enzymes. Incubations were carried out at 37°C. Aliquots were taken at 0 and 30 minutes and dasatinib was measured by LC/MS/MS. Study No./Document Control No. MAP005/354825/930003190

Human Recombinant Enzyme	Rate of Metabolism (pmol/min/pmol CYP)		
	1 µM	10 µM	100 µM
1A1	0.211	0.494	NR
1A2	0.007	0.302	3.239
2A6	0.043	0.574	3.278
2B6	0.062	0.203	NR
2C8	0.058	0.312	NR
2C9	0.068	0.775	2.253
2C19	0.043	0.279	6.176
2D6	0.036	0.545	1.380
2E1	0.113	0.119	0.407
3A4	0.232	2.708	4.460
3A5	0.023	0.305	1.247
4A11	0.136	0.362	8.372
FMO3	0.047	0.985	12.777
1B1	0.200	0.951	4.779

Additional Information: Many enzymes appear capable of metabolizing dasatinib, including CYP1A1, 2A6, 1B1, 2B6, 2C8, 2C9, 2E1, 4A11 and FMO3. Based on the relative expression of each enzyme in the human liver, it appears that dasatinib is primarily metabolized by CYP3A4.

NR = Not reported.

Study Description or Title: Mass Balance of Radioactivity after Oral Administration of [¹⁴C]Dasatinib to Male Rat, Monkey and Human

Test Article: [¹⁴C]Dasatinib

Study Type: Non-GLP

Location in Dossier: mba00096.pdf

Study No./Document Control Numbers: MBA00096/930010421 ca180-019 total radioactivity

MBA00097/930010419

MBA00129/930011551

Species/Strain Gender (M/F)/Number of animals Feeding condition Vehicle/Formulation Method of Administration Dose Analyte Assay Route/Time Study/Document Control No.	Rat/Sprague-Dawley M3 Fasted Overnight/Fed 4 h post-dose 80 mM sodium citrate buffer, pH 3.1 Oral gavage 15 mg/kg (60 µCi/kg) ¹⁴ C-Total Radioactivity Liquid Scintillation Counting PO/0-168 h MBA00096/930010421	Monkey/Cynomolgus M3 Fasted Overnight/Fed 4 h post-dose 80 mM sodium citrate buffer, pH 3.1 Oral gavage 10 mg/kg (30 µCi/kg) ¹⁴ C-Total Radioactivity Liquid Scintillation Counting PO/0-168 h MBA00097/930010419	Human M3 Fasted 10 h/Fed 4 h post-dose 25 mM sodium citrate buffer Oral 100 mg (120 µCi) ¹⁴ C-Total Radioactivity Liquid Scintillation Counting PO/0-216 h MBA00129/930011551
Species	Excretion of Radioactivity % of Dose (Mean ± SD)		
	Urine	Feces	Total
Rat	6.45 ± 0.82	76.39 ± 8.45	82.84 ± 9.11 ^a
Monkey	2.99 ± 1.55	76.82 ± 9.75	79.81 ± 11.0 ^b
Human	3.58 ± 1.17	85.32 ± 17.28	88.90 ± 17.79

Additional Information:

^a In the rat study, an additional 6.70% of the radioactive dose was found in cage rinse, cage wash, and cage wipe samples and approximately 0.31% of the dose remained in the carcass after 168 h, which brought the total recovery of radioactivity for this study to 89.8%.

^b In the monkey study, approximately 8.84% of additional radioactivity was found in cage debris, cage wash, and cage wipe samples, which brought the total recovery of radioactivity for this study to 88.6%.
SD = Standard deviation.

Study Description or Title: Biliary Excretion of Radioactivity following Oral and Intravenous Administration of [¹⁴C]Dasatinib to Bile-Duct Cannulated Sprague Dawley Rats and Intravenous Administration to Bile-Duct Cannulated Cynomolgus Monkeys

Test Article: [¹⁴C]Dasatinib

Study Type: N88-GLP

Location in Dossier: 930010331.pdf

Study No./Document Control Number: NA 930010331

Study No./Document Control Number: MB 400127930010309

maba00127.pdf

Species/Strain	Rat/Sprague Dawley; bile-duct cannulated	Monkey/Cynomolgus; bile-duct cannulated
Gender (M/F); Number of animals	M/F 2 per treatment	M/F
Feeding condition	Fasted Overnight and for duration of study	Fasted Overnight, Fed 4 h post-dose
Vehicle/Formulation	50 mM sodium acetate buffer, pH 4.0	80 mM sodium citrate buffer, pH 5.1
Method of Administration	Single dose oral gavage and IV	Single dose IV
Dose	10 mg/kg (60 µCi/kg)	2 mg/kg (30 µCi/kg)
Analyte	¹⁴ C-Total Radioactivity	¹⁴ C-Total Radioactivity
Assay	Liquid Scintillation Counting	Liquid Scintillation Counting
Route/Time	PO or IV/0-12 h	IV/0-72 h

Species	Route	Time Interval	% of Radioactive Dose Recovered (Mean ± SD) ^a				
			Urine	Bile	Feces	GI Tract (plus contents)	Total
Rat	PO	0-12 h	3.2	35.8	- ^b	53.0	92.0
	IV	0-12 h	12.0	67.4	-	-	79.4
Monkey	IV	0-72 h	9.89 ± 2.88	67.24 ± 16.40	13.67 ± 12.66	-	90.80 ± 2.56

Additional Information:

^a Standard Deviation was not determined for the rat study since only two animals were evaluated per group.

^b A dash (-) means that a sample was not collected or not analyzed.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

Acute toxicology:

Single doses of dasatinib in rats and monkeys resulted in overlapping toxicities in the GI tract, lymphocytic/ hematopoietic system, liver, and kidneys. Cardiotoxicity was evident in rats and presented with ventricular necrosis, valvular/ ventricular/ atrial hemorrhage (at ≥ 100 mg/kg, 600 mg/m²), and cardiac hypertrophy (mainly in males, at ≥ 30 mg/kg, 180 mg/m²). There was a tendency for increased systolic and diastolic blood pressure in monkeys. Considering the results of the single-dose safety pharmacology studies, \uparrow blood pressure was reported at ≥ 10 mg/kg (120 mg/m²).

Thrombocytopenia was seen in rats, however, hemorrhage and bruising was more evident in monkeys. Ecchymosis was observed in monkeys over numerous sites of the body (thorax, limbs, gingiva, head, and neck). Toxicities to the reproductive organs were observed in rats only (male reproductive system: single cell necrosis and hemorrhage in epididymis, hemorrhage and multinucleated cells in testes).

Repeat-dose toxicology

One- and 6/9-month oral toxicology studies in SD rats and Cynomolgus monkeys were reviewed. Toxicities in the animals were apparent at sub-therapeutic exposures.

- Toxicities in rats started to manifest at the lowest dose tested (1 mg/kg, 6 mg/m² in the 1-month study). This dose had an AUC of approximately 30-40 ng.hr/mL, which is about 0.1 fold the AUC reported in males and females at the recommended dose of 70 mg BID.
- Toxicities in monkeys started to manifest at the lowest dose tested (1 mg/kg, 12 mg/m² in the 1-month study). This dose had an AUC of approximately 15-35 ng.hr/mL, which is 0.06-0.1 fold the AUC reported in males and females at the recommended dose 70 mg BID.

Repeat-dose studies in rats and monkeys resulted in toxicities in multiple organs/tissues. Findings were seen in the GI tract, lymphocytic/ hematopoietic system, kidneys, heart, liver, adrenals, reproductive organs, thyroid, pancreas, lung, and bile duct. Electrolyte imbalance was also noted; which may be due to the nephrotoxicity and/or GI toxicity. Findings not listed below consist of: \downarrow RBC, \downarrow hemoglobin, and \uparrow reticulocytes as well as \uparrow WBC, \uparrow neutrophils, and \uparrow monocytes which may be secondary to internal injury/bleeding; \downarrow ALP, \downarrow globulin, \downarrow albumin, and \downarrow total protein which may be secondary to BW loss/malnutrition.

Studies in SD rats

Toxicities included:

- GI tract (throughout the tract): bloated/swollen abdomen, diarrhea, distention of the GI tract with gas/ fluid/ ingesta or digesta, edema, darkened serosa and mucosa; ulceration/perforation/hemorrhage; congestion/inflammation; squamous hyperplasia/ hyperkeratosis in stomach; villus alteration (e.g. blunting, fusion, branching) in duodenum, jejunum, and ileum; fibrosis of cecum (characterized by aggregates of hyalinized collagen); crypt ectasia/abscess/ edema of cecum
- Hematopoietic/lymphocytic system: hematopoietic cell depletion in bone marrow; ↓lymphocytes; lymphoid depletion in lymph nodes/ thymus/ spleen, hemorrhage/ congestion of mesenteric lymph node and thymus; ↓weight of thymus and spleen; reticuloendothelial hyperplasia and ↑medullary mast cells in mesenteric lymph node; fibrous adhesion in spleen
- Liver: hypertrophy; ↑triglycerides; ↑cholesterol; slightly ↑AST and ALT; inflammation
- Electrolytes: ↓Ca, ↓phosphorus, ↓Na, ↑Cl
- Heart: hypertrophy; fibrosis
- Adrenals: hypertrophy; hyperplasia, vacuolation of zona glomerulosa
- Male reproductive system: ↓size of seminal vesicles; reduced secretion of seminal vesicles; immature sperm in epididymis
- Female reproductive system: hypertrophy of ovaries; fluid filled uterus; dilatation of uterus; ↑ corpus lutea and cyst in ovaries, ↓ incidence of acyclic ovaries; ↓squamous metaplasia of endometrial glands
- Thyroid/ parathyroid: hypertrophy, ↑colloid of thyroid
- Pancreas: acinar atrophy
- Kidney: ↑urinary volume; tubular epithelia regeneration; tubular ectasia/ proteinosis; tubular epithelial hyaline droplets; fibrosis; electrolyte imbalance may be partially attributed to renal injury.
- Lung: minimal segmental medial arteriolar hyperplasia
- Pituitary: ↓weight
- Tongue: degeneration/necrosis, hyperkeratosis, hemorrhage, cyst
- Other: ↑or↓ platelets; chromorhinorrhea; labored breathing; necrosis in salivary gland

Studies in Cynomolgus monkeys

Repeated dosing of dasatinib in cynomolgus monkeys for 10 days, 1-month, or 9 months resulted in severe toxicities to the GI tract and to lymphocytes/ lymphoid system, regardless of the duration of the study. Renal toxicities were mainly evident by histologic examination. Although not strong, hepatotoxicity manifested in the 1- and 9-month toxicology studies.

Organ/tissues that were affected in the 9-month study but not in the shorter term repeat-dose studies included the following: bile duct (hyperplasia), lung (hyperplasia/hypertrophy, fibrosis, hemorrhage, and inflammation), adrenal medulla (mineralization), reproductive organs (♂: immature prostate/ seminal vesicle/ testis; ♀: mineralization and inflammation of uterus), pancreas (inflammation). Of note, multiple organs presented with vascular mineralization in the 9-month study, e.g. in heart, tongue, spleen, stomach, and pancreas.

Primary toxicologic findings observed in the monkeys included:

- GI tract: vomiting, diarrhea, gas/fluid-filled contents of the cecum and colon; red/liquid feces, ulceration/ hemorrhage, edema, inflammation, enterocyte vacuolation and villous fusion; flattening of superficial epithelium, rectal crypt abscess
- Lymphocytic system: ↓lymphocytes; thymic weight; lymphocytic depletion of thymus, spleen, and mesenteric lymph node
- Liver/ hepatobiliary: hypertrophy, slightly ↑AST and ALT, focal necrosis/ hepatocellular necrosis, ↑triglycerides; bile duct hyperplasia
- Heart: inflammation, hypertrophy, vascular mineralization
- Kidney: inflammation; degeneration of cortical tubular epithelial cells; dilatation of cortical tubules; tubular ectasia/proteinosis; cortical mineralization of tubules and glomeruli/ fibrosis; inflammation; ↑BUN
- Phosphorus: hypo-phosphatemia
- Lung: hyperplasia/hypertrophy, hemorrhage, fibrosis, inflammation
- Pancreas: inflammation
- Adrenal medulla: mineralization
- Male reproductive organs: immature prostate, seminal vesicle, and testis
- Female reproductive organ: mineralization and inflammation of uterus
- Multiple organs: vascular mineralization e.g. in heart, tongue, spleen, stomach, and pancreas

Genetic toxicology:

BMS-354825 was clastogenic to CHO cells, in the absence or presence of S9 metabolic activation: BMS-354825 was positive for the induction of structural chromosome aberrations in CHO cells.

Dasatinib was not mutagenic in the bacterial reverse mutation assays (Ames Test) using *S. typhimurium* strains TA98, TA100, TA1535, TA1537, and for *E. coli* strain WP2 *uvrA*. BMS-354825 did not cause chromosomal damage in the rat bone marrow micronucleus test, under the conditions of the study.

No genetic toxicology studies were conducted with the unique human metabolites.

Carcinogenicity:

No study was conducted.

Reproductive toxicology:

The effects of dasatinib on male and female fertility have not been studied. However, results of repeat-dose toxicity studies in multiple species indicate the potential for dasatinib to impair reproductive function and fertility. Effects evident in male animals included reduced size and secretion of seminal vesicles, and immature prostate, seminal vesicle, and testis. The administration of dasatinib resulted in uterine inflammation and mineralization in monkeys, and cystic ovaries and ovarian hypertrophy in rodents.

Dasatinib was evaluated for embryo-fetal toxicities in rats and rabbits. Dosing in both species was from implantation (GD 6 in rats and GD 7 in rabbits) to the end of organogenesis (GD15 in rats and GD 19 in rabbits). Rats were sacrificed on GD 20 and rabbits on GD 29.

Dasatinib was teratogenic in both species. Embryo-fetal effects, i.e. lethality (rats) and/or abnormalities (rats and rabbits), were present at doses that did not cause maternal toxicities. In addition, in both studies embryo-fetal lethality and/or abnormalities were seen at sub-therapeutic exposures.

In rats, the lowest dose (2.5 mg/kg/day or 15 mg/m²/day) resulted in embryo-fetal toxicities. This dose had maternal AUC of 105 ng/hr/mL (0.3 x the human AUC in females at the recommended dose of 70 mg BID). In rabbits the lowest dose (0.5 mg/kg/day or 6 mg/m²/day) caused embryo-fetal toxicities. This dose had a maternal AUC of 44 ng.hr/mL (0.1 x the human AUC in females at the recommended dose of 70 mg BID).

Embryo-fetal toxicities included the following:

Rats:

- Embryo-lethality, starting at the LD (2.5 mg/kg or 15 mg/m²): ↑resorption and ↓mean litter size
- Fetal abnormalities starting at the LD: malformations of the scapula or humerus (bent) and reduced ossification of the sternbrae and thoracic vertebral centra (irregularly/dumbbell shaped and/or reduced ossification site counts). Additional fetal abnormalities at the LMD (5 mg/kg or 30 mg/m²) included fluid-filled thoracic and abdominal cavities, edema (body), microhepatia (small liver), misshapen clavicles, bent radius and femur, wavy or nodulated ribs, and reduced ossification of the thoracic, lumbar, and sacral vertebrae (hypoplastic, not ossified, and/or incompletely ossified centra) and forepaw phalanges (reduced ossification site counts).

Rabbits:

- Fetal abnormalities starting at the LD: delays in ossification of the fetal lumbar vertebrae (bifid arches), pelvis (incomplete ossification or not ossified pubes), and possibly hyoid body (incomplete ossification or not ossified). Additional observations at the HD consisted of irregular ossification of the hyoid (angulated) and presence of 7th cervical ribs.

Special toxicology:

Dasatinib inhibits the activity of a number of SRC family members including LCK. Because LCK was shown to play a role T cell signaling (involved in graft rejection), the immunosuppressive potential of dasatinib to prevent graft rejection was tested in a murine model of T cell proliferation (murine lymphocyte response, MLR) and a murine model of graft rejection (neonatal heart to ear transplant). In the MLR model, administration of dasatinib, under the condition of the test, inhibited T-cell proliferation

in a dose dependent manner. Oral administration of dasatinib had immunosuppressive effects in the heart to ear transplant murine model of graft rejection, under the conditions tested. Reduced graft rejection was seen when dasatinib was administered continuously but not when it was given in the 5 days-on/ 2days-off schedule.

2.6.6.2 Single-dose toxicity

Study Title: single dose oral toxicity study in rats

Key findings: the following acute toxicities were observed:

- GI toxicity: liquid/red feces, hemorrhage/ulceration/edema in GI tract
- Hematopoietic/ Lymphocytic system: lymphocyte depletion, bone marrow cell depletion
- Thrombocytopenia
- Cardiac toxicity: ventricular necrosis; valvular, ventricular, and atrial hemorrhage; cardiac hypertrophy
- Hepatotoxicity: ↑liver weight, ↑AST, ↑triglycerides, ↓albumin, hepatocellular vacuolation, and necrosis
- Nephrotoxicity: tubular dilation, vacuolation, pyuria, ↑urinary volume, ↑RBC in urine
- Male reproductive system: single cell necrosis and hemorrhage in epididymis, hemorrhage and multinucleated cells in testes
- Pancreatic toxicity (possibly): single cell necrosis
- ↓RBC, ↑WBC, ↑neutrophils, and ↑monocytes appear to be secondary to internal injury and inflammation

Report no.: DS02138

Volume #, and page #: Item 5

Conducting laboratory and location: BMS/ Syracuse, NY

Date of study initiation: Aug 2002

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, radiolabel, and % purity: BMS354825, Batch No. C007A-354825-01;
— , pure

Formulation/vehicle: 80 mM sodium citrate, pH 3.0 - 3.5

Dosing:

Doses administered: 30, 100, or 300 mg/kg (180, 600, or 1800 mg/m²)

Single dose (to fasted rats) with a 2-week recovery period

Group Number	BMS-354825 (mg/kg)	Volume (ml/kg)	Concentration BMS-354825 (mg/ml)	Number of Animals
1	0 ^a	10	0	10 M, 10 F
2	30	10	3	10 M, 10 F
3	100	10	10	10 M, 10 F
4	300	10	30	10 M, 10 F

^a -80 mM citrate buffer, pH 3.0 - 3.5.

Table provided by the sponsor.

Species/strain: Rats/ Sprague Dawley

#/sex/group or time point (main study): at least 5/sex/group sacrificed on D4

The remaining rats were sacrificed on D16 (recovery group)

Any animals sacrificed or found dead prior to the D4 necropsy were considered part of the Day 4 necropsy.

Route/Volume: oral gavage, 10 mL/kg

Age: 10 weeks on treatment Day 1

Weight: 266-314 g (♂s) and 156-194 g (♀s)

Concentration verification

The assay results of the dosing solutions for stability and concentration verification were within 10% of target.

Observations:

Mortality: twice daily for mortality and moribundity.

Clinical Observations: prior to and approximately 3 hrs after dosing on Day 1.

Thereafter, once daily.

Body weight: pre-dose on Day 1 and approximately every 3 to 4 days.

Food consumption: approximately every 3 to 4 days.

Physical and ophthalmoscopic examinations: on Days -11, 3, 11 and 16.

Physical examinations included an assessment of general health status and neurologic and respiratory function.

Hematology/ serum chemistry: On Days 3 and 12, blood samples for scheduled clinical-pathology tests were obtained from the tail vein. On Days 4 and 16, blood samples for coagulation tests were obtained by cardiac puncture prior to necropsy. Animals were fasted overnight prior to scheduled blood collection.

Blood samples for clinical pathology were obtained by the same method, if possible, from moribund animals prior to necropsy.

Urinalysis: On Days 3 and 12, urine collected over an 18-hr period.

Urinary sediments were examined microscopically; output, specific gravity, pH, color and appearance, and qualitative determinations of glucose, protein, ketones, bilirubin, occult blood, and urobilinogen were determined.

Organ weight: only on animals that survived the scheduled necropsies (D4 and D16)

Adrenal glands	Ovaries	Spleen
Brain	Pituitary gland	Testes
Heart	Prostate gland	Thyroid gland
Kidneys	(with seminal vesicles)	Uterus (with cervix)
Liver		Thymus

Gross pathology: Day 4 and Day 16Histopathology:Tissues Obtained and Preserved in Fixative^a

Adrenal glands, aorta, bone, brain, cervix, epididymides, esophagus, eyes (with optic nerve), heart, identification number, kidneys, large intestine (cecum and colon), liver, lung, lymph nodes (mandibular and mesenteric), mammary gland^b, ocular accessory gland (harderian gland), ovaries, pancreas, parathyroid gland, peripheral nerve (sciatic), pituitary gland, prostate, salivary gland (mandibular), seminal vesicles, skeletal muscle (biceps femoris), skin (dorsal thorax), small intestine (duodenum, jejunum, ileum), spinal cord (entire cord), spleen, stomach (cardia, fundus, pylorus), testes, thymus, thyroid gland, tongue, trachea, urinary bladder, uterus, vagina.

^a Missing tissues were documented in the individual animal reports.

^b If tissue was not identified, the region was sampled.

In addition, all tissue masses and gross lesions were collected and fixed.

- Bone marrow smears were not prepared.
- All of the tissues listed above were fixed in 10% neutral buffered formalin, processed, sectioned, and stained with hematoxylin and eosin.
- All tissues collected from the control and HD animals, and from 6 ♂s and 7 ♀s in the MD group that were found dead or euthanatized on or before Day 4, were examined by light microscopy for drug-related or spontaneous lesions.
- Tissues examined from the 4 ♂s and 3 ♀s in the MD group that survived to the scheduled necropsy on Day 16, and in the LD animals were: heart, liver, mandibular and mesenteric lymph nodes, kidney, adrenal glands, bone marrow, stomach, duodenum, jejunum, ileum, cecum, colon, ocular accessory gland, spleen, and thymus
- Tissues examined in the 4 MD ♂s at Day 16 and in all LD ♂s: testes and epididymides;
- Tissues examined in the 3 MD ♀s at Day 16 and in all LD ♀s: ovaries, pancreas, and tongue; and in 1 low-dose female, uterus.
- Additional sections of heart from Animal Nos. 1102, 3110, 3202, and 3204 were stained with Massons trichrome and Prussian blue stains and examined microscopically. Massons trichrome helps to highlight the supporting collagenous stroma to determine the pattern of tissue injury

Toxicokinetics: Not done**Results:**Mortality:

Morbidity and mortality were attributed to:

- GI lesions (resulting in fluid and electrolyte loss and impairment of mucosal integrity)
- Bone-marrow and lymphoid depletion
- Multifocal myocardial necrosis and hemorrhage

Mortalities, including unscheduled sacrifices due to moribundity

	♂s	♀s
MD	4/10 (Days 3-4)	7/10 (Days 2-3)
HD	10/10 (Days 2-3)	10/10 (Days 2-3)

Clinical Observations:

LD, MD, and HD:

- mucous feces, soiled/rough haircoat, dehydration (♀s), chromodacryorrhea, and chromorhinorrhea

MD and HD:

- ↓activity, dehydration (♂s), hunched posture, pallor, surface hypothermia, ptosis, tremor (♀s), and absent feces (♀s)

Additional findings (low incidence):

- swollen ventral neck was observed from (1 MD ♂ on Days 9-15)
- rale (1 MD ♂ on Day 1 / 1 HD ♀ on Day 1)
- alopecia (1 MD ♂ on Days 1-15)

Body weight:

Changes in the BW compared to the corresponding control

	♂		♀	
	Days 1-4	Days 4-8	Days 1-4	Days 4-8
Control	↑18 g	↑21 g	↑10 g	↑11 g
LD	↑12 g	↑24 g	↑6 g	↑18 g
MD	↓6 g	↑25 g	↓2 g	↑22 g
*HD				

* Due to mortality only Day 1 BWs are available.

Food consumption:

Day 1-2:

- Dose-dependent ↓food consumption at LD, MD, and HD; statistically significant at all dose levels.

Day 3-5:

- ↓food consumption at MD (no data available for HD); statistically significant.

Days 5-15:

- LD and MD data were comparable to control.

Physical and ophthalmoscopic examinations:

No additional findings (see under clinical observations for test article-related findings)

Hematology

Day 3 data
(Numbers in parentheses represent remarkable Day 12 data)

		RBC	HGB	HCT	RET	PLT
LD	♂	—	—	—	*↓45%	*↓25%
	♀	—	—	—	—	↓30%
MD	♂	—	—	—	*↓45% (*↑100%)	*↓65% (*↑80%)
	♀	—	—	↓20%	↑50% (*↑90%)	↓60% (*↑80%)
HD	♂	*↓60%	*↓60%	*↓60%	*↑100%	*↓55%
	♀§					

* Statistically significant.

§ No data available for HD ♀s on Day 3.

Numbers in parentheses are remarkable Day 12 data. No D12 data available for HD animals.

		WBC	Neut	Lymph	Mono	Eos	Baso	LUC
LD	♂	—	↑100%	↓45% (↓25%)	↑55%	↓30%	—	—
	♀	—	↑2.5-fold	↓15% (*↓30%)	↑100%	—	(*↓50%)	↑2-fold
MD	♂	*↑90% (*↓25%)	*↑12-fold (*↑3.5-fold)	*↓45% (*↓35%)	*↑3-7-fold (*↑100%)	↓30%	—	*↑2-fold
	♀	↑80% (*↓50%)	↑8-fold	↓50% (*↓55%)	↑1.5-fold (*↓69%)	↓40% (*↓65%)	↓50% (*↓75%)	↑1.5-fold (*↓65%)
HD	♂	*↑60%	*↑10-fold	*↓65%	*↑3-fold	↓50%	*↓85%	↑1.5-fold
	♀§							

* Statistically significant.

§ No data available for HD ♀s on Day 3.

Numbers in parentheses are remarkable Day 12 data. No D12 data available for HD animals.

Day 12 data:

- No data available for HD due to mortality.
- Most findings appeared to be reversible.
- ↑Reticulocytes and ↓lymphocytes were persistent.

Day 4 coagulation parameters

Group	Sex	PT	APTT	FIB	Group	Sex	PT	APTT	FIB
		sec	sec	mg/dl			sec	sec	mg/dl
1m	Mean	18.14	12.50	297.6	1f	Mean	18.00	10.84	250.8
	S.D.	1.35	2.22	14.1		S.D.	0.72	1.22	18.2
	N	5	5	5		N	5	5	5
2m	Mean	19.14	12.26	395.2**	2f	Mean	18.40	11.70	373.4**
	S.D.	0.48	1.47	43.9		S.D.	0.42	1.26	44.6
	N	5	5	5		N	5	5	5
3m	Mean	17.13	16.20	509.3**					
	S.D.	1.46	3.46	45.0					
	N	3	3	3					

- ↑fibrinogen at LD (♂s and ♀s) and in MD ♀s (no data available for MD ♂s or HD animals)

- There appear a tendency for ↑aPTT as doses are increased, e.g. ↑30% for MD ♂s. Due to the small number of animals and lack of HD and MD (♀) data, findings are inconclusive.
- No remarkable finding on Day 16

Serum chemistry:

Day 3 data

		AST	GLC	BUN	TRIG	TP	ALB
LD	♂	*↑67%	—	—	—	—	*↓12%
	♀	↑90%	—	—	↑1.3-fold	—	↓20%
MD	♂	*↑1.2-fold	*↑20%	↑50%	*↑1.5-fold	*↓27%	*↓33%
	♀	↑1.5-fold	↑17%	↑100%	↑2.8-fold	↓25%	↓33%
HD	♂	*↑2.2 fold	*↑90%	*↑2-fold	↑55%	*↓32%	*↓42%
	♀§						

* Statistically significant.

§ No data available for HD ♀s on Day 3.

AST: aspartate aminotransferase, GLC: glucose, BUN: blood urea nitrogen, CHOL: cholesterol, TRIG: triglycerides, TP: total protein, ALB: albumin.

		GLOB	A/G	Ca	Phos	K	Na	Cl
LD	♂	—	*↓10%	*↓10%	*↓30%	*↓10%	—	—
	♀	—	↓9%	↓10%	↓20%	—	—	—
MD	♂	*↓20%	*↓10%	*↓40%	*↓60%	*↓12%	*↓6%	*↓7%
	♀	↓20%	↓15%	↓40%	↓50%	↓8%	↓3%	↓2%
HD	♂	*↓20%	*↓20%	*↓50%	*↓58%	*↓10%	*↓6%	*↓9%
	♀§							

* Statistically significant.

§ No data available for HD ♀s on Day 3.

- Some other changes, were not toxicologically significant and are not presented in the Table: ↑ALT in HD ♂s (statistically significant), and ↓ALP at LD (♂s and ♀s, statistically significant in ♂s), MD (♂s and ♀s, statistically significant in ♂s), and HD ♂s (statistically significant), ↓cholesterol in HD ♂s (↓50%, statistically significant)
- Base on Day 12 data for LD and MD, changes appeared to be reversible.

Urinalysis:

Day 3 data for males

BMS-354825, mg/kg	Blood		Bilirubin		Glucose	
	Incidence, males	Severity, range ^a	Incidence, males	Severity, range ^a	Incidence, males	Severity, range ^a
0 ^b	0/10	-	0/10	-	1/10	Tr
30	2/10	Tr	0/10	-	0/10	-
100	5/8	Tr - 3+	2/8	1+	4/8	Tr - 1+
300	1/4	Tr	2/4	1+	2/4	Tr - 1+

^a Range: trace (Tr), 1+, 2+, 3+, 4+

^b 80 mM citrate buffer, pH 3.0-3.5

Table provided by the sponsor.

Drug-related effects were observed at MD and HD on assessment Day 3 and included:

- ↑ Incidence of blood in urine at MD and bilirubin at MD and HD.
- ↑ Incidence of urinary glucose MD and HD (this was considered a secondary, stress-related change that was consistent with the observed increase in serum glucose).
- Based on the D12 data for LD and MD (no HD data available), the above urinalysis findings appeared to be reversible.

Day 3 data on urinary volume (VOL) and specific gravity (SPGR)

Group Sex		VOL ml	SPGR	Group Sex		VOL ml	SPGR
1m	Mean	4.60	1.0543	1f	Mean	2.71	1.0569
	S.D.	2.07	0.0162		S.D.	1.25	0.0251
	N	10	10		N	7	7
2m	Mean	8.30	1.0344	2f	Mean	5.50	1.0351
	S.D.	3.68	0.0197		S.D.	3.21	0.0176
	N	10	10		N	10	10
3m	Mean	8.38	1.0596	3f	Mean	15.00	1.0253
	S.D.	7.91	0.0312		S.D.	10.44	0.0240
	N	8	8		N	3	3
4m	Mean	8.00	1.0640				
	S.D.	6.22	0.0242				
	N	4	4				

- ↑Urinary volume (↑100% in ♂s and up to 6-fold in ♀s) was observed at all dose levels. However, the specific gravity remained the same (no change in the osmolality), suggesting increased total solutes/particles in the urine in the treatment groups. This finding suggests the possibility of dysfunctional kidney with impaired ability to concentrate urine.
- ↑Urinary volume persisted to Day 12 (↑100% in MD ♂s and ↑60% in MD ♀s, no HD data available)

Day 3 data on white blood cell (WBC), red blood cell (RBC), and epithelial cells (EPIT)

Group Sex		UWBC /HPF	URBC /HPF	EPIT /HPF	Group Sex		UWBC /HPF	URBC /HPF	EPIT /HPF
1m	Mean	0.0100	0.0000	0.0400	1f	Mean	0.0000	0.0000	0.0200
	S.D.	0.0316	0.0000	0.0516		S.D.	0.0000	0.0000	0.0447
	N	10	10	10		N	5	5	5
2m	Mean	0.0600	0.2000	0.0400	2f	Mean	0.0600*	0.0500	0.0700
	S.D.	0.0699	0.6325	0.0516		S.D.	0.0516	0.0972	0.0675
	N	10	10	10		N	10	10	10
3m	Mean	0.2375*	2.5875	0.3000	3f	Mean	0.0000	0.0000	0.3667
	S.D.	0.3420	7.0361	0.6908		S.D.	0.0000	0.0000	0.5508
	N	8	8	8		N	3	3	3
4m	Mean	0.1500	0.0250	0.0250					
	S.D.	0.0577	0.0500	0.0500					
	N	4	4	4					

HPF: high power field

- ↑urinary WBC at all dose levels in ♂s and at LD in ♀s.
- ↑WBC was also observed on D12 (at LD and MD; no data available for HD).

Organ weight:

Changes in the relative organ weights (organ : BW ratios), on Day 4 (or on Day 16):

		Adrenals	Heart	Liver	Spleen	Thymus	Thyroid
LD	♂	↑15% (↑14%)	↑9% (—)	— (—)	↓20% (—)	*↓50% (—)	↑12% (—)
	♀	↑35% (—)	↑5% (—)	*↑12% (—)	↓14% (—)	*↓45% (—)	↑40% (—)
MD	♂	*↑35% (*↑35%)	↑14% (↑9%)	— (*↑15%)	*↓54% (↓13%)	*↓67% (—)	↑24% (↑20%)
	§♀	unknown (—)	unknown (—)	unknown (*↑16%)	unknown (—)	unknown (—)	unknown (—)

* Statistically significant.

§ No data available for MD ♀s on D4.

—: no remarkable effect/ no effect.

Notes:

- No data available for HD animals.
- Numbers in parentheses represent Day 16 data.

Most findings were reversible. There appear to be delayed change in the liver weight in MD animals (liver hypertrophy in ♂s and ♀s).

Gross pathology:

Day 4:

- ↓Size of thymus at all doses
- ↓Size of spleen at MD and HD
- Red discoloration of stomach (glandular or nonglandular) at all doses
- Abnormal stomach contents (1 HD ♀)
- Red or black discoloration of duodenum, jejunum, ileum, cecum and/or colon at MD and HD

- Red or black discoloration of mandibular and/or mesenteric lymph nodes at MD and HD
- Tan discoloration of liver at MD and HD
- Red discoloration of ovaries at MD and HD
- Red discoloration of epididymides (1 HD ♂)

Day 16:

- White discoloration of epididymides at MD and HD
- ↓size of testis (1 MD ♂)

Gross findings observed at necropsy on or before Day 4 or on Day 16 that were observed in individual animals, and stated to be incidental and not drug related included: red discoloration of the hindlimb muscle in 1 MD ♂ (focal hemorrhage), red discoloration of the uterus in 1 HD ♀ (no microscopic correlate), distention of uterus with fluid in 2 MD ♀s (microscopically, consistent with proestrus), raised area on uterine horn of 1 LD ♀ (microscopically, focal uterine luminal dilatation).

Histopathology:

Day 4

	Dose(mg/kg)	Group 1		2		3		4	
		vehicle		30		100		300	
		M	F	M	F	M	F	M	F
Animals On Study		10	10	10	10	10	10	10	10
Animals Logged		5	5	5	5	6	7	10	10
Heart		5	5	5	5	6	7	10	10
Not Remarkable		4	5	4	5	1	2	1	3
Remarkable Observations		1	0	1	0	5	5	9	7
Infiltration, Mononuclear Cell; Ventricle		1	0	1	0	0	0	0	0
Necrosis, Coagulative; Ventricle		0	0	0	0	4	3	6 a	5
Hemorrhage; Atrium		0	0	0	0	0	0	1	0
Hemorrhage; Heart Valve		0	0	0	0	0	1	0	3
Hemorrhage; Ventricle		0	0	0	0	4	5 a	8 b	5
Thrombus; Aortic Valve		0	0	0	0	1	0	0	0
Large Intestine									
Cecum		5	5	5	5	6	7 *	10 *	10
Autolysis Only		0	0	0	0	2	5	8	4
Not Remarkable		5	5	5	5	3	0	1	4
Remarkable Observations		0	0	0	0	1	2	1	2
Depletion, Lymphoid		0	0	0	0	1	2	1	2
Colon		5	5	5	5	6	7 *	10 *	10
Autolysis Only		0	0	0	0	2	4	7	3
Not Remarkable		5	5	5	5	4	3	3	7
Liver (Continued)		5	5	5	5	6	7	10	10
Vacuolation		0	0	0	0	0	1	2	0
Infiltration, Mononuclear Cell		3	2	2	1	0	1	1	0
Necrosis, Coagulative		0	0	0	0	0	0	1	0
Necrosis, Single Cell		0	0	0	0	0	2	0	3
Pancreas		5	5	0 *	5	6	7	10	10
Not Remarkable		5	5	0	5	6	7	10	6
Remarkable Observations		0	0	0	0	0	0	0	4
Necrosis, Single Cell		0	0	0	0	0	0	0	4

Small Intestine								
Duodenum	5	5	5	5	6	7 *	10	10
Autolysis Only	0	0	0	0	2	4	6	3
Not Remarkable	5	5	5	5	0	0	0	0
Remarkable Observations	0	0	0	0	4	3	4	7
Hemorrhage	0	0	0	0	0	0	0	2
Enteropathy	0	0	0	0	4 b	3	4 b	7 b
Ileum	5	5	5	5	6	7 *	10 *	10
Autolysis Only	0	0	0	0	2	4	7	4
Not Remarkable	5	5	5	1	0	0	0	0
Remarkable Observations	0	0	0	4	4	3	3	6
Depletion, Lymphoid	0	0	0	0	1	2	1	2
Edema	0	0	0	4 a	0	0	0	0
Ulcer	0	0	0	0	0	0	0	1
Enteropathy	0	0	0	0	4 b	3	3	6 b
Jejunum	5	5	5	5	6	7 *	10 *	10
Autolysis Only	0	0	0	0	2	5	7	4
Not Remarkable	5	5	5	5	0	0	1	0
Remarkable Observations	0	0	0	0	4	2	2	6
Enteropathy	0	0	0	0	4 b	2	2	6 b
Stomach								
Autolysis Only	5	5	5	5	6	7	10	10
Not Remarkable	0	0	0	0	0	0	1	0
Remarkable Observations	5	5	2	3	2	1	0	7
Hemorrhage; Glandular Area	0	0	3	2	4	6	9	3
Hemorrhage; Nonglandular Area	0	0	1	1	0	0	3	0
Edema; Nonglandular Area	0	0	1	0	4	5 a	6 a	3
Hyperplasia; Nonglandular Area	0	0	3	2	0	0	0	0
Ulcer; Nonglandular Area	0	0	2	2	0	0	0	0
	0	0	0	0	2	6 a	5	3
Thyroid Gland								
Not Remarkable	5	5	0 *	0 *	6	7	10	10
Remarkable Observations	5	4	0	0	6	7	7	9
Cyst, Ultimobranchial	0	1	0	0	0	0	3	1
	0	1	0	0	0	0	3	1
Uterus								
Autolysis Only	0 *	5	0 *	1 *	0 *	7	0 *	10
Not Remarkable	0	0	0	0	0	1	0	1
Remarkable Observations	0	5	0	0	0	5	0	8
Dilatation	0	0	0	1	0	1	0	1
	0	0	0	1	0	1	0	1
Genital System - Male								
Epididymides	5	0 *	5	0 *	6	0 *	10	0 *
Not Remarkable	4	0	4	0	5	0	5	0
Remarkable Observations	1	0	1	0	1	0	5	0
Necrosis, Single Cell	0	0	0	0	0	0	1	0
Hemorrhage	0	0	0	0	1	0	5	0
Giant Cell, Multinucleated	0	0	0	0	0	0	2	0
Granuloma, Sperm	1	0	1	0	0	0	0	0

Testes	5	0 *	5	0 *	6	0 *	10	0 *
Not Remarkable	5	0	5	0	6	0	8	0
Remarkable Observations	0	0	0	0	0	0	2	0
Hemorrhage	0	0	0	0	0	0	1	0
Giant Cell, Multinucleated	0	0	0	0	0	0	2	0
Hematopoietic and Lymphatic System								
Bone Marrow	5	5	5	5	6	7	10	10
Not Remarkable	5	5	1	2	0	0	0	0
Remarkable Observations	0	0	4	3	6	7	10	10
Depletion	0	0	4 a	3	6 b	7 b	10 b	10 b
Lymph Nodes								
Mandibular Node	5	5	5	5	6	7	10	10
Missing	0	1	0	0	0	0	2	2
Autolysis Only	0	0	0	0	0	3	2	0
Not Remarkable	5	4	5	5	0	0	0	0
Remarkable Observations	0	0	0	0	6	4	6	8
Depletion, Lymphoid	0	0	0	0	6 b	4 a	4	8 b
Abscess	0	0	0	0	2	1	2	0
Mesenteric Node	5	5	5	5	6	7	10	10
Not Remarkable	5	5	4	5	0	0	0	0
Remarkable Observations	0	0	1	0	6	7	10	10
Depletion, Lymphoid	0	0	1	0	6 b	7 b	10 b	10 b
Spleen								
Missing	0	0	0	0	0	0	0	1
Not Remarkable	5	5	5	5	0	0	0	0
Remarkable Observations	0	0	0	0	6	7	10	9
Depletion, Lymphoid	0	0	0	0	6 b	7 b	10 b	9 b
Thymus								
Not Remarkable	5	5	0	2	0	0	0	0
Remarkable Observations	0	0	5	3	6	7	10	10
Depletion, Lymphoid	0	0	5 b	3	6 b	7 b	10 b	10 b
Edema	0	0	0	0	0	0	1	0
Ocular Accessory Gland								
Not Remarkable	5	5	5	5	3	0	3	0
Remarkable Observations	0	0	0	0	3	7	7	10
Pigment	0	0	0	0	3	7 b	7 a	10 b
Kidneys								
Autolysis Only	0	0	0	0	1	1	1	2
Not Remarkable	2	3	1	1	1	1	4	2
Remarkable Observations	3	2	4	4	4	5	5	6
Dilatation; Cortical Tubules	0	0	0	0	3	4	3	6 a
Nephropathy, Progressive Murine	3	2	4	4	3	2	5	2
Vacuolation	0	0	0	0	0	2	1	5

* Fisher's test not calculated (less than 4 tissues)

a: p<0.05; b: p<0.01

Day 16 (no HD data is available):

	Group		1		2		3	
	Dose(mg/kg)		vehicle		30		100	
	Sex		M	F	M	F	M	F
Animals On Study	10	10	10	10	10	10	10	10
Animals Logged	5	5	5	5	4	4	3	3
Heart								
Heart	5	5	5	5	4	4	3	3 *
Not Remarkable	5	5	4	5	3	3	1	1
Remarkable Observations	0	0	1	0	1	1	2	2
Infiltration, Mononuclear Cell	0	0	1	0	0	0	0	0
Infiltration, Macrophage; Ventricle	0	0	0	0	1	1	2	2
Digestive System								
Liver								
Liver	5	5	5	5	4	4	3	3 *
Not Remarkable	2	0	1	0	0	0	0	0
Remarkable Observations	3	5	4	5	4	4	3	3
Vacuolation	0	3	1	5	0	0	3	3
Extramedullary Hematopoiesis	0	0	1	0	4	4	3	3
Infiltration, Mononuclear Cell	3	5	4	3	2	2	2	2
Genital System - Female								
Uterus								
Uterus	0 *	0 *	0 *	0 *	0 *	0 *	1 *	1 *
Remarkable Observations	0	0	0	0	0	0	1	1
Dilatation	0	0	0	0	0	0	1	1
Genital System - Male								
Epididymides								
Epididymides	5	0 *	5	0 *	4	4	0 *	0 *
Not Remarkable	4	0	4	0	0	0	0	0
Remarkable Observations	1	0	1	0	4	4	0	0
Infiltration, Mononuclear Cell	0	0	1	0	0	0	0	0
Infiltration, Neutrophil	0	0	0	0	3	3	a	0
Granuloma, Sperm	1	0	0	0	4	4	a	0
Testes								
Testes	5	0 *	5	0 *	4	4	0 *	0 *
Not Remarkable	5	0	5	0	3	3	0	0
Remarkable Observations	0	0	0	0	1	1	0	0
Degeneration	0	0	0	0	1	1	0	0

Summary of the study:

BMS-354825 was administered as free base in citrate buffer, pH 3.0-3.5, orally as a single dose to SD rats at doses of 30, 100, or 300 mg/kg (180, 600, or 1800 mg/m²).

High mortality was observed at MD and HD. All HD rats were found dead or sacrificed in moribund condition by study Day 3. At MD, 4/10 ♂s and 7/10 ♀s were found dead or sacrificed in moribund condition by Day 4. Morbidity and mortality were attributed to gastrointestinal lesions resulting in fluid and electrolyte loss and impairment of mucosal integrity, bone-marrow and lymphoid depletion, and multifocal myocardial necrosis and hemorrhage.

Clinical signs of toxicity were observed at all dose levels but were most evident at MD and HD. They included mucous feces, chromodacryorrhea, and chromorhinorrhea, inactivity, hunched posture, pallor, surface hypothermia, ptosis, and tremor.

Acute GI toxicity/ enteropathy was evident at all dose levels, as demonstrated by red/liquid feces and hemorrhage, ulceration, and edema in the GI tract. GI toxicity was reversible.

Hematology parameters revealed anemic condition of animals on Day 3, which may be secondary to internal bleeding, as observed on histopathology for several tissues/organs. In addition, ↑WBC, neutrophils, and monocytes observed on Study Day 3 appeared to be due to internal injury/ inflammation. ↓lymphocytes on Day 3 was further confirmed by lymphocytic depletion observed in lymph nodes, thymus, and spleen on Day 4. Although most hematology findings appeared to be reversible, ↓lymphocyte count was persistent until the following assessment day (Day 12).

Acute changes in the coagulation parameters included ↓platelets (all doses) and ↑fibrinogen (LD and MD; no data available for the HD). These findings appeared to be reversible as shown by the limited data available on Day 12 (platelets) and D16 (fibrinogen). There appeared to be a tendency for ↑aPTT as doses were increased, e.g. ↑30% for MD ♂s. However, due to the small number of animals and lack of all HD data and some MD data, findings are inconclusive.

Acute hepatotoxicity was demonstrated by ↑AST, ↑triglycerides, and ↓albumin, at all dose levels on Day 3, as well as vacuolation and necrosis in the liver seen on Day 4. Increased liver weight was seen as early as Day 4; however, in the absence of the HD data and the MD data for ♀s on Day 4, a final conclusion on liver hypertrophy cannot be made. Liver vacuolation progressed from D4 (14% of MD ♀s affected) to D16 (100% of MD ♀s, 100% of LD ♀s and 20% of LD ♂s affected).

In the heart, minimal to mild hemorrhage and/or coagulative necrosis were observed at Days 2 - 4 at MD and HD. Hemorrhage occurred in the ventricular myocardium, atrial myocardium, and heart valves, with greatest frequently in the subendocardial ventricular myocardium. Hemorrhages of the ventricular myocardium were focal to multifocal, and often associated with foci of coagulative necrosis and infiltrates of neutrophils. Based on the limited data available on D16, histopathology findings in the heart (e.g. hemorrhage of atrium, ventricle, and valve, and aortic valve thrombus) appeared to be reversible.

Acute nephrotoxicity was demonstrated by Day 4 histopathology findings (tubular dilation and tubular epithelial cell vacuolation at MD and HD), as well as by D3 urinalysis data, e.g. ↑volume, which suggested inability of the kidneys to concentrate the urine, resulting in increased volume with no change in osmolality. Histopathology findings in conjunction with ↑urinary epithelial cell and ↑urinary RBCs are indicative of tubular damage. ↑Urinary WBC/pyuria and ↑RBCs may be indicative of acute glomerulonephritis. The electrolyte loss seen in serum chemistry might be due to multiple factors, such as renal toxicity and/or GI toxicity. Nephrotoxicity appeared to be reversible based on the limited D16 histopathology results. ↑Urinary volume was still observed on D12 with magnitude comparable to D3 (in ♂s).

Acute toxicities in the male reproductive organs consisted of: single cell necrosis and hemorrhage in epididymis at MD and HD (mainly at HD), hemorrhage and multinucleated cells in testes at HD. Since these findings were seen mainly or exclusively at HD and no HD data were available on D16, the reversibility of these toxicities remains unknown.

In the Harderian gland (ocular accessory gland), the amount of porphyrin pigment was mildly to moderately increased on Days 2-3 at MD and HD.

Other acute findings possibly associated with dasatinib treatment included: reversible single cell necrosis in pancreas (4/10 HD ♀s; reversibility is unknown) and ↑adrenal weight.

In conclusion, acute toxicities of dasatinib consisted of:

- GI toxicity: liquid/red feces, hemorrhage/ulceration/edema in GI tract.
- Hematopoietic/ Lymphocytic system: lymphocyte depletion, bone marrow cell depletion
- Thrombocytopenia
- Cardiac toxicity: ventricular necrosis; valvular, ventricular, and atrial hemorrhage; cardiac hypertrophy
- Hepatotoxicity: ↑liver weight, ↑AST, ↑triglycerides, hepatocellular vacuolation, and necrosis, (↓albumin)
- Nephrotoxicity: tubular dilation, vacuolation, pyuria, ↑urinary volume (no change in specific gravity), ↑RBC in urine
- Male reproductive system: single cell necrosis and hemorrhage in epididymis, hemorrhage and multinucleated cells in testes
- Pancreatic toxicity (possibly): single cell necrosis
- Harderian gland: ↑porphyrin pigment
- ↓RBC, ↑WBC, ↑neutrophils, and ↑monocytes appear to be secondary to internal injury and inflammation

Study Title: Single-dose oral toxicity study in monkeys

Key findings: All HD animals were sacrificed moribund on Day 1 (♀s) or on Day 2 (♂s). The following acute toxicities were observed during this study:

- Hypothermia/ ↓body temperature
- GI tract: vomiting, red/liquid feces; abnormal content on gross pathology; hemorrhage in tongue, stomach, duodenum, ileum, jejunum; single cell necrosis/ edema/ neutrophil infiltration in stomach; enteropathy in the ileum characterized by villous atrophy and the presence of disorganized and stratified apical villous epithelium.
- Hematopoietic/ Lymphocytic system: single cell necrosis in bone marrow; ↓lymphocytes; lymphoid depletion in cecum, colon, ileum, stomach, mesenteric and mandibular lymph nodes, and in spleen
- Skin: hemorrhage; bruising/ ecchymosis over numerous sites of the body (thorax, limbs, gingiva, head, and neck)

- Liver: ↑ALT, ↑AST
- Kidneys: minimal cortical tubular dilatation
- Cardiovascular (possibly related): murmur in 1 MD ♂ on Day 14; tendency for ↑diastolic and systolic blood pressure.
- Other: ↓activity; ↑WBC neutrophils, monocytes, and fibrinogen appeared to be secondary to internal injury and inflammation.

Report no.: DS02147

Volume #, and page #: Item 5

Conducting laboratory and location: BMS/ Syracuse, NY

Date of study initiation:

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, radiolabel, and % purity: BMS354825, Batch No. C007A-354825-01;
— pure

Formulation/vehicle: 80 mM sodium citrate, (final pH ~3.2)

Dosing:

Doses administered: 15, 25, or 45 mg/kg (180, 300, or 540 mg/m²); single dose (Day 4 and Day 15 necropsies)

Group Number	Single Dose		Concentration	Number of Animals
	BMS-354825 (mg/kg)	Volume (ml/kg)	BMS-354825 (mg/ml)	
1	0*	5	0	2M, 2F
2	15	5	3	2M, 2F
3	25	5	5	2M, 2F
4	45	5	9	2M, 2F

* 80 mM sodium citrate, pH~3.2

Species/strain: monkeys/Cynomolgus

#/sex/group (main study): 2/sex/group

Route/Volume: Oral

Age: ~3-4 years; at the time of dosing

Weight: 4.2-5.8 kg (♂s) and 2.7-3.5 (♀s)

Concentration verification

The assay results of the dosing solutions for stability and concentration verification were within 10% of target (—) of the nominal concentration).

Observations:

Mortality: twice daily for mortality

Clinical Observations: prior to and after dosing on Day 1. Thereafter, each animal was observed at least once daily.

Body weight: recorded on Days 1, 3, 8, and 13

Food consumption: daily beginning at least 3 days prior to the start of dosing. For 3 of 12 drug-treated monkeys, pre-study food intake was obtained for only 2 days.

Physical and ophthalmoscopic examinations: prior to study inception and on Days 3 and 14.

Physical examinations included an assessment of general health status, neurologic function and status, heart and lung auscultation, temperature, pulse rate, and respiratory rate. Ophthalmologic examination included examination of external structures of the eye as well as a fundic examination by direct ophthalmoscopy.

EKG: prior to study inception and on Days 1 and 11. Electrocardiogram tracings were analyzed for abnormalities in the cardiac rhythm and heart rate. QT intervals were evaluated after correcting for rate by Van de Water's method.

Blood pressure, heart rate, and arterial oxygen saturation:

Indirect blood pressures were obtained pre-study and during Week 2.

With the exception of one LD monkey, blood pressures were not obtainable for unknown reasons from drug-treated monkeys on Day 1 with the equipment used. The absence of blood pressure data at that time (~ 2 hr post-dose) did have some impact on the cardiovascular assessment of the drug in monkeys. However, EKG tracings with heart rates were obtained on all monkeys at approximately 2 hr post-dose.

As a result of extended efforts to obtain blood pressure data on drug-treated monkeys, arterial oxygen saturation data were obtained, in all but one instance, after the 4-hr blood sampling for toxicokinetics, and not at 2 hr post-dose. The time change for collection of this data does not appear to adversely affect the results for oxygen saturation.

Hematology/ serum chemistry:

Pre-dose (all animals); Day 1 (HD ♀s); Day 2 (Control, LD, and MD ♂s and ♀s, and HD ♂s).

The D1/D2 sampling schedules were decided based on the poor physical conditions of the HD animals: unscheduled sacrifices in HD ♀s occurred on D1 and in HD ♂s occurred on Day 2. Animals were fasted overnight prior to scheduled blood collection, except for samples collected on Day 2.

Urinalysis: on Days 3 and 14 (18-hr collection): from control, LD, and MD animals.

Parameters evaluated: urinary sediments were examined microscopically; output, specific gravity, pH, color and appearance, and qualitative determinations of glucose, protein, ketones, bilirubin, occult blood, and urobilinogen.

Organ weight: not done

Gross pathology:

At scheduled necropsy (Days 4 and 15) and at unscheduled necropsies (Day 1: HD ♀s and Day 2: HD ♂s). Gross examination of organs and tissues listed below.

Tissues Obtained and Preserved in Fixative

Adrenal glands	Lymph nodes	Skin (dorsal thorax)
Aorta	(mandibular and	Small intestine
Bone and bone marrow	mesenteric)	(duodenum, jejunum, ileum)
(sternum, rib, femur)	Mammary gland	Spinal cord
Brain	Ocular accessory	(cervical, lumbar)
Cervix	gland (lacrima gland)	Spleen
Epididymides	Ovaries	Stomach (cardia,
Esophagus	Pancreas	fundus, pylorus)
Eyes (with optic nerve)	Parathyroid gland	Testes
Gallbladder	Peripheral nerve (sciatic)	Thymus
Heart	Pituitary gland	Thyroid gland
Kidneys	Prostate gland	Tongue
Large intestine	Salivary gland	Tonsils
(cecum, colon)	(mandibular)	Trachea
Liver	Seminal vesicles	Urinary bladder
Lung	Skeletal muscle	Uterus
	(psoas)	Vagina

In addition, all tissue masses and gross lesions were collected and preserved. Additional tissues were examined at the discretion of the Study Pathologist.

Histopathology:

Samples of organs/tissues listed above and all gross lesions were collected and preserved in 10% neutral buffered formalin.

Sections of kidney, large intestine (cecum, colon), liver, lung, lymph nodes (mesenteric, mandibular), small intestine (duodenum, jejunum, ileum), spleen, stomach, and suspected drug-related gross lesions were processed, sectioned, stained with hematoxylin and eosin, and examined microscopically. Additional sections of skin from animal nos. 2101, 4101, and 4102 were stained with Masson's trichrome and phosphotungstic acid hematoxylin and examined microscopically.

Toxicokinetics: Approximately 1.2-ml blood samples was collected from all drug-treated animals at approximately 1, 2, 4, 8, 12, and 24 hr after dosing.

Results:

Mortality: all HD animals were sacrificed moribund on Day 1 (HD ♀s) or on Day 2 (HD ♂s)

Clinical Observations:

At LD, MD, and HD:

↓Activity, surface hypothermia with ↓body temperature on Day 1, dehydration, and ecchymosis over numerous sites of the body (thorax, limbs, gingiva, head, and neck). Ecchymosis was first noted within 2 hours of dose administration and was reversible by Day 8 at LD and MD.

Additional signs at MD and HD:

Stool changes (soft, liquid, bloody) and pale mucous membranes (gingiva).

Signs at HD only:

↑Muscle tone, tremors, abnormal behavior (animal under perch of cage with hindlimbs over head), emesis/vomitus (with or without blood)

Day 1: Several monkeys had significantly ↓body temperature.

Day 3: Minimal to marked s.c. hemorrhage (bruising) at LD and MD (no HD data available due to mortality). Heart murmur was noted in 1 MD ♂ (this finding was stated to be related to Ketamine anesthetic).

Day 14: One LD monkey had a focal perivascular hemorrhage adjacent to the retinal blood vessels near the optic disc. Heart murmur was noted in 1 MD ♂ (same animal who had murmur on Day 3).

Body weight: no data available for HD on Day 3, due to mortality

↓BW of 0.4 g in MD ♀s on Day 3

Food consumption: ↓food consumption at MD and HD (up to 60% compared to pre-study data)

Physical and ophthalmoscopic examinations: see under clinical observations

EKG: The electrocardiogram for 1 LD ♀ on Day 1 and 1 MD ♂ monkey on Day 11 were not of sufficient quality to calculate and evaluate corrected QT (QTc) intervals. No qualitative abnormalities were observed in these two electrocardiograms. Corrected QT intervals were within normal limits for all other evaluated monkeys and there were no significant abnormalities detected in any group compared to predose electrocardiograms.

Blood pressure, heart rate, and arterial oxygen saturation:

No drug effect in the percent of arterial oxygen saturation.

Blood pressures were not attainable on Day 1.

Diastolic and systolic blood pressures and mean arterial blood pressure were increased on Day 11 in LD and MD ♂s (no data available for HD animals due to mortality).

Because increases were non-dose-dependent and assessments included 1 animal/sex/group, a test article-related effect remains unclear for this study.

Blood pressures in ♂s on Day 11

		Diastolic (mm Hg)	Systolic (mm Hg)	Mean arterial
C	n=1	40	96	54
LD	n=1	84	157	108
MD	n=1	66	143	80

C: control; LD: low-dose; MD: mid-dose.

Hematology:

Due to the moribund condition of the animals, all HD ♀s were sacrificed on Day 1. Because of the lack of Day1 control data, results for the HD ♀s are not presented in Table below. Since Day 1 data were on fasted animals and Day 2 data were on non-fasted animals, a comparison of the HD ♀s to Day 2 controls may be inappropriate.

Findings appear to be reversible based on the minimal D14 data (no animals at HD and only 1 animal/sex/group for each LD and MD).

Changes in the hematology parameters on Day 2 (non-fasted) compared to Day 2 control groups (non-fasted):

		WBC	LYMPH	NEUT	MONO	LUC	% RET
MD	♂ N=2 on D2	↑30%	↓35%	↑50%	↑3-fold	↑20%	—
	♀ N=2 on D2	—	—	—	↑40%	↑45%	↓40%
HD	♂ N=2 on D2	↑1.3-fold	↓42%	↑2-fold	↑↑3.5-fold	↑1.7-fold	↓63%
	♀ N=1 on D1						

WBC: white blood cells; LYMPH: lymphocytes; NEUT: neutrophils; MONO: monocytes; LUC: large unstained cells; RET: reticulocytes.

—: No effect/ no remarkable effect.

Changes at the LD were either not remarkable or not toxicologically significant.

Coagulation parameters:

- ↑fibrinogen on Day 2 at MD (♀s: ↑27%) and at HD (No ♀ data; ♂s:↑30%).
- Based on the minimal animal data, this finding was reversible.

Serum chemistry

Due to the moribund condition of the animals, all HD ♀s were sacrificed on Day 1. Because of the lack of Day1 control data, results for the HD ♀s are not presented in Table below. Since Day 1 data were on fasted animals and Day 2 data were on non-fasted animals, a comparison of the HD ♀s to Day 2 controls may be inappropriate.

Based on the minimal Day 14 data, findings appear to be reversible. ↓GGT for MD animals was 20-29% on D14.

Changes in the serum chemistry parameters on Day 2 (non-fasted) compared to Day 2 control groups (non-fasted):

		AST	ALT	ALP	GGT	BUN	CREAT
MD	♂ N=2 on D2	↑4.5-fold	↑1.6-fold	—	↓26%	↑68%	↑24%
	♀ N=2 on D2	↑87%	—	↑38%	↓24%	—	—
HD	♂ N=2 on D2	↑1.1-fold	↑65%	↓25%	↓42%	↑73%	↑3%
	♀ N=1 on D1						

AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; GGT: gamma glutamyl transpeptidase; BUN: blood urea nitrogen; CREAT: creatinine.

—: No effect/ no remarkable effect.

Changes at the LD were either not remarkable or not toxicologically significant.

		TBIL	CA	PHOS	NA	K	CL
MD	♂ N=2 on D2	—	↓23%	↓5%	↓3%	↓23%	↓3%
	♀ N=2 on D2	—	↓10%	↓34%	—	↓24%	—
HD	♂ N=2 on D2	↑50%	↓32%	↓20%	↓7%	↓25%	↓5%
	♀ N=1 on D1						

Urinalysis: No clear drug-related effect.

Organ weight: Not done.

Gross pathology:

Drug-related gross findings on or before Day 4 consisted of:

- Hemorrhage in the skin and/or subcutis at all doses on Days 1, 2, and 4
- Hemorrhage in the stomach and tongue of one HD ♂ and ♀
- Hemorrhage and white discoloration in the gingiva of both HD ♀s
- Red discoloration of the small intestine (1 ♂) and/or stomach at HD
- Red or abnormal contents of the small and/or large intestine in the HD ♀s.
- Red discoloration in the ovaries and uterus of 1 HD ♀.

Day 15 (recovery):

- The above findings were reversible.

White discoloration of the gingiva in HD ♀s on Day 1 was said to be attributed to inadequate peripheral vascular perfusion and was stated to be secondary to drug-induced moribundity. Red discoloration of the mandibular lymph node in one of these HD ♀s females was stated to be secondary to the hemorrhage observed in the tongue.

Histopathology:

D1-D4 data:

	Dose (mg/kg)	Group 1		Group 2		Group 3		Group 4	
		Vehicle		15		25		45	
		M	F	M	F	M	F	M	F
		2	2	2	2	2	2	2	2
	Sex								
	Animals On Study	1	1	1	1	1	1	2	2
	Animals Logged								
Cecum:									
	Depletion, Lymphoid	0	0	0	0	1	1	2	2
Colon									
	Depletion, Lymphoid	0	0	0	0	1	1	2	2
Duodenum									
	Hemorrhage	0	0	0	0	0	0	0	1
Ileum									
	Depletion, Lymphoid	0	0	0	0	0	0	2	0
	Enteropathy	0	0	0	0	0	0	1	0
	Hemorrhage	0	0	0	0	0	0	1	2
Jejunum									
	Hemorrhage	0	0	0	0	0	0	0	2
Stomach									
	Depletion, Lymphoid	0	0	0	0	1	1	2	2
	Edema	0	0	0	0	0	1	0	0
	Hemorrhage	0	0	0	0	0	0	2	2
	Infiltration, Neutrophil	0	0	0	0	0	0	1	0
	Necrosis, Single Cell; Fundus	0	0	0	0	0	0	0	1
Tongue									
	Hemorrhage	0	0	0	0	0	0	1	1
Bone marrow									
	Necrosis, Single Cell	0	0	0	0	0	0	0	1
Lymph node; Mandibular									
	Missing	0	0	0	0	0	0	0	1
	Depletion, Lymphoid	0	0	0	0	1	1	2	1
Lymph node; Mesenteric									
	Depletion, Lymphoid	0	0	0	0	0	1	2	2
	Pigment, Hemosiderin	1	1	0	1	1	0	1	1
Spleen									
	Depletion, Lymphoid	0	0	0	0	1	1	2	2
Skin									

Hemorrhage _____ 0 0 1 0 1 1 2 0

Kidneys

Cyst _____ 0 0 1 0 0 0 0 0
 Dilatation _____ 0 0 0 0 0 0 0 2
 Infiltration, Mononuclear Cell _____ 1 0 1 1 0 1 2 1

Day 15 data:

No clear drug related effect.

Toxicokinetics:

Day 1

Dose (mg/kg)	Cmax (ng/ml)		Tmax (hr)		AUC* (ng.hr/ml)	
	Male	Female	Male	Female	Male	Female
15	757	1492	1	1	2225	2760
25	1079	828	1.5	1.5	5373	3801
45	1763	2107	1	2	7771	8745
1:1.7:3 Dose Ratio	1:1.4:2.3	1:0.6:1.4			1:2.4:3.5	1:1.4:3.2

* AUC calculated from time zero to the time of last measurable concentration, equal to 24 hr/ ranging between 12 and 24 hr.

- Systemic exposure following single oral doses of BMS-354825 was dose proportional increasing the dose from LD to MD and was less than dose proportional increasing the dose from MD to HD
- There were no apparent sex-related differences in the systemic exposure.
- Tmax was 1-2 hrs.

Summary of the study:

Cynomolgus monkeys were dosed with dasatinib at 15, 25, or 45 mg/kg (180, 300, or 540 mg/m²). Because of the moribund condition of several animals, the hematology and serum chemistry assessments originally planned for Day 3, were performed on Day 2; urinalysis was performed on day 3. Scheduled sacrifices were conducted on Days 4 and 15.

All HD animals were sacrificed moribund on Day 1 (HD ♀s) or on Day 2 (HD ♂s). Clinical signs of toxicity included: vomiting (HD); tremors (HD); ↑muscle tone (HD); abnormal behavior (HD); red/liquid feces (MD and HD) pale mucous membrane of gingival (MD and HD); ↓activity (all doses); hypothermia (all doses), dehydration (all doses), and ecchymosis over numerous sites of the body (thorax, limbs, gingiva, head, and neck; all dose levels).

Reduced body temperature was noticed on Day 1, minimal to marked s.c. hemorrhage/bruising was observed on Day 3 (LD and MD; no HD data available) and heart murmur in 1 animal (1 MD ♂) recorded on Day 14.

MD and HD animals had ↓ food consumption. This resulted in body weight loss or ↓ BW gain, e.g. ↓0.4 g in MD ♀s on Day 3 (no data available for HD animals on Day 3).

No clear drug effect was noted on EKG parameters, heart rate, or arterial oxygen saturation. There was a tendency for ↑ diastolic and systolic blood pressures on Day 11. Because increases were non-dose-dependent and assessments included 1 animal/sex/group (LD and MD only), a test article-related effect remains unclear for this study. However, based on the safety pharmacology studies, dasatinib has the potential to ↑ systolic and diastolic pressure shortly after administration of the drug.

↓ Lymphocyte counts were observed in MD and HD ♂s on Day 2 (no HD ♀ data available), which correlated with lymphoid depletion noted on histopathology. ↑ WBC, ↑ neutrophil, ↑ monocyte, and ↑ fibrinogen appeared to be secondary to internal injury/inflammation observed in multiple organs. Based on the minimal Day 15 data, consisting of no HD animals and only 1/sex/group for LD and HD, changes in the hematology parameters were reversible. Serum chemistry parameters revealed the potential for dasatinib to cause hepatotoxicity, as indicated by ↑ AST (up to 4.5-fold) and ↑ ALT (up to 1.5-fold) at MD and HD. There were no gross or histopathology correlates in the liver. Although changes were noted at LD for some of the hematology and serum chemistry parameters, these changes were not considered toxicology significant.

No clear drug-related effect was observed on urinalysis. Organ weights were not reported.

Drug-related gross pathology findings on Days 1-4 (including unscheduled sacrifices) consisted of:

- Hemorrhage in the skin and/or subcutis at all doses on Days 1, 2, and 4
- Hemorrhage in the stomach and tongue of one HD ♂ and ♀
- Hemorrhage and white discoloration in the gingiva of both HD ♀s
- Red discoloration of the small intestine (1 ♂) and/or stomach at HD
- Red or abnormal contents of the small and/or large intestine in the HD ♀s.
- Red discoloration in the ovaries and uterus of 1 HD ♀.

Drug related histopathology findings on Days 1-4, including the unscheduled sacrifices were:

- Lymphoid depletion: in cecum and colon at MD (1/1 ♂ and 1/1 ♀) and HD (2/2 ♂s and 2/2 ♀s); in ileum (2/2 HD ♂s), in stomach at MD (1/1 ♂ and 1/1 ♀) and HD (2/2 ♂s and 2/2 ♀s), in mesenteric lymph node at MD (1/1 ♀) and HD (2/2 ♂s and 2/2 ♀s), in mandibular lymph node at MD (1/1 ♂ and 1/1 ♀) and HD (2/2 ♂s and 1/2 ♀), in spleen at MD (1/1 ♂; 1/1 ♀) and HD (2/2 ♂s and 2/2 ♀s)

- GI tract: hemorrhage in tongue (1/2 HD ♂ and 1/2 HD ♀), duodenum (1/2 HD ♀), ileum (1/2 HD ♂ and 2/2 HD ♀s), jejunum (2/2 HD ♀s), and stomach (2/2 HD ♂s and 2/2 HD ♀s); single cell necrosis in fundus of stomach (1/2 HD ♀); neutrophil infiltration in stomach (1/2 HD ♂); edema in stomach (1/1 MD ♀); minimal enteropathy in the ileum of 1/2 HD ♂ and was characterized by villous atrophy and the presence of disorganized and stratified apical villous epithelium that displaced the apical lamina propria.
- Skin: hemorrhage (1/1 LD ♂, 1/1 MD ♂, 1/1 MD ♀, 2/2 HD ♂s)
- Kidneys: minimal dilatation of cortical tubules (2/2 HD ♀s), mononuclear cell infiltration (in all groups including control)
- Bone marrow: single cell necrosis (1/2 HD ♀)

Based on the minimal Day 15 data, gross and histopathology findings appear to be reversible.

Toxicokinetics:

- Systemic exposure following single oral doses of BMS-354825 was dose proportional increasing the dose from LD to MD and was less than dose proportional increasing the dose from MD to HD
- There were no apparent sex-related differences in the systemic exposure.
- Tmax was 1-2 hrs.

In conclusion acute toxicities in monkeys consisted of the following:

- GI tract: vomiting, red/liquid feces; abnormal content on gross pathology; hemorrhage in tongue, stomach, duodenum, ileum, jejunum; single cell necrosis/ edema/ neutrophil infiltration in stomach; enteropathy in the ileum characterized by villous atrophy and the presence of disorganized and stratified apical villous epithelium.
- Hematopoietic/ Lymphocytic system: single cell necrosis in bone marrow; ↓lymphocytes; lymphoid depletion in cecum, colon, ileum, stomach, mesenteric and mandibular lymph nodes, and in spleen
- Skin: hemorrhage; bruising/ ecchymosis over numerous sites of the body (thorax, limbs, gingiva, head, and neck)
- Liver: ↑ALT, ↑AST
- Kidneys: minimal cortical tubular dilatation
- Cardiovascular (possibly related): murmur in 1 MD ♂ on Day 14; tendency for ↑diastolic and systolic blood pressure.
- Other: ↓activity; ↓body temperature; ↑WBC neutrophils, monocytes, and fibrinogen appeared to be secondary to internal injury and inflammation.

Summary of single-dose toxicity studies of oral dasatinib in rats and monkeys

Single doses of dasatinib in rats and monkeys resulted in overlapping toxicities in the GI tract (red/liquid feces, hemorrhage and edema in the GI tract, also hemorrhage in the

tongue in monkeys), lymphocytic/ hematopoietic system (lymphoid depletion; bone marrow depletion in rats and single cell necrosis in monkeys), liver (\uparrow AST and ALT in monkeys; single cell necrosis, hypertrophy and \uparrow triglycerides in rats), kidneys (tubular dilatation in both species and additional findings in rats, e.g. tubular epithelial vacuolation, \uparrow urinary volume, \uparrow urinary RBC and WBC). Cardiotoxicity was evident in rats and presented with ventricular necrosis, valvular/ ventricular/ atrial hemorrhage, and cardiac hypertrophy. There was a tendency for increased systolic and diastolic blood pressure in monkeys. However, because of low number of animals, limited groups available for evaluation and a non-dose-dependent effect, contribution of dasatinib to this finding is not clear.

Thrombocytopenia was seen in rats but not monkeys. In addition, toxicities to the reproductive organs were observed in rats only (male reproductive system: single cell necrosis and hemorrhage in epididymis, hemorrhage and multinucleated cells in testes). In addition, low incidence of single cell necrosis in the pancreas of rats may be related to dasatinib treatment.

Toxicities were in general more pronounced in rats. Presence of higher number of animals for data evaluation in the rat single dose study might have contributed to the more pronounced toxicities in rats.

Hemorrhage/bruising was more evident in monkeys. Ecchymosis was observed in these animals over numerous sites of the body (thorax, limbs, gingiva, head, and neck). Red discoloration and focal hemorrhage in the hindlimb muscle in 1 MD ♂ observed in the single dose rat study was stated to be incidental and not drug-related.

Study in Sprague-Dawley rats:

Doses of dasatinib administered: 30, 100, or 300 mg/kg (180, 600, or 1800 mg/m²); single doses. Scheduled necropsies: Days 4 and 16.

Toxicokinetic parameters were not evaluated.

High mortality was observed at MD and HD. All HD rats were found dead or sacrificed in moribund condition by study Day 3. At MD, 4/10 ♂s and 7/10 ♀s were found dead or sacrificed in moribund condition by Day 4. Morbidity and mortality were attributed to GI lesions resulting in fluid and electrolyte loss and impairment of mucosal integrity, bone-marrow and lymphoid depletion, and multifocal myocardial necrosis and hemorrhage.

The following acute toxicities were observed in this study:

- GI toxicity: liquid/red feces, hemorrhage/ulceration/edema in GI tract
- Hematopoietic/ Lymphocytic system: lymphocyte depletion, bone marrow cell depletion
- Thrombocytopenia
- Cardiac toxicity: ventricular necrosis; valvular, ventricular, and atrial hemorrhage; cardiac hypertrophy

- Hepatotoxicity: ↑liver weight, ↑AST, ↑triglycerides, hepatocellular vacuolation, and necrosis, (↓albumin)
- Nephrotoxicity: tubular dilation, vacuolation, pyuria, ↑urinary volume (no change in specific gravity), ↑RBC in urine
- Male reproductive system: single cell necrosis and hemorrhage in epididymis, hemorrhage and multinucleated cells in testes
- Pancreatic toxicity (possibly related): single cell necrosis
- Harderian gland: ↑porphyrin pigment
- ↓RBC, ↑WBC, ↑neutrophils, and ↑monocytes appear to be secondary to internal injury and inflammation

Study in Cynomolgus monkeys:

Doses of dasatinib administered: 15, 25, or 45 mg/kg (180, 300, or 540 mg/m²); single doses. Scheduled sacrifices were conducted on Days 4 and 15.

Dose (mg/kg)	C _{max} (ng/ml)		T _{max} (hr)		AUC* (ng.hr/ml)	
	Male	Female	Male	Female	Male	Female
15	757	1492	1	1	2225	2760
25	1079	828	1.5	1.5	5373	3801
45	1763	2107	1	2	7771	8745
1:1.7:3 Dose Ratio	1:1.4:2.3	1:0.6:1.4			1:2.4:3.5	1:1.4:3.2

* AUC calculated from time zero to the time of last measurable concentration, equal to 24 hr/ ranging between 12 and 24 hr.

All HD animals were sacrificed moribund on Day 1 (HD ♀s) or on Day 2 (HD ♂s).

Acute toxicities in monkeys consisted of the following:

- GI tract: vomiting, red/liquid feces; abnormal content on gross pathology; hemorrhage in tongue, stomach, duodenum, ileum, jejunum; single cell necrosis/ edema/ neutrophil infiltration in stomach; enteropathy in the ileum characterized by villous atrophy and the presence of disorganized and stratified apical villous epithelium.
- Hematopoietic/ Lymphocytic system: single cell necrosis in bone marrow; ↓lymphocytes; lymphoid depletion in cecum, colon, ileum, stomach, mesenteric and mandibular lymph nodes, and in spleen
- Skin: hemorrhage; bruising/ ecchymosis over numerous sites of the body (thorax, limbs, gingiva, head, and neck)
- Liver: ↑ALT, ↑AST
- Kidneys: minimal cortical tubular dilatation
- Cardiovascular (possibly related): murmur in 1 MD ♂ on Day 14; tendency for ↑diastolic and systolic blood pressure.

- Other: ↓activity; ↓body temperature; ↑WBC neutrophils, monocytes, and fibrinogen appeared to be secondary to internal injury and inflammation.

2.6.6.3 Repeat-dose toxicity

Study DS02047 -- Two-Week Oral Exploratory Study in Rats

This study was not reviewed.

In this exploratory non-GLP study, BMS-354825 was administered in 50 mM sodium acetate buffer, pH 4.2-4.6, orally by gavage to groups of 6 rats/sex at daily doses of 1, 15, or 30 mg/kg (6, 90, or 180 mg/m²) for 2 weeks. A vehicle-control group received 50 mM sodium acetate buffer, pH 4.2-4.6, alone. Endpoints included toxicokinetics, survival, clinical signs, body weights, food intake, physical examinations (including ophthalmologic and neurologic assessments), T-cell dependent antibody response to keyhole limpet hemocyanin (KLH), splenic lymphocyte phenotyping, clinical pathology, gross pathology, histopathology and electron microscopy. Scheduled necropsies were conducted on Day 15.

At 15 and 30 mg/kg, dose-related findings consisted of (1) chromorrhinorrhea, soiled/rough haircoat, bloated/swollen abdomen (MD ♀s), dehydration, and soft stool; (2) decreased size of the thymus; (3) distention of the GI tract with gas, fluid, and/or ingesta or digesta, red discoloration of the mesenteric lymph node (4) enteropathy (small and large intestines), edema of the large intestine, and lymphoid depletion of the mandibular and mesenteric lymph nodes, spleen, and thymus.

Additional drug-related findings at the MD (mortality/moribundity precluded the assessment at the HD) included (1) ↓9-15% in platelet counts, hemoglobin, and hematocrit; ↑59-64% in platelet counts and ↑69-109% in reticulocyte counts, and in ♀s, ↑5-6% in mean corpuscular volume and mean corpuscular hemoglobin; (2) ↓29-46% in ALP and ↓18-24% in total protein, albumin, and globulins, and ↑1.3 - 2.3-fold in triglycerides; (3) ↓30-50% in splenic lymphocyte subpopulations (T-cells and B-cells); (4) ↑18% in absolute liver (♀s) weights and ↑16-22% in adrenal weights, and ↓13% in kidney (♂s), ↓25-38% in thymus, and ↓25-26% in spleen weights; (5) at necropsy, abnormal contents of the colon (hard feces). Since diarrhea was noted clinically and enteropathy was observed microscopically, GI loss was considered the primary cause of decreased serum protein values. Despite the decreases in splenic lymphocyte subpopulations and weights at the intermediate dose, BMS-354825 at doses of up to 15 mg/kg did not have any adverse effect on the ability of rats to respond to a T-cell-dependent antigen (KLH).

At the HD, additional findings consisted of (1) mortality (all rats found dead or euthanized on Day 8 [♂s] or Day 9-12 [♀s]); (2) ↓activity, surface hypothermia, pallor, diarrhea, hunched posture, ptosis, and/or thin appearance; (3) ↓body-weight gain (♀s), body-weight loss (♂s, ↓12%), and ↓food consumption; (4) ↓size of spleen and, in ♂s,

red discoloration of the small intestine; (5) bone-marrow depletion. In the bone marrow, depletion of hematopoietic elements ranged from minimal to moderate in severity. Lymphoid depletion in the spleen and thymus and hematopoietic depletion in the bone marrow was more severe in the ♂s than ♀s. This finding is consistent with and may have contributed to the increased severity of clinical observations and survival in the HD ♂s.

There were no drug-related ophthalmologic alterations.

Toxicokinetics:

Dose-related ↑systemic exposure to BMS-354825 was observed on Day 1 and Day 14, and was generally greater than dose-proportional. Mortality precluded assessment at the high dose on Day 14. At HD, females had greater exposures than males on Day 1, but no sex-related differences in exposure were evident at LD or MD on Days 1 or 14. At both the LD and MD, exposure parameters in males and females decreased by 17 - 50% on Day 14, compared to Day 1. At the low and intermediate doses, less than 1% of the total dose was excreted in the urine.

In conclusion, daily oral administration of BMS-354825 for 2 weeks was generally well tolerated in rats at doses up to 15 mg/kg (90 mg/m²). Severe toxicity and lethality occurred in all animals at 30 mg/kg (180 mg/m²). No significant effects occurred at 1 mg/kg (6 mg/m²). The following toxicities were observed:

- Hematopoietic/Lymphocytic system (more severe in ♂s): hematopoietic cell depletion in bone marrow; lymphoid depletion in lymph nodes, thymus, and spleen, red discoloration of mesenteric lymph node, ↓weight of thymus and spleen
- GI tract: bloated/swollen abdomen, diarrhea, distention of the GI tract with gas, fluid, and/or ingesta or digesta, edema of the large intestine, red discoloration of the small intestine
- Liver: ↑triglycerides, ↓albumin, hypertrophy
- Adrenals: ↑weight
- Other: ↑or↓ platelets, chromorhinorrhea, ↓RBC, ↓hemoglobin, ↑reticulocytes, ↓ALP, ↓globulin, ↓total protein

Study title: One-Month Intermittent Dose Oral Toxicity Study in Rats

Key study findings: 17 unscheduled deaths occurred in the study, (10 HD ♂, 5HD ♀, 2LD ♀); 13 were attributed to enteropathy, 2 to gavage errors and 2 were not determined.

The following toxicities were observed:

- GI tract: liquid or non-formed feces; distention and fluid/gas filled lumens, darkened serosa and mucosa; perforation/hemorrhage; congestion/inflammation;

- Hematopoietic/ Lymphocytic system: ↓lymphocytes; lymphoid depletion of mesenteric lymph node, spleen, and thymus; congestion of mesenteric lymph node and thymus; hemorrhage in thymus; ↓weight of spleen
- Liver: hypertrophy; ↑cholesterol; slightly ↑AST and ALT; (↓albumin); inflammation (in an unscheduled sacrifice at LD)
- Heart: hypertrophy
- Adrenals: hypertrophy (♂s only)
- Reproductive system: small seminal vesicles; reduced secretion of seminal vesicles; immature sperms in epididymis; dilatation of uterus.

Study no: DS02158

Volume #, and page #: Item 5

Conducting laboratory and location: —

Date of study initiation: 8/29/02

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, radiolabel, and % purity: BMS354825, Batch # C007A-354825-01,
— pure

Formulation/vehicle: 80 mM sodium citrate

Dosing:

Species/strain: Sprague Dawley Rats

#/sex/group or time point (main study): 15/sex/dose (n=10 terminal sacrifice (including unscheduled deaths and moribunds/n=5 recovery sacrifice)

Satellite groups used for recovery: 5/sex/group for recovery

Age: 4-8 weeks at initiation

Weight: 100 to 300 grams

Doses in administered units: 0, 1, 15, and 25 mg/kg (6, 90, 150 mg/m²)

DailyX5 followed by 2 day recovery for 28 days. Actual doses administered were 0, 0.9, 13.2, and 25mg/kg

Route/Volume: oral gavage, 5 mL/kg

Group Number	Daily Dose		Concentration	Number of Animals ^a
	BMS-354825 (mg/kg)	Volume (mL/kg)	BMS-354825 (mg/mL)	
1	0	5	0	15M, 15F
2	1	5	0.2	15M, 15F
3	15	5	3	15M, 15F
4	25	5	5	15M, 15F

^a The first ten rats/sex/group were designated for terminal sacrifice (including unscheduled deaths and moribunds). Animals designated for recovery sacrifice (five rats/sex/group) underwent 2 weeks of recovery following dose administration.

Observations and times:

Clinical signs: Twice daily for mortality and moribundity; once daily cageside observations for each animal; abnormal findings recorded

Body weights: Once prior to treatment, on the first day and weekly thereafter and upon termination

Food consumption: Weekly

Ophthalmoscopy: not performed

EKG: not performed

Hematology: Prior to last toxicokinetic sampling (Day 26) and prior to recovery necropsy

Clinical chemistry: Prior to last toxicokinetic sampling (Day 26) and prior to recovery necropsy

Urinalysis: not performed

Gross pathology: Following last toxicokinetic sampling (Day 26) or following recovery period

Organs weighed: adrenals, brain, heart, kidneys, liver, lung, ovary, pituitary gland, prostate, spleen, testis

Histopathology: Following last toxicokinetic sampling (Day 26) or following recovery period

Toxicokinetics: Days 1 and 26; 5 animals/sex/group bled at 1, 2, 4, 8, 12, 24 hours post-dose. The first surviving 5/sex/group bled at 1 and 8 hours post-dose; the second surviving 5/sex/group bled at 2 and 13 hours post-dose and the surviving designated recovery animals bled at 4 and 24 hours post-dose.

Histopathology Inventory:

Study	DS02158
Species	Rat DX5, 4wks
Adrenals	X*
Aorta	
Bone Marrow smear	
Bone (femur)	X
Brain	X*
Cecum	X
Cervix	
Colon	X
Duodenum	X
Epididymis	X
Esophagus	X
Eye	X
Fallopian tube	
Gall bladder	
Gross lesions	X
Harderian gland	
Heart	X*
Ileum	X
Injection site	
Jejunum	X
Kidneys	X*
Lachrymal gland	

Larynx	
Liver	X*
Lungs	X*
Lymph nodes, cervical	
Lymph nodes mandibular	
Lymph nodes, mesenteric	X
Mammary Gland	X
Nasal cavity	
Optic nerves	
Ovaries	X*
Pancreas	X
Parathyroid	X
Peripheral nerve	
Pharynx	
Pituitary	X*
Prostate	X*
Rectum	X
Salivary gland	X
Sciatic nerve	X
Seminal vesicles	X
Skeletal muscle	X
Skin	X
Spinal cord	X
Spleen	X*
Sternum	X
Stomach	X
Testes	X*
Thymus	X
Thyroid	X
Tongue	X
Trachea	X
Urinary bladder	X
Uterus	X
Vagina	X
Zymbal gland	

X, histopathology performed

*, organ weight obtained

Results:

Mortality:

Scheduled Sacrifice	♂				♀			
	C	LD	MD	HD	C	LD	MD	HD
	10	10	10	10	10	10	10	10
Gavage Related						1		1
Enteropathy/ lymphoid depletion				10				3
Undetermined						1		1

Unscheduled deaths in HD ♂s occurred at Days 7, 10, 13, 15, 18, 21(two), 25 and 26. Unscheduled deaths in ♀s which were attributed to enteropathy and lymphoid depletion occurred at Day 7 (two) and Day 19, whereas both deaths of undetermined cause occurred on Day 22. With the exception of the undetermined deaths and gavage related deaths, moderate to severe enteropathy was present, along with lymphoid depletion and hypocellular appearance of the bone marrow. Minimal inflammation of the kidney and liver were noted in the LD female for which cause of death could not be determined, although enteropathy of the jejunum was minimal and lymphoid depletion and hypocellular appearance of the bone marrow was not present, the company did not rule out the possibility that the death was drug related.

Clinical signs:

Primarily, clinical signs were observed in HD animals. Signs included changes in excretion (few, liquid or non-formed feces), swelling of the abdomen, and rough coat.

		Cycle 1		Cycle 2				Cycle 3				Cycle 4				Recovery (Days 27-44)			
		Dosing (1-5)		Recovery (6-7)		Dosing (8-12)		Recovery (13-14)		Dosing (15-19)		Recovery (20-21)		Dosing (22-26)		Recovery (27)			
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Appearance	Hunched					1HD						2HD						1HD	
	Swollen, vent/abdo													1HD					
	Thin		1LD		1LD 2HD	2HD		1HD		1HD	3HD		1HD						
Behavior	Other-distended abdomen			1HD		1HD		2HD		1HD		4HD		1HD					
	Hypoactive				1LD	1HD		2HD		1HD		3HD		2HD					
Discharge	Recumbent			1HD	1HD			1HD											
	Nasal-cloudy		1LD		1LD														
Excretion	Red-oral		1LD		1LD														
	Few Feces			2HD	1LD 3HD	2HD	1HD	2HD		4HD	1HD	5HD	1HD	3HD		2HD	2HD	2HD	
	Liquid Feces					1HD		1HD				1HD							
Eyes	Non-formed feces	2HD		2HD	2HD	2HD	1LD	1HD		4HD	1HD	6HD	1HD	3HD		1HD	1HD	1HD	
	Discolored											1HD							
	Red Discharge		1LD																
Respiration	Clear Discharge							1HD											
	Squinted-periorbital												1HD						
Skin and Pelage	Irregular				1LD							1HD							
	Labored			1HD	1LD 1HD							1HD		1HD					
Skin and Pelage	Brown Hair-Scrotum			1HD															
	Rough Hair		1LD	1HD	1LD			1HD		1HD	1HD	3HD		2HD				1HD	
	Pale					1HD		1HD				1HD		1HD					
	Cold to touch			1HD	1HD			1HD				1HD							

Body weights:

A dose dependant decrease in body weight in males was evident following drug administration in males. This effect was partially reversed following the recovery period, as shown in the table below. Changes in body weight were not remarkable ($\pm 5\%$) in females.

Change in body weight as a percent of Control			
♂	LD	MD	HD
Week 2	0	↓2	↓10*
Week 3	↓1	↓2	↓9*
Week 4	↓2	↓6	↓20*
Week 5	↓6	↓8*	↓20*
Week 6	↓7	↓8*	↓12*

*statistically different from control value ($p \leq 0.05$)

Food consumption:

LD, MD, and HD ♂s had significantly decreased food consumption during weeks 1-3 and increased, at HD during weeks 5 and 6. Females had significantly ↓consumption during week one.

	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 1	↓3	↓13*	↓37*	↓5	↓1	↓19*
Week 2	↓8*	↓8*	↓14*	↑4	↑1	↑4
Week 3	↓10*	↓11*	↓37*	↓1	↓2	↑4
Week 5	↓8*	↓4	↑15*	↓8	↓5	↑5
Week 6	↓9*	↓9*	↑11*	↓9	↓9	↓4

Clinical chemistry and Hematology:

The table below summarizes significant changes in clinical chemistry and hematology at week 4. All changes were reversible at week 6 with the exception of RBC in males and MCV in females. In males, additional changes were observed in MHC (↑11%) and MCV (↑11%) at six weeks.

	♂			♀		
	LD	MD	HD	LD	MD	HD
RBC	-	↓7	↓12	-	-	↓9
HCT	-	↓5	↓9	-	-	-
HGB	-	↓5	↓9	-	-	-
Platelets	-	↑49	↑48	-	↑54	↑85
MCV	-	-	-	-	-	↑8
Neutrophils	-	↑131	↑154	-	↑138	↑213
Lymphocytes	-	-	↓47	-	-	-
Monocytes	-	↑100	↑100	-	-	↑200
Eosinophils	-	-	-	-	↑200	↑200
BUN	-	-	↑45	-	-	-

ALP	-	↓21	↓50	-	↓21	↓21
AST	-	↑23	↑69	-	-	↑19
ALT	-	-	↑55	-	-	-
In Phosphorus	-	-	↓10	-	-	-
Albumin	-	↓9	↓18	-	↓9	↓9
Cholesterol	-	-	↑40	-	-	↑40

RBC: red blood cells; HCT: hematocrit; HGB: hemoglobin; MCV: mean corpuscular volume; BUN: blood urea nitrogen; ALP; alkaline phosphatase; AST: aspartate aminotransferase; ALT: alanine aminotransferase; In Phosphorus: inorganic phosphorus.

Organ weights:

Primary organs affected included the Adrenal, liver, prostate and spleen. Percent changes are detailed in the table below. Irreversible decreases in spleen, and prostate were observed following both MD and HD. An increase in adrenal weight was detected in males, following the MD increases were reversible, however increases were still evident at two weeks post-dosing following HD. Irreversible increases in weight were observed in males and females following the HD and in males following the MD.

Note: Thymus weights were not taken.

Changes in the relative organ weights (organ: BW)

	MD		HD	
	♂	♀	♂	♀
Adrenal	*↑25%	—	NK (*↑28%)	—
Heart	↑6% (*↑2%)	*↑16% (*↑17%)	NK (↑5%)	↑5% (↑10%)
Kidney	—	— (↑9%)	NK	— (↑7%)
Liver	↑14% (↑5%)	↑7% (*↑17%)	NK (*24%)	*↑14% (*↑24%)
Pituitary	—	—	NK	↓25%
Spleen	*↓35%	*↓28%	NK	*↓47%

* Statistically significant.

NK: not known (no data available for HD ♂s).

—: no remarkable effect/ no effect.

Note: numbers in parentheses represent changes at the end of the recovery period.

Gross pathology:

Drug related macroscopic changes were noted primarily in animals treated with 13.2 and 25 mg/kg. In males and females aberrations were noted in the stomach, cecum, duodenum, ileum, jejunum, and colon. These included distention and fluid/gas filled lumens, combined with darkened serosa and mucosa. The changes detected were reversible and not detected in the recovery sacrifice groups. Additionally dark, mottled mesenteric lymph nodes were detected in animals treated with 25mg/kg.

		TERMINAL SACRIFICE				RECOVERY SACRIFICE			
		C	LD	MD	HD	C	LD	MD	HD
Adrenal Cortex- Dark					1M				
Bone Deformed								1M	
Cecum	Distended				5M 5F				
	Lumen, material				2M 2F				
	Lumen, fluid				3M 3F				
	Wall, thickened				2M				
Colon	Distended				1F				
	Lumen, fluid				1F				
Duodenum	Mucosa-dark				1M				
	Distended				3M 1F				
	Lumen Filled				2M 1F				
	Lumen-Material				1M				
	Serosa-Dark				1M				
Esophagus Perforated			1F		1F				
Ileum	Distended				4M 1F				
	Lumen, material				2M				
	Mucosa, dark				2M				
	Lumen, filled				2M 1F				
Jejunum	Distended				5M 3F				
	Lumen, gas				2F				
	Lumen, material				3M				
	Mucosa, dark				2M 1F				
	Lumen, fluid				2M 1F				
	Serosa, dark				1M				
	Intussuscepted				3M				
Lung Failure to collapse							1F		
Lymph Node Mesenteric	Dark				2M 1F				
	Mottled				1M				
	Gelatinous				1M				
	Enlarged				1F				
Rectum	Lumen, fluid				1F				
	Distended				1F				
Seminal Vesicle Small		1M			2M				
Spleen Small					5M				
Stomach	Dark area				4M 1F				
	Distended				2M 1F				
	Lumen Gas				1M				

	Mucosa, dark				1M			
	Lumen-filled				1M 1F			
Urinary Bladder								
	Lumen-fluid				1M			
Uterus	Distended				1F	1F		1F
	Lumen-fluid				1F	1F		1F

C: control; LD: low-dose; MD: mid-dose; HD: high-dose

Histopathology:

Unscheduled deaths occurred in HD ♂s and were attributed to moderate enteropathy, severe lymphoid depletion, and bone marrow hypocellularity. Unscheduled deaths in females were attributed to enteropathy and lymphoid depletion. Reversible mild to moderate enteropathy and lymphoid depletion of the thymus was reported in MD animals. Minimal inflammation of the kidney and liver were noted in the LD female for which cause of death could not be determined, although enteropathy of the jejunum was minimal and lymphoid depletion and hypocellular appearance of the bone marrow was not present, the company did not rule out the possibility that the death was drug related.

		TERMINAL SACRIFICE				RECOVERY SACRIFICE			
		C	LD	MD	HD	C	LD	MD	HD
Cecum	Edema				3M				
	Submucosa, hemorrhage				1M				
	Congestion				3M 2F				
Colon									
	Congestion				3M 2F				
Duodenum	Enteropathy			9M 8F	9M 9F				
	Congestion				4M 4F				
Epididymis	Immature sperm forms				3M				
Esophagus	Submucosa, hemorrhage	2M	1F						
	Injury, gavage		1F		1F				
	Serosa, chronic inflammation				1F				
Heart	Cardiomyopathy degenerative	1M			1M	2M			
	Epicardium, chronic active inflammation		1F						
Ileum	Enteropathy			10M 10F	5M 9F				
	Congestion				2M 4F				
	Hemorrhage				1M				
Jejunum	Enteropathy			10M 8F	9M 10F				
	Congestion				7M 4F				

		TERMINAL SACRIFICE				RECOVERY SACRIFICE			
		C	LD	MD	HD	C	LD	MD	HD
Kidney	Nephropathy, chronic progressive	10M 5F			9M 2F	1F			1F
	Tubule, microconcretion		1F						
Liver	Inflammation, chronic	8M 5F	1F			5M 5F			5M 5F
	Hemorrhage	2M							
	Distorted architecture	1M							
	Arteritis	1M							
Lymph Node-mesenteric	Congestion				9M 9F				
	Depletion, lymphoid		1F		1M				
Lung	Alveolus, macrophages, vacuolated	1M							1M 1F
	Pleura, chronic inflammation		1F						
Marrow, Femur	Hypocellular				10M 3F				
	Congestion, hemorrhage				10M 4F				
Marrow, Sternum	Hypocellular				10M 3F				
	Congestion, hemorrhage				10M 3F				
Pancreas Depletion, zymogen granules					7M				
Rectum Congestion					2M 1F				
Salivary Gland Necrosis					2M				
Seminal Vesicle Reduced secretion					4M				
Skin Edema					4M				
Spleen	Depletion, lymphoid				10M 6F				
	Extramedull. hematopoiesis increased								4M
Stomach-GL	Congestion				4M				
	Erosion				1M 1F				
	Focal Hemorrhage				1M 1F				
Thymus	Depletion, Lymphoid		1F	8M 7F	8M 3F				
	Edema		1F	8M 1F	8M 3F				
	Hemorrhage/hemoglobin crystals		1F	2F	2M 2F				
	Inflammation-chronic, active		1F						

		TERMINAL SACRIFICE				RECOVERY SACRIFICE			
		C	LD	MD	HD	C	LD	MD	HD
	Congestion				6M 3F				
	Necrosis				1F				
Urinary Bladder	Hemorrhage				1M				
Uterus	Dilatation	1F			2F	2F			2F

M: male; F: female.

Toxicokinetics:

- Systemic exposure to BMS-354825 was generally dose proportional
- Exposures were similar between males and females.
- An overall decrease in exposure occurred upon repeated cycles in both sexes at MD and HD.

BMS-354825							
Dose [mg/kg/day]	Study Day	C _{max} (ng/mL)		T _{max} (h)		AUCT ^a (ng.h/mL)	
		Male	Female	Male	Female	Male	Female
0.9	1	7.9	9.8	4	4	34	45
	26	6.6	13.4	4	2	32	41
15	1	96.1	88.4	8	8	937	920
	26	57.7	51.8	8	2	827	705
25	1	102.1	184.2	8	4	1315	1737
	26	49	63.8	4	4	951	944
Ratios							
0.9:15:25 Dose Ratio	1	1:12.2:12.9	1:9.0:18.8			1:27.6:38.7	1:20.4:38.6
	26	1:8.7:7.4	1:3.9:4.8			1:25.8:29.7	1:17.2:23

^a Calculated from time zero to the time of last measurable concentration. equal to 24 h. ranging between 8 to 24 h.

Summary of individual study findings:

17 unscheduled deaths occurred in the study, (10 HD ♂, 5HD ♀, 2LD ♀); 13 were attributed to enteropathy, 2 to gavage errors and 2 were not determined. Unscheduled deaths at HD: six males and two females found and four males and one female sacrificed in moribund condition. Mortalities were generally due to enteropathy and lymphoid depletion. Clinical signs of toxicity in these animals included hunched appearance, hypoactive behavior, labored or irregular respiration, swelling and paleness and stool changes. One death occurred in a LD ♀. Minimal inflammation of the kidney and liver were noted in the LD female for which cause of death could not be determined. Although enteropathy of the jejunum was minimal and lymphoid depletion and hypocellular appearance of the bone marrow was not present, the possibility that the death was drug related cannot be excluded.

Drug-related in-life findings at MD and HD included decreased body weight (♂s at MD and HD) and food consumption. ♂s at MD and HD had significantly ↓mean food

consumption values during Weeks 1-3 and HD ♀s had significantly ↓food consumption values during Week 1. Food consumption were increased during the recovery period, Weeks 5-6.

Drug related hematology findings at MD and HD included minimally ↓RBC (♂ and ♀), hemoglobin (♂) and hematocrit (♂). ↓lymphocytes (↓47%) was observed in HD ♂s. Additional findings included reversible ↑ in neutrophils (↑130%-210% in MD and HD ♂s and ♀s), monocytes (↑100% in MD ♂s and ♀ and ↑200% in HD ♀), eosinophils (↑200% in MD and HD ♀s), and platelet (↑50% in MD ♂s and ♀s and ↑50-85% in HD ♂s and ♀s). Drug related changes in hematology were reversible, with the exception of RBC in ♂s and MCV in ♀s. In ♂s, additional changes were observed in MHC (↑11%) and MCV (↑11%) at the end of the recovery period.

Drug-related changes in serum chemistry parameters at the end of the treatment period (Week 4) included ↓ALP (up 50% in MD/HD ♂s and ♀s), ↑cholesterol (40% in HD ♂s and ♀s), ↑BUN (↑45% in HD ♂s), ↑ALT (↑55% in HD ♂s), ↑AST (↑69% in HD ♂s), and ↓albumin (↓9-18%). There were no differences in serum chemistry data between control and treated groups at the end of the recovery period.

Drug-related organ weight changes at the end of the treatment period consisted of ↑weight of adrenals (♂s), heart (♂s and ♀s), and liver (♂s and ♀s) and ↓weight of spleen (♂s and ♀s). Cardiac hypertrophy was not reversible as shown by relative heart weights at Week 6 comparable to those recorded at Week 4. Liver hypertrophy progressed through the recovery period. Slightly ↑weight of kidneys, not previously noted at the end of the treatment, was observed at the end of the recovery period.

Reversible dose related enteropathy was observed in both males and females at MD and HD in the duodenum, jejunum and ileum and were characterized by disorganization of the enterocytes in the superficial portion of the mucosa with fusion of the villi and decreased villus to crypt ratio. Reversible lymphoid depletion and hemorrhage in the thymus were also noted at these doses. Additional drug-related macroscopic changes at HD included distention of the stomach, small intestine, and cecum, colon and rectum; red discoloration of the mesenteric lymph node; and, in males, red discoloration of the stomach and small intestine, intussusception of the small intestine and decreased size of the spleen. Microscopically, hypocellularity of the bone marrow occurred only in unscheduled deaths whereas reversible lymphoid depletion of the spleen, hemorrhage of the stomach (1 ♂ and 1 ♀) and reversible edema in the cecum (♂s) occurred in HD animals scheduled for the terminal necropsy.

Toxicokinetics:

- Systemic exposure to BMS-354825 was generally dose proportional
- Exposures were similar between males and females.
- An overall decrease in exposure occurred upon repeated cycles in both sexes at MD and HD.

In summary, the following toxicities were observed:

- GI tract: liquid or non-formed feces; distention and fluid/gas filled lumens, darkened serosa and mucosa; perforation/hemorrhage; congestion/inflammation;
- Hematopoietic/ Lymphocytic system: ↓lymphocytes; lymphoid depletion of mesenteric lymph node, spleen, and thymus; congestion of mesenteric lymph node and thymus; hemorrhage in thymus; ↓weight of spleen
- Liver: hypertrophy; ↑cholesterol; slightly ↑AST and ALT; (↓albumin); inflammation (in an unscheduled sacrifice at LD)
- Heart: hypertrophy
- Adrenals: hypertrophy (♂s only)
- Reproductive system: small seminal vesicles; reduced secretion of seminal vesicles; immature sperms in epididymis; dilatation of uterus; fluid-filled uterus (also seen in control).
- Other: labored breathing; necrosis in salivary gland; minimally ↑kidney weight at the end of recovery period; minimal inflammation in kidney in one unscheduled death (LD ♀). ↓RBC, ↑neutrophils, ↑monocytes, and ↑platelets appear to be secondary to internal injury/bleeding and inflammation.

Study title: BMS-354825: Six-Month Oral Toxicity Study in Rats

Key study findings: The HD was not tolerated and resulted in unscheduled deaths. Mortalities were higher in ♂s than ♀s, although there were no apparent gender-dependent differences in exposure. Toxicities were seen in the following tissues/organs/parameters: GI tract, liver, heart, adrenal glands, hematopoietic system, female reproductive system, pancreas, thyroid/parathyroid, tongue, coagulation parameters, and electrolytes. Because of histopathology findings at LD, a NOAEL could not be identified.

Report #: DS03072

— **Study #:** 6108-408

Volume #, and page #: Item 5

Conducting laboratory and location:

Date of study initiation: 25 April 2003

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity:

Test Article	Lot Number	Storage	Purity (%)
BMS-354825	RD71123-45	Room temperature and protected from light	— as is)

Vehicle Control: 80 mM sodium-citrate buffer

Methods

Doses: 9, 24, and 90/60/48 mg/m²/day x 6 months (26 weeks)

Group	No. of Animals		Dose Level ^a (mg/kg/day)	Dose Concentration ^a (mg/mL)
	Male	Female		
Toxicity Animals ^b				
1 (Vehicle Control) ^c	25	25	0	0
2 (Low)	25	25	1.5	0.3
3 (Mid)	25	25	4	0.8
4 (High)	25	25	15/10/8 ^e	3/2/1.6 ^e
Toxicokinetic Animals ^d				
5 (Low)	11	11	1.5	0.3
6 (Mid)	11	11	4	0.8
7 (High)	11	11	15/10/8 ^e	3/2/1.6 ^e
Sentinel Animals ^f				
8	10	10	0	0

a Animals were dosed at a volume of 5 mL/kg.

b Toxicity animals designated for recovery sacrifice (the last five animals/sex/group) underwent 4 weeks of recovery following 26 weeks of dose administration.

c Group 1 animals were dosed with the vehicle control article only.

d Two animals/sex/group served as replacement animals to compensate for mortality.

e Animals in the high-dose groups were dosed 15 mg/kg/day (3 mg/mL) during Weeks 1 through 7, 10 mg/kg/day (2 mg/mL) during Weeks 8 through 16, and 8 mg/kg/day (1.6 mg/mL) beginning at Week 17.

f Animals were designated for health screen testing only.

Table provided by the sponsor.

Species/strain: rat/ Sprague-Dawley

Number/sex/group or time point: see Table above

Route, formulation, volume: oral (gavage), solution, 5 mL/kg

Satellite groups used for toxicokinetics or recovery: see Table above

5/sex/group saved for a 4-week recovery after 26 weeks of dosing

Age: 54 days at initiation

Weight: ♂s: 220-255 g at initiation

♀s: 156-184 g

Concentration Verification of Dosing Formulations

For all samples, the mean concentration of BMS-354825 was acceptable (90 to 110% of the theoretical concentration).

Observations and times:

Mortality: twice daily

Clinical signs: once daily for cageside observations

Detailed observations: prior to initiation of treatment, weekly during the treatment, and the day of scheduled sacrifice

Body weights: prior to initiation of treatment, on the 1st day of treatment, then weekly thereafter. BWs were not reported for Group 8 (sentinel animals), because these animals were only used for monitoring health conditions in the animal room. BWs were not reported for TK animals.

Food consumption: weekly

Ophthalmoscopy: not done

EKG: not done

Hematology: during Weeks 4, 13, 26, and 31. Blood was also collected from toxicity animals sacrificed at an unscheduled interval. Additional hematology sampling for platelet aggregation was conducted for the first 5 surviving toxicity animals at the terminal sacrifice and all surviving animals at the recovery sacrifice.

Clinical chemistry: during Weeks 4, 13, 26, and 31. Blood was also collected from toxicity animals sacrificed at an unscheduled interval.

Urinalysis: during Weeks 4, 13, 26, and 31. Overnight collection. The following parameters were evaluated:

appearance/color	pH
bilirubin	protein
blood	specific gravity
glucose	urobilinogen
ketones	volume
microscopic examination of sediment	

Gross pathology: at necropsy (after 26 weeks of dosing and after an additional 4 weeks of recovery)

Organ weights: at necropsy

adrenal (2)	prostate
brain	seminal vesicles (2)
heart	spleen
kidney (2)	testis (2)
liver	thymus
ovary (2)	thyroid (2) with parathyroid
pituitary gland	uterus with cervix

Histopathology: Adequate Battery: yes (x), no ()

Peer review: yes (x), no ()

Bone marrow smears were prepared but not evaluated.

The following tissues (when present) from each toxicity animal were preserved in 10% neutral-buffered formalin.

adrenal (2)	ovary (2)
aorta	pancreas
brain	peripheral nerve (sciatic)
cecum	pituitary gland
cervix	prostate
colon	rectum
duodenum	salivary gland [mandibular (2)]
epididymis (2)	seminal vesicle (2)
esophagus	skeletal muscle (biceps femoris)
eye (2)	skin
femur with bone marrow (articular surface of the distal end)	spinal cord (cervical, thoracic, and lumbar)
Harderian gland	spleen
heart	sternum with bone marrow
ileum	stomach (glandular and nonglandular)
jejunum	testis (2)
kidney (2)	thymus
lesions	thyroid (2) with parathyroid
liver	tongue
lung with mainstem bronchi	trachea
lymph node (mesenteric and mandibular)	urinary bladder
mammary gland (females)	uterus
optic nerve	vagina

- Preserved tissues, listed above, from control and HD terminal sacrifice animal and each toxicity animal that died or was sacrificed at an unscheduled interval were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically.
- Target organs identified microscopically as the adrenal, cecum, duodenum, ileum, jejunum, lung with mainstem bronchi, lymph node (mesenteric), ovary, thyroid, and uterus, were processed and examined from LD and MD animal from the terminal sacrifice and each animal from the recovery sacrifice.
- Macroscopic lesions were processed and examined microscopically from the LD and MD terminal sacrifice and recovery sacrifice animals.

Unscheduled Sacrifices: Necropsies were performed on all toxicity animals that died or were sacrificed at an unscheduled interval. Animals to be sacrificed were bled for clinical pathology if possible.

Toxicokinetics: on study Days 1, 90, and 181. Blood sampling (0.5 mL) at the following time-points: 1, 2, 4, 8, 12, and 24 hrs post-dose.

Because of excess mortality, blood was collected from the first 3 (Day 90) or 2 (Day 181) Group 7 males approximately 1, 4, and 12 hours post-dose and the next 3 animals approximately 2, 8, and 24 hours post-dose.

Results

Mortality: 9 HD ♂s, 2 HD ♀s (main groups) and 7 HD TK ♂s, during the treatment period, starting from Day 23 and continued until Day 160. In addition, 1 control ♀ and 1 ♂ given 4 mg/kg/day (MD) died during clinical pathology blood collection on Days 86 and 177, respectively.

Clinical signs prior to death included: swollen abdomen, hunched posture (males), thin appearance, irregular respiration, fecal abnormalities (few, liquid, or non formed feces), red haircoat, and rough haircoat (♂s). Most of these animals lost substantial body weight in the period before death.

Clinical pathology tests were performed for 3 HD ♂s and 2 HD ♀s sacrificed because of poor health. The most prominent findings for these animals were consistent with dehydration (e.g., high red blood cell count, hemoglobin, hematocrit, and urea nitrogen), inflammation (e.g., high absolute neutrophil and monocyte counts), low absolute lymphocyte count, and protein loss (e.g., low total protein due to low albumin and globulin).

Incidence of macroscopic findings in unscheduled deaths:

		SEX: -----MALE-----				-----FEMALE-----			
		GROUP: -1- -2- -3- -4- -1- -2- -3- -4-							
GENERAL COMMENT (GC)	NUMBER EXAMINED:	0	0	1	9	1	0	0	2
ANIMAL THIN		0	0	0	1	0	0	0	0
DISCHARGE-ORAL		0	0	0	2	0	0	0	0
GI TRACT-LIGHT FLUID CONTENT		0	0	0	2	0	0	0	0
DISCHARGE-NASAL		0	0	0	2	0	0	0	0
GI TRACT-LARGE LUMEN		0	0	0	2	0	0	0	0
FLUID-THORACIC CAVITY		0	0	0	6	0	0	0	2
NO MACROSCOPIC LESIONS		0	0	0	1	1	0	0	0
DISCHARGE-OCULAR		0	0	0	1	0	0	0	0
LN, MESENTERIC (MS)	NUMBER EXAMINED:	0	0	1	9	1	0	0	2
	NOT REMARKABLE:	0	0	0	8	1	0	0	2
DIFFUSELY RED		0	0	1	1	0	0	0	0
SPLEEN (SP)	NUMBER EXAMINED:	0	0	1	9	1	0	0	2
	NOT REMARKABLE:	0	0	1	7	1	0	0	2
SMALL ADHESION(S)		0	0	0	1	0	0	0	0
		0	0	0	1	0	0	0	0
DUODENUM (DU)	NUMBER EXAMINED:	0	0	1	9	1	0	0	2
	NOT REMARKABLE:	0	0	1	7	1	0	0	2
DIFFUSELY RED THICKENED WALL		0	0	0	1	0	0	0	0
		0	0	0	1	0	0	0	0
JEJUNUM (JE)	NUMBER EXAMINED:	0	0	1	9	1	0	0	2
	NOT REMARKABLE:	0	0	1	7	1	0	0	2
DIFFUSELY RED THICKENED WALL		0	0	0	1	0	0	0	0
		0	0	0	1	0	0	0	0
ILEUM (IL)	NUMBER EXAMINED:	0	0	1	9	1	0	0	2
	NOT REMARKABLE:	0	0	1	7	1	0	0	2
DIFFUSELY RED THICKENED WALL		0	0	0	1	0	0	0	0
		0	0	0	1	0	0	0	0
CECUM (CE)	NUMBER EXAMINED:	0	0	1	9	1	0	0	2
	NOT REMARKABLE:	0	0	1	6	1	0	0	2
DIFFUSELY RED GELATINOUS THICKENED WALL		0	0	0	1	0	0	0	0
		0	0	0	2	0	0	0	0
		0	0	0	1	0	0	0	0

One additional finding in high-dose males that died during the course of the study was minimal segmental medial arteriolar hyperplasia in the lungs.

Clinical signs:

HD:

- Swollen abdomen, red haircoat, Fecal abnormalities (few and liquid, mainly in ♂s)
- Findings were reversible upon recovery

Body weights:

- Mean body weights of HD ♂s (15/10/8 mg/kg/day) were significantly lower than controls from Weeks 2-9 and during Week 19.
- The week-to-week body weight change was significantly lower in HD ♂s during Weeks 1, 3, and 25 and significantly higher during Weeks 8, 9, 13, 19, and 26.

Overall BW changes (g)

	Control	LD	MD	HD
Treatment Period				
Week 1-27 ♂s	243	244	256	231
Week 1-27 ♀s	110	114	128*	126*
Recovery Period				
Week 27-31 ♂s	-1	-3	-13*	-16*
Week 27-31 ♀s	-4	-3	-3	-12

* Statistically significant.

Food consumption:

↓ Food consumption in HD ♂s during the first 2 weeks of the study but significantly higher than controls from Weeks 5-12 and Weeks 16-18.

Ophthalmoscopy: Not done

EKG: Not done

Hematology:

Table below is the summary of hematology and coagulation changes. Numbers in parentheses represent the highest changes during the week(s) indicated, e.g. changes in the RBC levels in MD ♂s were ↓7%, ↓10%, and ↓12% for Weeks 4, 13, and 26, respectively, when compared to the corresponding controls. This information has been represented as 4, 13, 26 (↓12%).

Parameter	LD (1.5 mg/kg/day)		MD (4 mg/kg/day)		HD (15/10/8 mg/kg/day)	
	♂	♀	♂	♀	♂	♀
RBC	26 (*↓5%)	26 (*↓4%)	4, 13, 26 (*↓12%)	4, 13, 26 (*↓11%)	4, 13, 26 (↓*16%)	4, 13, 26 (*↓19%)
Hb	—	—	4, 13, 26 (*↓4%)	13, 26 (*↓6%)	4, 13, 26 (*↓6%)	4, 13, 26 (*↓10%)
Hct	—	—	13, 26	13, 26	4, 13, 26	4, 13, 26

			(*↓6%)	(*↓6%)	(*↓8%)	(*↓10%)
MCV	4, 26 (*↑4%)	—	4, 13, 26 (*↑7%)	4, 13, 26 (*↑6%)	4, 13, 26 (*↑11%)	4, 13, 26 (*↑10%)
MCH	4, 26 (*↑3%)	—	4, 13, 26 (*↑8%)	4, 13, 26 (*↑6%)	4, 13, 26 (*↑12%)	4, 13, 26 (*↑11%)
Ret (Abs)	—	—	—	—	4 (*↑45%)	4, 26 (*↑35%)
WBC	26 (*↓15%)	4 (↓14%)	4, 13, 26 (*↓18%)	4 (↓18%)	13, 26 (*↓22%)	—
Neut (Abs)	—	—	—	—	4 (*↑123%)	4, 13, 26 (*↑127%)
Lymph (Abs)	26 (*↓14%)	4 (↓15%)	4, 13, 26 (*↓23%)	4 (*↓20%)	4, 13, 26 (*↓33%)	4 (*↓20%)
Mono (Abs)	—	—	—	—	4, 13 (*↑63%)	4, 13 (*↑50%)
Platelets	—	—	4, 13, 26 (*↑25%)	4, 13, 26 (*↑19%)	4, 13, 26 (*↑65%)	4, 13, 26 (*↑61%)
Platelet aggregation†	—	—	26 (↓25%)	26 (*↓33%)	26 (*↓68%)	26 (*↓73%)
aPTT	—	—	4, 26 (*↓10%)	26 (↓11%)	4, 13, 26 (*↓15%)	4, 13, 26 (*↓16%)
Fibrinogen	—	—	4, 13, 26 (*↑18%)	13, 26 (*↑20%)	—	4, 13, 26 (*↑52%)

RBC: Red blood cell; Hb: Hemoglobin; Hct: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; Ret (Abs): Absolute reticulocyte; WBC: White blood cell; Neut (Abs): Absolute neutrophil; Lymph (Abs): Absolute lymphocyte; Mono (Abs): Absolute monocyte; PT: Prothrombin time; aPTT: Activated partial thromboplastin time; —: No drug-related effect.

* Statistically significant.

† Platelet aggregation was not measured during Weeks 4 and 13.

Recovery (Week 31):

Findings were in general reversible. The following changes appeared to be relatively high after 4 weeks of recovery. “*” indicates statistically significant changes, when compared to corresponding controls.

- MCV in HD ♀s (*↑7%)
- Abs lymphocytes in HD ♀s (*↓25%)
- Abs monocytes in HD ♀s (↑40%)
- Fibrinogen in MD and HD ♀s (*↑35% and *↑47%, respectively)

Clinical chemistry:

**APPEARS THIS WAY
ON ORIGINAL**

Parameter	LD (1.5 mg/kg/day)		MD (4 mg/kg/day)		HD (15/10/8 mg/kg/day)	
	♂	♀	♂	♀	♂	♀
BUN	—	—	—	4, 13, 26 (*↓21%)	—	4, 13, 26 (*↓13%)
Creatinine	—	—	—	13, 26 (*↓13%)	4 (↓14%)	4, 13, 26 (*↓14%)
Total protein	26 (*↓5%)	26 (*↓6%)	4, 13, 26 (*↓9%)	13, 26 (*↓14%)	4, 13, 26 (*↓26%)	4, 13, 26 (*↓19%)
Albumin	—	—	4, 13, 26 (*↓12%)	4, 13, 26 (↓10%)	4, 13, 26 (*↓26%)	4, 13, 26 (↓26%)
Globulin	—	26 *↓11%)	—	13, 26 (*↓18%)	4, 13, 26 (*↓25%)	4, 13, 26 (*↓14%)
Cholesterol	—	—	4, 13, 26 (*↑16%)	—	4, 13 (↑*10%)	4, 13, 26 (*↑39%)
Triglycerides	—	—	—	—	4, 13 (*↑338%)	4, 13, 26 (*↑323%)
AST	13 (*↑15%)	4 (*↑14%)	4, 13, 26 (*↑25%)	4, 13, 26 (*↑25%)	4, 13, 26 (*↑39%)	4, 13, 26 (*↑31%)
ALT	—	26 (*↓22%)	26 (*↓23%)	26 (*↓36%)	26 (*↓26%)	26 (*↓26%)
ALP	—	26 (*↓14%)	13, 26 (*↓25%)	4, 13, 26 (*↓32%)	4, 13, 26 (*↓46%)	4, 13, 26 (*↓43%)
Ca	—	4, 26 (*↓3%)	26 (*↓3%)	4, 13, 26 (*↓5%)	4, 13, 26 (*↓10%)	4, 13, 26 (*↓8%)
Pi	—	4 (*↓6%)	—	4 (*↓7%)	—	4 (*↓11%)
Na	—	—	—	4 (*↓2%)	—	4, 13 (*↓3%)
Cl	—	—	—	—	26 (*↑2%)	26 (*↑2%)

BUN: Blood urea nitrogen; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; Ca: calcium; Pi: Inorganic phosphorus; Na: Sodium; Cl: Chloride.

* Statistically significant.

Changes were in general reversible. The following changes appeared to be persistent after 4 weeks of recovery period.

- Phosphorus in HD ♂s and ♀s (*↑27% and *↑31%, respectively)
- Na in HD ♂s and ♀s (*↑3% and *↑4%, respectively)
- Cl in HD ♀s (*↑3%)
- Triglycerides in HD ♀s (*↑50%)

Urinalysis:

Parameter	1.5 mg/kg/day		4 mg/kg/day		15/10/8 mg/kg/day	
	Male	Female	Male	Female	Male	Female
Urine volume	-	-	-	-	4 (+75%)	4 (+72%)
Urine specific gravity	-	-	-	-	4 (-27%)	-
Urine pH	-	-	-	-	4, 26 (-3%)	26 (-5%)

- = No drug-related effect.

a Entries in this table indicate the week numbers at which the parameter was affected; the value in parentheses is the maximum difference from control at the indicated week(s).

Table submitted by the sponsor.

Organ weights:

Organ	1.5 mg/kg/day		4 mg/kg/day		15/10/8 mg/kg/day	
	Male	Female	Male	Female	Male	Female
Terminal Sacrifice (Week 27)						
Liver	-	-	+15%	+16%	+17%	+44%
Heart	+7%	+10%	+22%	+20%	+25%	+33%
Adrenal Glands	-	-	+27%	-	+31%	+7%
Thyroid/Parathyroid Glands	-	-	+45%	+37%	+58%	+46%
Ovaries	N/A	-	N/A	+28%	N/A	+48%
Pituitary Gland	-	-	-	-18%	-16%	-22%
Recovery Sacrifice (Week 31)						
Liver	-	-	-	+14%	-	+30%
Heart	-	-	+19%	+19%	+6%	+16%

N/A = Not applicable.

- = No drug-related effect

a Entries in this table indicate the percent difference from control for absolute organ weights.

Table submitted by the sponsor.

Relative (organ: BW) changes in the organ weights at the end of the treatment period

	LD		MD		HD	
	♂	♀	♂	♀	♂	♀
Liver	—	—	*↑14%	*↑8%	*↑21%	*↑40% (*↑21%)
Heart	*↑9%	*↑7% (↑5%)	*↑21% (*↑16%)	*↑12% (↑8%)	*↑29% (*↑13%)	*↑27% (↑10%)
Adrenals	—	—	*↑25% (↑17%)	—	*↑33% (*↑25%)	—
Thyroid/Parathyroid	— (↑19%)	—	*↑43% (↑58%)	*↑27%	*↑63% (↑58%)	*↑40%
Seminal vesicle	—	N/A	↓9%	N/A	↓18%	N/A
Ovaries	N/A	↑14%	N/A	↑20% (↑26%)	N/A	↑42% (↑13%)
Pituitary	—	*↓13%	—	*↓23%	↓12%	*↓25%

Organ weight/BW ratios in each group compared to that in corresponding controls. Numbers in parentheses represent changes on Week 31 (recovery group).

Most findings were reversible. The following relative weights were still remarkable at the end of the 4-week recovery period:

- ↑Thyroid/parathyroid: in MD and HD ♂s.
- ↑Heart: in MD and HD ♂s respectively
- ↑Adrenals: in MD and HD ♂s
- ↑Ovaries: in MD and HD ♀s

Gross pathology:

At terminal sacrifice, drug-related macroscopic findings included:

- Enlarged gastrointestinal tract lumen at HD (4/11 ♂s and 1/18 ♀s)
- Diffusely red or mottled mesenteric lymph nodes at MD and HD
- Light foci/red foci/ raised area in stomach (MD and HD ♂s)

- Large ovaries in MD and HD ♀s
- Fluid-filled uteri in ♀s at all dose levels.

	SEX: -----MALE-----				-----FEMALE-----			
	GROUP: -1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
LN, MESENTERIC (MS)	NUMBER EXAMINED: 20 20 19 11 19 20 20 18							
	NOT REMARKABLE: 20 20 16 7 19 19 12 10							
DIFFUSELY RED	0	0	1	0	0	0	3	2
MOTTLED	0	0	2	4	0	1	5	6
STOMACH, NONGL (SU)	NUMBER EXAMINED: 20 20 19 11 19 20 20 18							
	NOT REMARKABLE: 20 20 19 9 19 20 20 18							
LIGHT FOCUS(1)/AREA(S)	0	0	0	1	0	0	0	0
RAISED AREA(S)	0	0	1	0	0	0	0	0
RED FOCUS(1)/AREA(S)	0	0	0	1	0	0	0	0
OVARY (OV)	NUMBER EXAMINED: 0 0 0 0 19 20 20 18							
	NOT REMARKABLE: 0 0 0 0 19 20 18 16							
LARGE CYST(S)	0	0	0	0	0	0	1	1
	0	0	0	0	0	0	1	1
UTERUS (UT)	NUMBER EXAMINED: 0 0 0 0 19 20 20 18							
	NOT REMARKABLE: 0 0 0 0 14 14 14 9							
LARGE, DIFFUSE	0	0	0	0	2	4	0	4
LUMEN FILLED WITH FLUID	0	0	0	0	4	6	6	7

Recovery sacrifice:

- Fluid-filled uteri
- Erosion/ulceration (1/5 MD ♂s)

	SEX: -----MALE-----				-----FEMALE-----			
	GROUP: -1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
STOMACH, NONGL (SU)	NUMBER EXAMINED: 5 5 5 5 5 5 5 5							
	NOT REMARKABLE: 5 5 4 5 5 5 5 5							
EROSION/ULCERATION	0	0	1	0	0	0	0	0
UTERUS (UT)	NUMBER EXAMINED: 0 0 0 0 5 5 5 5							
	NOT REMARKABLE: 0 0 0 0 5 4 4 4							
LARGE, DIFFUSE	0	0	0	0	0	1	1	1
LUMEN FILLED WITH FLUID	0	0	0	0	0	1	1	1

Histopathology:

Drug-related microscopic observations were seen in the:

- GI tract: duodenum, jejunum, and ileum at LD, MD, and HD, in the cecum at MD and HD
- Mesenteric lymph node at LD, MD, and HD
- Adrenal cortex at LD, MD, and HD
- Thyroid at LD, MD and HD
- Ovary and uterus at MD and HD
- Tongue at HD
- Pancreas at HD
- Spleen at mainly HD
- Heart at HD

	SEX: -----MALE-----				-----FEMALE-----			
	GROUP: -1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
NUMBER:	20	20	19	11	19	20	20	18

THYROID (TY)	NUMBER EXAMINED:	20	20	19	11	19	20	20	18
	NOT REMARKABLE:	19	18	7	1	12	15	0	1
--COLLOID, INCREASED		1	2	12	10	1	5	20	17
HEART (HT)	NUMBER EXAMINED:	20	0	0	11	19	0	0	18
	NOT REMARKABLE:	7	0	0	4	13	0	0	11
--FIBROSIS		0	0	0	2	0	0	0	1
TONGUE (TO)	NUMBER EXAMINED:	20	0	0	11	19	0	0	18
	NOT REMARKABLE:	18	0	0	9	19	0	0	16
--INFILTRATE, LYMPHOHISTIOCYTIC		2	0	0	0	0	0	0	0
--DEGENERATION/NECROSIS, SKELETAL MUSCLE		0	0	0	1	0	0	0	1
--HYPERKERATOSIS		0	0	0	2	0	0	0	0
--HEMORRHAGE		0	0	0	0	0	0	0	1
--CYST, INTRAEPITHELIAL, BLOOD-FILLED		0	0	0	0	0	0	0	1
SPLEEN (SP)	NUMBER EXAMINED:	20	0	1	11	19	0	0	18
	NOT REMARKABLE:	20	0	0	7	16	0	0	12
--DEPLETION, LYMPHOCYTIC, WHITE PULP		0	0	0	0	0	0	0	1
--HEMATOPOIESIS, EXTRAMEDULLARY, INCREASED		0	0	0	3	1	0	0	2
--ADHESIONS, FIBROUS		0	0	1	1	0	0	0	1
--HYPERPLASIA, LYMPHOCYTIC		0	0	0	0	2	0	0	0
--HYPERPLASIA, LYMPHOCYTICULAR, RED PULP		0	0	0	0	0	0	0	1
--INFLAMMATION, LYMPHOHISTIOCYTIC, CAPSULAR/SUBCAPSULAR		0	0	0	0	0	0	0	1
THYMUS (TH)	NUMBER EXAMINED:	20	0	0	11	19	0	0	18
	NOT REMARKABLE:	20	0	0	10	17	0	0	16
--DEPLETION, LYMPHOCYTIC		0	0	0	0	0	0	0	1
--CONGESTION/HEMORRHAGE		0	0	0	1	2	0	0	3
LN, MESENTERIC (MS)	NUMBER EXAMINED:	20	20	14	11	19	20	20	18
	NOT REMARKABLE:	10	4	0	0	15	5	0	0
--HEMORRHAGE		2	5	18	10	0	9	12	17
--HYPERPLASIA, RETICULOENDOTHELIAL		0	14	15	11	3	4	15	17
--DEPLETION, LYMPHOCYTIC		2	0	2	5	0	0	1	0
--MAST CELLS, INCREASED, MEDULLARY		0	9	9	10	0	11	14	11
--INFILTRATE, NEUTROPHILIC, FOCAL		0	0	0	0	1	0	0	0
ADRENAL, CORTEX (AC)	NUMBER EXAMINED:	20	20	19	11	19	20	20	18
	NOT REMARKABLE:	4	0	0	0	3	3	0	0
--VACUOLATION, ZONA GLOMERULOSEA		13	20	19	11	15	15	19	18
--HYPERTROPHY/HYPERPLASIA, FOCAL		4	2	2	1	2	0	3	3
PANCREAS (PA)	NUMBER EXAMINED:	20	0	0	11	19	0	0	18
	NOT REMARKABLE:	16	0	0	10	16	0	0	15
--ATROPHY, ACINAR		1	0	0	1	0	0	0	3
STOMACH, GL (ST)	NUMBER EXAMINED:	20	0	1	11	19	0	0	18
	NOT REMARKABLE:	20	0	1	10	19	0	0	17
--EDEMA, SUBMUCOSAL		0	0	0	1	0	0	0	0
--INFILTRATE, LYMPHOHISTIOCYTIC		0	0	0	0	0	0	0	1
STOMACH, NONGL (SU)	NUMBER EXAMINED:	20	0	1	11	19	0	0	18
	NOT REMARKABLE:	19	0	0	9	19	0	0	18
--ULCERATION		0	0	0	1	0	0	0	0
--HYPERPLASIA, SQUAMOUS, NONGLANDULAR		0	0	1	2	0	0	0	0
--INFLAMMATION, CHRONIC-ACTIVE		1	0	1	2	0	0	0	0
--HYPERKERATOSIS, NONGLANDULAR MUCOSA		0	0	1	1	0	0	0	0
DUODENUM (DU)	NUMBER EXAMINED:	20	20	19	11	19	20	20	18
	NOT REMARKABLE:	20	15	10	0	19	14	12	1
--VILLUS ALTERATION (CHARACTERIZED BY VILLAR BLUNTING, VILLAR FUSION, VILLAR BRANCHING, EPITHELIAL HYPERPLASIA, AND/OR APICAL MICROPAPILLARY PROJECTIONS)		0	5	9	11	0	6	0	17
--INFLAMMATION, ACUTE		0	0	0	0	0	0	1	0
JEJUNUM (JE)	NUMBER EXAMINED:	20	20	19	11	19	20	20	18
	NOT REMARKABLE:	20	16	14	3	19	19	14	9
--VILLUS ALTERATION (CHARACTERIZED BY VILLAR BLUNTING, VILLAR FUSION, VILLAR BRANCHING, EPITHELIAL HYPERPLASIA, AND/OR APICAL MICROPAPILLARY PROJECTIONS)		0	4	5	9	0	1	6	9

ILEUM (IL)	NUMBER EXAMINED:	20	20	18	11	19	19	20	18
	NOT REMARKABLE:	19	16	9	2	19	16	15	4
--VILLUS ALTERATION (CHARACTERIZED BY VILLUS BLUNTING, VILLUS FUSION, VILLUS BRANCHING, EPITHELIAL HYPERPLASIA, AND/OR APICAL MICROVILLARY PROJECTIONS)		0	4	9	9	0	3	4	14
--INFLAMMATION, ACUTE		0	0	0	0	0	0	1	0
--INFLAMMATION, GRANULOMATOUS		1	0	0	0	0	0	0	0
CECUM (CE)	NUMBER EXAMINED:	20	20	19	11	19	20	19	18
	NOT REMARKABLE:	20	19	14	0	19	19	15	0
--FIBROSIS (CHARACTERIZED BY FOCAL TO MULTIFOCAL SMALL AGGREGATES OF DENSE HYALINIZED COLLAGEN WITHIN THE LAMINA PROPRIA)		0	0	4	11	0	0	1	16
--CRYPT ECTASIA/ABSCESS		0	1	1	10	0	1	3	18
--INFLAMMATION, ACUTE		0	0	0	0	0	0	0	1
--EDEMA, SUBMUCOSAL		0	0	1	3	0	0	0	0
OVARY (OV)	NUMBER EXAMINED:	0	0	0	0	19	20	20	18
	NOT REMARKABLE:	0	0	0	0	0	0	0	0
--CORPUS LUTEUM (=0, 1=1-5, 2=6-10, 3=11-15, 4=16-20, 5=20)		0	0	0	0	13	14	20	16
--CYST		0	0	0	0	0	1	2	3
--ACYCLIC		0	0	0	0	6	6	0	2
UTERUS (UT)	NUMBER EXAMINED:	0	0	0	0	19	20	20	18
	NOT REMARKABLE:	0	0	0	0	4	10	11	8
--DILATATION		0	0	0	0	10	9	9	10
--METAPLASIA, SQUAMOUS, ENDOMETRIAL GLANDS		0	0	0	0	7	4	2	1
--CYST, ENDOMETRIAL		0	0	0	0	0	0	1	0

Recovery:

It should be noted that many of the organs and tissues were not examined at the recovery period. Those included: bone, bone marrow, eye, nerve (optic, sciatic), skeletal muscle, spinal cord, brain, liver, trachea, esophagus, parathyroid, heart, tongue, spleen, thymus, aorta, pituitary, pancreas, colon, rectum, harderian gland, salivary gland, mammary gland, urinary bladder, cervix, vagina, prostate, seminal vesicles, testis, epididymis.

	SEX: -----MALE-----				-----FEMALE-----			
	GROUP: -1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
	NUMBER:	5	5	5	5	5	5	5
KIDNEY (KD)	NUMBER EXAMINED:	0	0	0	1	0	0	0
	NOT REMARKABLE:	0	0	0	0	0	0	0
--INFILTRATE, LYMPHOHISTIOCYTIC		0	0	0	1	0	0	0
--REGENERATION, TUBULAR EPITHELIUM		0	0	0	1	0	0	0
--ECTASIA/PROTEINOSTS, TUBULAR		0	0	0	1	0	0	0
--THICKENING, BASEMENT MEMBRANE		0	0	0	1	0	0	0
--HYALINE DROPLETS, TUBULAR EPITHELIUM		0	0	0	1	0	0	0
--FIBROSIS		0	0	0	1	0	0	0
--CYST		0	0	0	1	0	0	0
LUNG (LU)	NUMBER EXAMINED:	5	5	5	5	5	5	5
	NOT REMARKABLE:	3	2	1	3	3	4	1
--HYPERPLASIA, MEDIAL, ARTERIOLAR		0	0	1	0	0	0	0
--INFILTRATE, MACROPHAGE, ALVEOLAR		1	3	2	2	2	1	3
--HEMORRHAGE		1	0	1	0	0	0	1
THYROID (TY)	NUMBER EXAMINED:	5	5	5	5	5	5	5
	NOT REMARKABLE:	5	4	2	3	3	3	1
--COLLOID, INCREASED		0	1	3	2	2	2	4
--INFILTRATE, LYMPHOHISTIOCYTIC		0	0	0	1	0	0	0
--INFILTRATE, FOAMY MACROPHAGES, INTRAFOLLICULAR		0	0	0	0	1	0	1

LN, MESENTERIC (MS)	NUMBER EXAMINED:	5	5	5	5	5	5	5
	NOT REMARKABLE:	3	1	0	2	3	1	3
--HEMORRHAGE		0	0	0	1	1	1	0
--HYPERPLASIA, RETICULOENDOTHELIAL		2	4	2	3	1	1	0
--MST CELLS, INCREASED, MEDULLARY		0	2	4	2	0	2	2
ADRENAL CORTEX (AC)	NUMBER EXAMINED:	5	5	5	5	5	5	5
	NOT REMARKABLE:	2	1	0	0	3	0	0
--VACUOLATION, ZONA GLOMERULOSA		3	3	5	5	2	5	5
--HYPERTEPHY/HYPERPLASIA, FOCAL		0	2	0	0	1	0	1
STOMACH, GL (ST)	NUMBER EXAMINED:	0	0	1	0	0	0	0
	NOT REMARKABLE:	0	2	0	0	0	0	0
--EROSION		0	0	1	0	0	0	0
--INFLAMMATION, ACUTE		0	0	1	0	0	0	0
STOMACH, NONGL (SG)	NUMBER EXAMINED:	0	2	1	0	0	0	0
	NOT REMARKABLE:	0	0	0	0	0	0	0
--HYPERKERATOSIS, NONGLANDULAR MUCOSA		0	0	1	0	0	0	0
--CYST, INTRAEPITHELIAL		0	0	1	0	0	0	0
DUODENUM (DU)	NUMBER EXAMINED:	5	5	5	5	5	5	5
	NOT REMARKABLE:	5	5	4	4	5	5	5
--VILLUS ALTERATION (CHARACTERIZED BY VILLAR BLUNTING, VILLAR FUSION, VILLAR BRANCHING, EPITHELIAL HYPERPLASIA, AND/OR APICAL MICRO-PAPILLARY PROJECTIONS)		0	0	1	1	0	0	0
CECUM (CE)	NUMBER EXAMINED:	5	5	5	5	5	5	5
	NOT REMARKABLE:	5	5	5	1	5	4	1
--FIBROSIS (CHARACTERIZED BY FOCAL TO MULTIFOCAL SMALL AGGREGATES OF DENSE HYALINIZED COLLAGEN WITHIN THE LAMINA PROPRIA)		0	0	0	4	0	0	3
--CRYPT ECYASIA/ABSCESS		0	0	0	1	0	0	3
OVARY (OV)	NUMBER EXAMINED:	0	0	0	0	5	5	5
	NOT REMARKABLE:	0	0	0	0	0	0	0
--CORPUS LUTEUM (=0, 1=1-5, 2=6-10, 3=11-15, 4=16-20, 5>20)		0	0	0	0	1	2	4
--ACYCLIC		0	0	0	0	4	2	1
UTERUS (UT)	NUMBER EXAMINED:	0	0	0	0	5	5	5
	NOT REMARKABLE:	0	0	0	0	0	3	4
--DILATATION		0	0	0	0	4	1	2
--METAPLASIA, SQUAMOUS, ENDOMETRIAL GLANDS		0	0	0	0	2	1	0

Toxicokinetics:

Dose (mg/kg/day)	Study Day (Week)	C _{max} (ng/mL)		T _{max} (hr)		AUC _T ^a (ng/mL·hr)	
		Male	Female	Male	Female	Male	Female
1.5	Day 1	11.9	11.2	4.0	4.0	72.6	62.9
1.5	Week 13	8.3	12.0	4.0	2.0	48.2	114.2
1.5	Week 26	15.8	24.6	2.0	2.0	87.7	104.1
4	Day 1	39.8	34.7	4.0	4.0	250.9	244.3
4	Week 13	46.7	29.7	2.0	4.0	241.0	194.1
4	Week 26	29.8	52.9	2.0	2.0	322.7	416.3
15	Day 1	116.1	92.3	8.0	4.0	1335.2	1077.0
10	Week 13	49.0	82.1	8.0	2.0	564.2	972.6
8	Week 26	120.3	109.6	2.0	2.0	551.4	777.3
Ratios							
1:2.7:10	Day 1	1:3.3:9.8	1:3.1:8.2	-	-	1:3.5:18.4	1:3.9:17.1
1:2.7:6.7	Week 13	1:5.6:5.9	1:2.5:6.8	-	-	1:5.0:11.7	1:1.7:8.5
1:2.7:5.3	Week 26	1:1.9:7.6	1:2.2:4.5	-	-	1:3.7:6.3	1:4.0:7.5

a Calculated from time zero to the time of last measurable concentration, ranging between 8 to 24 hours.

Table submitted by the sponsor.

- Because HD animals were given different doses of dasatinib, a conclusive comparison of LD and MD to HD cannot be made.
- Systemic exposure to BMS-354825, following daily administration of 1.5 mg/kg/day (LD) and 4-mg/kg/day (MD) for 26 weeks was approximately dose proportional.
- At the high-dose, systemic exposure to BMS-354825 was slightly greater than dose proportional.
- There was no clear evidence of drug accumulation over the course of the study. There were instances of \uparrow and \downarrow in exposure (C_{max} and AUC) after repeated dosing at LD and MD. The AUCs were slightly higher after repeated dosing in LD and MD $\text{\textcircled{M}}$ s and $\text{\textcircled{F}}$ s (Week 13 or 26 compared to Day 1).
- There were no clear gender-dependent effects on exposure.
- T_{max} was mostly 2-4 hrs, with the exception of the HD $\text{\textcircled{M}}$ s, which had T_{max} of 8 hrs on Day 1 and Week 13.
- $T_{1/2}$ was not reported.

Summary of the study:

Doses of 1.5, 4, and 15 mg/kg (9, 24, and 90 mg/m², respectively) selected for this study were based on the results of a 1-month oral toxicity study in rats with BMS-354825 administered on a 5-day-on, 2-day-off repeating schedule and a 2-week exploratory study in rats with BMS-354825. In the present study, a daily dose of 15 mg/kg induced severe toxicity (mortality, swollen abdomen, and body weight loss) during the first 7 weeks of the study; as a result, the dose was lowered to 10 mg/kg on Week 8. After 9 weeks of daily dosing at 10 mg/kg, severe toxicity continued, and the dose was lowered to 8 mg/kg on Week 17 for the remainder of the study.

Unscheduled sacrifices occurred at HD, mainly in $\text{\textcircled{M}}$ s and included 9 HD $\text{\textcircled{M}}$ s, 2 HD $\text{\textcircled{F}}$ s from the main groups and 7 HD TK $\text{\textcircled{M}}$ s, during the treatment period, starting as early as Day 23. Mortality was higher in $\text{\textcircled{M}}$ s, although there were no clear gender-dependent effects on exposures. Clinical signs prior to death included: swollen abdomen, hunched posture, thin appearance, irregular respiration, fecal abnormalities (few, liquid, or non formed feces), red haircoat, and rough haircoat. Most of these animals lost substantial body weight in shortly before death. Gross pathology of unscheduled sacrifices revealed findings mainly in the GI tract (e.g. large lumen, red/thickened wall) and in the lymph node (red). Clinical pathology tests were performed for 3 HD $\text{\textcircled{M}}$ s and 2 HD $\text{\textcircled{F}}$ s sacrificed because of poor health. The most prominent findings for these animals were consistent with dehydration (e.g., \uparrow BUN), inflammation (e.g., \uparrow absolute neutrophil and monocyte counts), \downarrow absolute lymphocyte count, and protein loss (e.g., low total protein due to low albumin and globulin). One additional finding in HD males that died during the course of the study was minimal segmental medial arteriolar hyperplasia in the lungs.

Clinical signs were observed at HD, was more evident in $\text{\textcircled{M}}$ s, and consisted of swollen abdomen, red haircoat and fecal abnormalities

Overall, ↓BW gain in HD ♂s and ↑BW gain in HD ♀s was observed during the period (Weeks 1-27). Animals that died lost weight shortly prior to death.

Hematology parameters revealed changes in the RBC and lineages (e.g. ↓RBC, hemoglobin, and hematocrit), ↑neutrophils and monocytes, ↑platelets and fibrinogen, all of which appear to be secondary to internal injury, such as GI ulceration, and blood loss. ↓lymphocytes was also noted and correlated with microscopic pathology findings of lymphocytic depletion. Most changes in the hematology parameters were statistically significant and were seen at MD and HD and appeared to be comparable between ♂s and ♀s. The following hematology findings were persistent to the end of the recovery period:

- Abs lymphocytes in HD ♀s (*↓25%)
- Abs monocytes in HD ♀s (↑40%)
- Fibrinogen in MD and HD ♀s (*↑35% and *↑47%, respectively)

Statistically significant ↑ in triglycerides of more than 3-fold was seen in HD ♂s and ♀s, during the treatment period. This finding together with a slight ↑ in cholesterol and AST (not toxicologically significant) might be suggestive of the potential for hepatotoxicity. ↓Total protein, ↓ALP, ↓albumin observed in clinical pathology appears to be secondary to ↓BW/malnutrition. Other changes in the serum chemistry parameters included, ↓Ca, ↓phosphorus, ↓Na, and ↑chloride. Since multiple factors are responsible for these findings (e.g. renal problem, parathyroid dysfunction, GI toxicity including diarrhea), the exact mechanism of this imbalance is unknown at this time. The following changes appeared to be persistent after 4 weeks of recovery period.

- Phosphorus in HD ♂s and ♀s (*↑27% and *↑31%, respectively)
- Na in HD ♂s and ♀s (*↑3% and *↑4%, respectively)
- Cl in HD ♀s (*↑3%)
- Triglycerides in HD ♀s (*↑50%)

An ↑ in urine volume (~75%) was accompanied by ↓urine specific gravity (27% in ♂s).

Hypertrophy of the liver (♂s and ♀s), heart (♂ and ♀s), adrenals (♂s), thyroid/parathyroid (♂s and ♀s), and ovaries were most evident at MD and HD as shown by statistically significant ↑ in the relative weights of these organs. Although most findings were reversible, the following relative weights were still remarkable at the end of the 4-week recovery period:

- ↑Thyroid/parathyroid: in MD and HD ♂s.
- ↑Heart: in MD and HD ♂s
- ↑Adrenals: in MD and HD ♂s
- ↑Ovaries: in MD and HD ♀s

Gross pathology findings were noted in the GI lumen (HD), lymph nodes (MD and HD), stomach (MD and HD), ovaries (MD and HD) and uterus (LD, MD, and HD). These findings correlated with histopathology findings. Microscopic examination revealed toxicities in the following organs/tissues:

- Thyroid: increased colloid (LD, MD, and HD ♂s and ♀s) (correlating with increased thyroid/parathyroid weights)
- Heart: fibrosis (HD ♂s and ♀s)
- Tongue: degeneration/necrosis, hyperkeratosis, hemorrhage, cyst (HD ♂s and /or ♀s)
- Spleen: lymphocytic depletion, hematopoiesis (HD ♂s and/or ♀s); fibrous adhesion (MD and HD ♂s)
- Thymus: lymphocytic depletion, congestion/hemorrhage (HD ♂s and/or ♀s)
- Mesenteric lymph node: hemorrhage, reticuloendothelial hyperplasia, lymphocytic depletion, ↑medullary mast cells (LD, MD, HD ♂s and ♀s)
- Adrenal cortex: vacuolation of zona glomerulosa, hypertrophy/hyperplasia (LD, MD, and HD ♂s and ♀s)- (correlating with increased adrenal weights)
- Pancreas: acinar atrophy (HD ♂s and ♀s)
- GI tract: stomach, duodenum, jejunum, ileum, cecum: squamous nonglandular hyperplasia, inflammation, hyperkeratosis, villus alterations (e.g. blunting, fusion, branching, epithelial hyperplasia, micropapillary projections), fibrosis, crypt abscess, edema (LD, MD, and HD ♂s and ♀s)
- Ovary: ↑corpus lutea and cyst (LD, MD, and HD), ↓ incidence of acyclic ovaries (MD and HD)
- Uterus (seen with high incidence in control): ↓squamous metaplasia of endometrial glands (LD, MD, and HD)

Drug-related microscopic findings were generally dose-related in incidence and/or severity, and were at least partially reversible following the 4-week recovery period.

TK:

- Because HD animals were given different doses of dasatinib, a conclusive comparison of LD and MD to HD cannot be made.
- Systemic exposure to BMS-354825, following daily administration of 1.5 mg/kg/day (LD) and 4-mg/kg/day (MD) for 26 weeks was approximately dose proportional.
- At the high-dose, systemic exposure to BMS-354825 was greater than dose proportional.
- There was no clear evidence of drug accumulation over the course of the study. The AUCs were slightly higher after repeated dosing in LD and MD ♂s and ♀s (Week 13 or 26 compared to Day 1)
- There were no clear gender-dependent effects on exposure.
- T_{max} was mostly 2-4 hrs
- $T_{1/2}$ was not reported.

In conclusion, dasatinib at daily doses of 1.5 and 4 mg/kg/day (9 and 24 mg/m²/day) with the mean AUC < 416 ng.hr/mL, had acceptable toxicity profiles when dosed for 26 weeks. Dasatinib at a daily dose of 15 mg/kg (90 mg/m²/day, mean AUC= 1077-1335 ng.hr/mL) or 10 mg/kg (60 mg/m²/day, mean AUC= 564-972 ng.hr/mL) was not tolerated when dosed for 7 weeks, resulting in mortality. The intestinal tract appeared to

be the primary target of BMS-354825, as reflected by the clinical observations, clinical-pathology changes, and gross and microscopic changes. Overall the effects of dasatinib were detected in: the GI tract, liver, heart, adrenal glands, hematopoietic system, female reproductive system, pancreas, thyroid/parathyroid, and tongue. It should be noted that because of the electrolyte imbalance there is an increased potential of drug-induced cardiac toxicity.

Because of histopathology findings at LD, a NOAEL could not be identified.

Toxicities observed during this study are summarized below:

- GI tract: swollen abdomen; liquid feces; enlarged lumen; red foci /ulceration/ edema/ squamous hyperplasia/ hyperkeratosis in stomach; villus alteration (e.g. blunting, fusion, branching) in duodenum, jejunum, and ileum; fibrosis of cecum (characterized by aggregates of hyalinized collagen); crypt ectasia/abscess; edema of cecum; inflammation
- Hematopoietic/ lymphocytic system: ↓lymphocytes; red/mottled mesenteric lymph node; lymphoid depletion in spleen, thymus, and mesenteric lymph node; congestion and/or hemorrhage in thymus and mesenteric lymph node; reticuloendothelial hyperplasia and ↑medullary mast cells in mesenteric lymph node; fibrous adhesion in spleen
- Liver: hypertrophy; ↑cholesterol; ↑triglycerides; (↓albumin)
- Electrolytes: ↓Ca, ↓phosphorus, ↓Na, ↑Cl
- Heart: fibrosis, hypertrophy
- Adrenal glands: vacuolation of zona glomerulosa, hypertrophy/hyperplasia
- Female reproductive system: hypertrophy; fluid filled uterus; ↑corpus lutea and cyst in ovaries, ↓ incidence of acyclic ovaries; ↓squamous metaplasia of endometrial glands
- Male reproductive system: ↓size of seminal vesicles
- Pancreas: acinar atrophy
- Thyroid/parathyroid: hypertrophy; increased colloid
- Kidney: tubular epithelia regeneration; tubular ectasia/ proteinosis; tubular epithelial hyaline droplets; fibrosis; cyst (note: only one HD ♂ was microscopically evaluated for findings in kidney; no corresponding control is available); ↑urinary volume.
- Pituitary: ↓weight
- Tongue: degeneration/necrosis, hyperkeratosis, hemorrhage, cyst
- Other: ↓RBC, ↑neutrophils, ↑monocytes; ↑platelets; and ↑fibrinogen appear to be secondary to internal injury/blood loss and inflammation.

Overall summary of the repeat-dose toxicology studies in rats:

Overall, toxicities were more apparent in ♂s than ♀s. The most severe toxicities consisted of those seen in the GI tract (diarrhea, inflammation/ ulceration, villi abnormalities, etc) and the lymphocytic system (depletion). In summary, the effects of dasatinib were detected in the: GI tract, liver, heart, adrenal glands, hematopoietic system, reproductive systems (mainly female), pancreas, thyroid/parathyroid, and

tongue. Toxicities in the thyroid and female reproductive system manifested in the chronic (6-month) repeat-dose toxicology only.

Findings such as changes in the RBC (↓) and lineages, ↑reticulocytes, ↑neutrophils, ↑platelets and fibrinogen, observed in both short term and chronic studies appeared to be secondary to internal injury/bleeding and inflammation.

2-week exploratory study (based on the summary data submitted):

Doses administered were: 1, 15, and 30 mg/kg/day (6, 9, and 180 mg/m²/day) x 2 weeks.

Dose (m/kg)	Study Day	C _{MAX} (nM)		AUC (0-24hr)	
		M	F	M	F
1	1	55	37	143	104
15	1	339	575	2970	3210
30	1	536	1384	5953	10474
1	14	15	14	72	86
15	14	137	175	1842	1900
30	14	-	-	-	-

Dose Ratio	Study Day	C _{MAX} Ratio		AUC Ratio	
		M	F	M	F
1:15:30	1	1:6.2:9.7	1:15.5:37.4	1:20.8:41.6	1:30.9:100.7
1:15	14	1:9.1	1:12.5	1:25.6	1:22.1

No data available at 30 mg/kg on day 14 due to mortality.

Severe toxicity and lethality occurred in all animals at 30 mg/kg (180 mg/m²). No significant effects occurred at 1 mg/kg (6 mg/m²). The following toxicities were observed:

- Hematopoietic/Lymphocytic system (more severe in ♂s): hematopoietic cell depletion in bone marrow; lymphoid depletion in lymph nodes, thymus, and spleen, red discoloration of mesenteric lymph node, ↓weight of thymus and spleen
- GI tract: bloated/swollen abdomen, diarrhea, distention of the GI tract with gas, fluid, and/or ingesta or digesta, edema of the large intestine, red discoloration of the small intestine
- Liver: hypertrophy; ↑triglycerides, (↓albumin)
- Adrenals: ↑weight
- Other: ↑or↓ platelets; chromorrhinorrhea; ↓RBC, ↓hemoglobin, and ↑reticulocytes appear to be secondary to internal injury/bleeding; ↓ALP, ↓globulin, ↓albumin, and ↓total protein might be secondary to BW loss/malnutrition

1-month repeat-dose study:

Doses administered: 1, 15, and 25 mg/kg (6, 90, and 150 mg/m²) 5days-on 2-days-off x 4 cycles, followed by a 2-week recovery period.

BMS-354825							
Dose [mg/kg/day]	Study Day	C _{max} (ng/mL)		T _{max} (h)		AUCT ^a (ng.h/mL)	
		Male	Female	Male	Female	Male	Female
0.9	1	7.9	9.8	4	4	34	45
	26	6.6	13.4	4	2	32	41
15	1	96.1	88.4	8	8	937	920
	26	57.7	51.8	8	2	827	705
25	1	102.1	184.2	8	4	1315	1737
	26	49	63.8	4	4	951	944
Ratios							
0.9:15:25 Dose Ratio	1	1:12.2:12.9	1:9.0:18.8			1:27.6:38.7	1:20.4:38.6
	26	1:8.7:7.4	1:3.9:4.8			1:25.8:29.7	1:17.2:23

^a Calculated from time zero to the time of last measurable concentration, equal to 24 h, ranging between 8 to 24 h.

17 unscheduled deaths occurred in the study, (10 HD ♂, 5HD ♀, 2LD ♀); 13 were attributed to enteropathy, 2 to gavage errors and 2 were not determined. Higher mortality was seen in ♂s than ♀s. Mortality was generally due to enteropathy and lymphoid depletion. Test article-related effects were seen in the following organs/tissues/ parameters:

In summary, the following toxicities were observed:

- GI tract: liquid or non-formed feces; distention and fluid/gas filled lumens, darkened serosa and mucosa; perforation/hemorrhage; congestion/inflammation;
- Hematopoietic/ Lymphocytic system: ↓lymphocytes; lymphoid depletion of mesenteric lymph node, spleen, and thymus; congestion of mesenteric lymph node and thymus; hemorrhage in thymus; ↓weight of spleen
- Liver: hypertrophy; ↑cholesterol; slightly ↑AST and ALT; (↓albumin); inflammation (in an unscheduled sacrifice at LD)
- Heart: hypertrophy
- Adrenals: hypertrophy (♂s only)
- Reproductive system: small seminal vesicles; reduced secretion of seminal vesicles; immature sperms in epididymis; dilatation of uterus.
- Other: labored breathing; necrosis in salivary gland; minimally ↑kidney weight at the end of recovery period; minimal inflammation in kidney in one unscheduled death (LD ♀); ↓ALP and ↓albumin might be secondary to ↓BW/malnutrition

6-month toxicology study:

Doses administered: (1.5, 4, and 15/10/8 mg/kg/day) 9, 24, and 90/60/48 mg/m²/day x 6 months (26 weeks).

Dose (mg/kg/day)	Study Day (Week)	C _{max} (ng/mL)		T _{max} (hr)		AUC _T ^a (ng/mL·hr)	
		Male	Female	Male	Female	Male	Female
1.5	Day 1	11.9	11.2	4.0	4.0	72.6	62.9
1.5	Week 13	8.3	12.0	4.0	2.0	48.2	114.2
1.5	Week 26	15.8	24.6	2.0	2.0	87.7	104.1
4	Day 1	39.8	34.7	4.0	4.0	250.9	244.3
4	Week 13	46.7	29.7	2.0	4.0	241.0	194.1
4	Week 26	29.8	52.9	2.0	2.0	322.7	416.3
15	Day 1	116.1	92.3	8.0	4.0	1335.2	1077.0
10	Week 13	49.0	82.1	8.0	2.0	564.2	972.6
8	Week 26	120.3	109.6	2.0	2.0	551.4	777.3
Ratios							
1:2.7:10	Day 1	1:3.3:9.8	1:3.1:8.2	-	-	1:3.5:18.4	1:3.9:17.1
1:2.7:6.7	Week 13	1:5.6:5.9	1:2.5:6.8	-	-	1:5.0:11.7	1:1.7:8.5
1:2.7:5.3	Week 26	1:1.9:7.6	1:2.2:4.5	-	-	1:3.7:6.3	1:4.0:7.5

a Calculated from time zero to the time of last measurable concentration, ranging between 8 to 24 hours.

Higher mortality was observed in ♂s than ♀s (appeared to be mainly due to enteropathy and lymphoid depletion). Clinical signs of toxicity were more evident in ♂s. Test article-related effects were seen in the following organs/tissues/parameters:

- GI tract: swollen abdomen; liquid feces; enlarged lumen; red foci /ulceration/ edema/ squamous hyperplasia/ hyperkeratosis in stomach; villus alteration (e.g. blunting, fusion, branching) in duodenum, jejunum, and ileum; fibrosis of cecum (characterized by aggregates of hyalinized collagen); crypt ectasia/abscess/ edema of cecum; inflammation
- Hematopoietic/ lymphocytic system: ↓lymphocytes; red/mottled mesenteric lymph node; lymphoid depletion in spleen, thymus, and mesenteric lymph node; congestion and/or hemorrhage in thymus and mesenteric lymph node; reticuloendothelial hyperplasia and ↑medullary mast cells in mesenteric lymph node; fibrous adhesion in spleen
- Liver: hypertrophy, ↑triglycerides, ↑cholesterol, (↓albumin)
- Electrolytes: ↓Ca, ↓phosphorus, ↓Na, ↑Cl
- Heart: hypertrophy, fibrosis
- Adrenals: hypertrophy/ hyperplasia, vacuolation of zona glomerulosa
- Female reproductive system: hypertrophy of ovaries; ↑corpus lutea and cyst in ovaries, ↓ incidence of acyclic ovaries; ↓squamous metaplasia of endometrial glands; fluid filled uterus
- Male reproductive system: ↓size of seminal of vesicles
- Thyroid/ parathyroid: hypertrophy, ↑colloid of thyroid
- Pancreas: acinar atrophy
- Kidney: ↑urinary volume; tubular epithelia regeneration; tubular ectasia/ proteinosis; tubular epithelial hyaline droplets; fibrosis; cyst (note: only one HD ♂ was microscopically evaluated for findings in kidney; no corresponding control is available); electrolyte imbalance may be partially attributed to renal injury.
- Lung: minimal segmental medial arteriolar hyperplasia

- Pituitary: ↓weight
- Tongue: degeneration/necrosis, hyperkeratosis, hemorrhage, cyst
- Other: ↓protein, ↓albumin, and ↓ALP may be secondary to BW loss/malnutrition; ↓RBC, ↑neutrophils, ↑monocytes; ↑platelets; and ↑fibrinogen appear to be secondary to internal injury/blood loss and inflammation.

Study DS02062 -- Ten-Day Oral Exploratory Toxicity Study in Monkeys (930003269)

Note: This study has not been reviewed.

In this exploratory non-GLP study BMS-354825 was administered in 50 mM sodium acetate buffer, pH 4.2 - 4.6, orally by gavage to groups of one monkey per sex at daily doses 1, 10, or 15 mg/kg (12, 120, or 180 mg/m²) for two cycles (5-days-on 2-days-off for a total of 10 doses). Two additional monkeys received 25 mg/kg for 2 to 3 days or 62.5 mg/kg as a single dose. A vehicle-control group received 50 mM sodium acetate buffer vehicle (pH 4.2-4.6), orally at 5 mL/kg. Endpoints included toxicokinetics, survival, clinical signs, physical examinations (including ophthalmologic, neurologic, and electrocardiographic assessments, arterial oxygen saturation and blood pressure), body weight, food consumption, clinical pathology (Days 5 and 11/12), gross pathology, and histopathology. Scheduled necropsies were conducted on Day 13.

Dose-related increases in systemic exposure to BMS-354825 were observed on Days 1 and 12 and were greater than dose-proportional. On Day 12, exposures at 1, 10, and 15 mg/kg were decreased in both males and females compared to Day 1. Mortality at 25 and 62.5 mg/kg precluded assessment at these doses on Day 12. At 1, 10, and 15 mg/kg, and in the female at 25 mg/kg, less than 1% of the total dose of BMS354825 was excreted in the urine on Day 1. In the males at 25 mg/kg, and in the males and females at 62.5 mg/kg, the percentage of the BMS-354825 dose excreted in the urine on Day 1 was greater than 1%, and in the female at 62.5 mg/kg, 22% of the total dose was excreted in the urine. At 15 mg/kg, the percentage of the BMS-354825 dose excreted in the urine increased 107- and 220-fold in the male and female, respectively, from Day 1 to Day 12. At 1 and 10 mg/kg, the percentage of the total dose excreted in the urine remained less than 1% on Day 12.

Dose (mg/kg)	Study Day	C _{MAX} (µM)		AUC (0-24 hr)		% Dose in Urine as BMS-354825	
		M	F	M	F	M	F
1	1	0.03	0.03	0.17	0.06	0.08	0.10
10	1	0.88	0.73	2.71	1.69	0.05	0.03
15	1	1.16	0.74	3.39	2.68	0.03	0.02
25 ^a	1	1.88	2.26	14.53	9.67	1.31	0.17
62.5 ^a	1	4.68	5.83	17.35	21.77	1.40	22.1
1	12	<LLQ	<LLQ	<LLQ	<LLQ	0.89	<LLQ
10	12	0.34	0.30	1.55	1.00	0.03	0.06
15	12	0.14	1.04	1.30	1.97	3.20	4.40

Dose Ratio	Study Day	C _{MAX} Ratio		AUC Ratio	
		M	F	M	F
1:10:15:25:62.5	1	1:29.3:38.7:62.7:156	1:24.3:24.7:75.3:194.3	1:15.9:19.9:85.5:102.1	1:28.2:44.7:161.2:362.8

^a Animals did not survive to day 12.

Note: For doses of 1 and 15 mg/kg, LLQ was 0.001 µM and for doses of 10, 25, and 62.5 mg/kg, LLQ was 0.008 µM.

Table provided by the sponsor.

Animals administered vehicle or BMS-354825 at 1, 10, or 15 mg/kg survived to the scheduled necropsy on Day 13. All groups including the vehicle control had soft and liquid feces. Because of drug-related morbidity, animals that received 25 mg/kg were euthanized and necropsied on Day 3 (male), and Day 4 (female); the females that received a single dose of 62.5 mg/kg was euthanized and necropsied on Day 2. Dosing was discontinued after a single dose due to GI toxicity in the male that received 62.5 mg/kg, and this animal was returned to stock after recovery on Day 4. Clinical observations at 25 and 62.5 mg/kg included decreased activity, mucous feces, and pale mucous membranes. Hunched posture and vomitus were observed in the female at 25 mg/kg. Additional observations at 62.5 mg/kg included abdominal bloating and, in the female, surface hypothermia, pale and thin appearance, and bloody feces. In females, mucous feces, and vomitus occurred at 15 mg/kg, and vomitus and bloody feces occurred at 10 mg/kg. In the male at 1 mg/kg, bloody feces occurred on Day 4. Drug-related decreases in food consumption occurred in the female at 15 mg/kg and in both sexes at 25 and 62.5 mg/kg.

Drug-related clinical and anatomic pathology findings occurred at doses > 10 mg/kg and consisted of changes in the leukocyte count, total protein and albumin, GI tract, and lymphoid organs and kidney. At 10 mg/kg drug-related changes consisted of increases in total leukocyte and segmented neutrophil counts on Day 11. At 15 and 25 mg/kg, minimal enteropathy was observed in the small intestine. Enteropathy was characterized by accumulation of small clusters (generally less 10 cells) of disorganized enterocytes along the villous apex, along with enterocyte vacuolation and villous fusion. An additional drug-related change at 15, 25, and 62.5 mg/kg was generally dose-related

lymphoid depletion in the spleen. At 15 mg/kg, drug-related changes included (1) decreases in total protein and albumin on Days 5 and 11/12; (2) decreases in spleen weights; (3) in the female, increases in monocyte counts on Day 5 and increases in leukocyte and segmented neutrophil counts on Days 5 and 12; (4) lymphoid depletion in the thymus; (5) single-cell necrosis of luminal epithelial cells in the stomach and large intestine. An additional finding in the females at 25 and 62.5 mg/kg was single-cell necrosis in the thymus. At 25 mg/kg, drug-related observations consisted of focal red discoloration in the stomach and, in the male, diffuse red discoloration in the duodenum; in the female, lymphoid depletion in large intestinal lymphoid nodules and mesenteric lymph node, and edema in the ileum. In the female at 62.5 mg/kg, findings consisted of (1) diffuse red discoloration in the small intestine; (2) red contents in the stomach, small intestine, and large intestine; (3) edema, hemorrhage, and ulceration in the small intestine and neutrophilic infiltration in the colon; (4) dilatation of cortical tubules and degeneration of cortical tubular epithelial cells in the kidney.

The majority of changes in serum chemistry and hematologic parameters were secondary to GI toxicity (i.e., emesis, soft and liquid feces, and small intestinal enteropathy, resulting in fluid and protein losses). Increases in total leukocyte, segmented neutrophil, and monocyte counts were attributed to tissue damage associated with GI inflammation.

There were no drug-related alterations in electrocardiograms, nervous system function, respiratory and heart rate or sounds, blood pressure, arterial oxygen saturation or ophthalmologic examinations.

According to the summary of the study, toxicities appeared to be in the following:

- GI tract: vomiting, red/liquid feces, ulceration, hemorrhage, edema, inflammation, enterocyte vacuolation and villous fusion.
- Lymphocytic system: lymphoid depletion
- Kidney: degeneration of cortical tubular epithelial cells; dilatation of cortical tubules
- ↑WBC, neutrophils, and monocytes appear to be secondary to the internal injury/inflammation

Study title: One-Month Intermittent Dose Oral Toxicity Study in Monkeys.

Key study findings: There were no unscheduled deaths during the study. The following organs/tissues/parameters were affected;

- GI tract: vomiting, diarrhea, gas/fluid-filled contents of the cecum and colon
- Lymphocytic system: lymphoid depletion in spleen and thymus; ↓thymic weight
- Liver: slightly ↑AST and ALT, ↓albumin, liver hypertrophy, focal necrosis (in 1 ♂)
- Heart: inflammation, hypertrophy
- Kidney: inflammation
- Phosphorus: hypo-phosphatemia

- ↑WBC, neutrophils, and monocytes appear to be secondary to the inflammation seen in multiple organs and possible internal injuries.

Study no: DS02159

Volume #, and page #: Item 5

Conducting laboratory and location:

Date of study initiation: 09/04/02

GLP compliance: yes

QA report: yes (X) no ()

Drug, lot #, radiolabel, and % purity: BMS354825, Batch C007A-354825-01; pure

Formulation/vehicle: 80 mM sodium citrate

Dosing:

Species/strain: Cynomolgous monkey

#/sex/group or time point (main study): 4/sex/group

Satellite groups used for toxicokinetics or recovery: 4/sex/group for toxicokinetics; 1/sex/group for recovery

Age: 2-7 years

Weight: 1.5-5 kg

Doses in administered units: 0, 1, 5 and 15 mg/kg (12, 60, and 180 mg/m²)
5-days-on 2-days-off, for 28 days.

Route/Volume: nasogastric intubation, 5 mL/kg

Group Number	Daily Dose		Concentration	Number of Animals ^a
	BMS-354825 (mg/kg)	Volume (ml/kg)	BMS-354825 (mg/ml)	
1	0	5	0	4M, 4F
2	1	5	0.2	4M, 4F
3	5	5	1	4M, 4F
4	15	5	3	4M, 4F

^a The first three monkeys/sex/group were designated for terminal sacrifice. Animals designated for recovery sacrifice (one monkey/sex/group) underwent 2 weeks of recovery following dose administration.

Table provided by the sponsor.

Observations and times:

Clinical signs: Twice daily (am and pm) for mortality and moribundity; once daily cageside observations for each animal; abnormal findings recorded. Once weekly and day of sacrifice detailed observations will be made for each animal.

Body weights: Once prior to treatment, on the first day and weekly thereafter and upon termination

Physical Examination: Prior to initiation of treatment and during week 4. Exams will be performed on anesthetized animals, including HR, respiration rate and body temperature

Food consumption: daily, qualitatively

Ophthalmoscopy: Prior to initiation of treatment and during week 4. Exams will be performed on anesthetized animals

EKG: Prior to initiation of treatment and during week 4 approximately 1 hour postdose. Exams will be performed on anesthetized animals, using 10 leads

Hematology: Twice prior to initiation of treatment and at scheduled sacrifice.

Clinical chemistry: Twice prior to initiation of treatment and at scheduled sacrifice.

Urinalysis: Twice prior to initiation of treatment and at scheduled sacrifice.

appearance/color	pH
bilirubin	protein
blood	specific gravity
glucose	urobilinogen
ketones	volume
microscopic examination of sediment	

Gross pathology: Following 4 cycles of dosing, 3 animals will be fasted overnight, anesthetized , exsanguinated and necropsied. The remaining animal will be euthanized in the same manner two weeks later

Organs weighed: adrenals, brain, epididymis, heart, kidneys, liver with gall bladder, ovary, spleen, testis, thymus, and thyroid with parathyroid

Histopathology: Following 4 cycles of dosing for terminal sacrifice group and two weeks later for recovery subject

Histopathology Inventory:

Study	DS02159
Species	Monkey DX5, 4wks
Adrenals	X*
Aorta	
Bone Marrow smear	
Bone (femur)	X
Brain	X*
Cecum	X
Cervix	X
Colon	X
Duodenum	X
Epididymis	X*
Esophagus	
Eye	
Fallopian tube	
Gall bladder	X*
Gross lesions	X
Harderian gland	
Heart	X*
Ileum	X
Injection site	
Jejunum	X

Kidneys	X*
Lachrymal gland	
Larynx	
Liver	X*
Lungs	X
Lymph nodes, cervical	
Lymph nodes mandibular	
Lymph nodes, mesenteric	X
Mammary Gland	
Nasal cavity	
Optic nerves	
Ovaries	X*
Pancreas	X
Parathyroid	X*
Peripheral nerve	
Pharynx	
Pituitary	
Prostate	
Rectum	X
Salivary gland	
Sciatic nerve	
Seminal vesicles	X
Skeletal muscle	
Skin	
Spinal cord	
Spleen	X*
Sternum	
Stomach	X
Testes	X*
Thymus	X*
Thyroid	X*
Tongue	
Trachea	
Urinary bladder	X
Uterus	X
Vagina	X
Zymbal gland	

X, histopathology performed

*, organ weight obtained

Toxicokinetics: Days 1 and 26; at 1, 2, 4, 8, 12, 24 hours post-dose.

Results:

Mortality: no unscheduled deaths

Clinical signs:

At HD, drug-related changes included sporadic vomitus, a reversible decrease in mean body weight gain (Weeks 1-4) and, in 1 ♂, hunched posture, and thin and

dehydrated appearance. Otherwise, clinical signs were primarily limited to changes in excretion, including liquid, nonformed feces, or lack thereof and qualitative changes in food consumption.

		Cycle 1				Cycle 2				Cycle 3				Cycle 4				Recovery (Days 29-44)		
		Dosing (1-5)		Recovery (6-7)		Dosing (8-12)		Recovery (13-14)		Dosing (15-19)		Recovery (20-21)		Dosing (22-26)		Recovery (27-28)		♂	♀	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀			
Appearance	Dehydrated																			
	Hunched posture											1HD								
	Prolapsed rectum					1HD														
	Thin														1HD					
Discharge	Vomitus w/ food		1HD			1HD	1HD			1HD	1HD	1HD						1HD		1LD
	Menstruating		1HD															1HD		
Excretion	Liquid Feces	1HD				3HD	1MD	1HD	2MD	2HD	2HD	2HD		2HD	2HD	3HD	1HD	1HD	1LD	
	No Feces											1HD								
	Non-formed Feces	3HD	2MD	1HD	2MD	1LD	1MD	2C	2LD	2MD	1C	2MD	2MD	1MD	2C	3HD	1C	3MD	1HD	1LD
Skin and Pelage	Alopecia-dorsal	1HD				1HD													1HD	
	Red peri-orbital-rt													1MD						
Food Consump	Low	3C	1C	4C	1LD	2C	1LD	2C	2LD	3C	4LD	3C	2C	4C	3C	3C	1C	1C	1LD	1LD
	None	1LD	2LD	2LD	2MD	2LD	2MD	1LD	2MD	3LD	3MD	4LD	3LD	3LD	3LD	3LD	1MD	1MD	1MD	

C control; LD: low-dose; MD: mid-dose; HD: high-dose

Body weights:

3 animals, 1 ♂ and 2 ♀s, in the HD group experienced weight loss (shown below). Weight loss was evident at the beginning of the second cycle and persisted through the fourth cycle. There was no evidence of weight loss at week 6 or week seven.

- Male-↓ 0.5kg (25%)
- Female-↓ 0.1kg (~5%)
- Female- ↓ 0.4kg (20%)

Body Temperature (BT): no drug-related effect on BT.

Respiration: no drug-related effect on respiration rate.

Heart Rate: no drug-related on HR.

Food consumption: See table of clinical signs.

Ophthalmoscopy: No significant changes noted in any groups.

Electrocardiography: Changes were not remarkable

		Control	1mg/kg	5mg/kg	15mg/kg
HR	♂	Baseline	179 ± 22.5	182 ± 36.5	176 ± 46.1
					153 ± 22.2

(BPM ± SEM)	♀	Week 4	174 ± 13.0	179 ± 26.1	173 ± 15.8	144 ± 12.0
		Baseline	185 ± 33.4	176 ± 10.2	164 ± 27.4	170 ± 17.9
		Week 4	179 ± 24.2	167 ± 9.0	177 ± 18.7	182 ± 12.4
P-R interval (s ± SEM)	♂	Baseline	0.07 ± 0.012	0.07 ± 0.012	0.07 ± 0.012	0.08 ± 0.010
		Week 4	0.08 ± 0.000	0.08 ± 0.01	0.08 ± 0.010	0.07 ± 0.012
	♀	Baseline	0.08 ± 0.05	0.07 ± 0.010	0.07 ± 0.012	0.08 ± 0.010
		Week 4	0.07 ± 0.010	0.07 ± 0.012	0.07 ± 0.010	0.07 ± 0.012
QRS Interval (s ± SEM)	♂	Baseline	0.04 ± 0.000	0.04 ± 0.000	0.04 ± 0.000	0.04 ± 0.000
		Week 4	0.04 ± 0.000	0.04 ± 0.000	0.04 ± 0.000	0.04 ± 0.000
	♀	Baseline	0.04 ± 0.000	0.04 ± 0.005	0.05 ± 0.001	0.04 ± 0.000
		Week 4	0.04 ± 0.000	0.04 ± 0.000	0.04 ± 0.000	0.04 ± 0.000
Q-T Interval (s ± SEM)	♂	Baseline	0.21 ± 0.010	0.21 ± 0.030	0.20 ± 0.028	0.23 ± 0.019
		Week 4	0.20 ± 0.000	0.20 ± 0.028	0.21 ± 0.019	0.23 ± 0.019
	♀	Baseline	0.21 ± 0.025	0.22 ± 0.016	0.22 ± 0.023	0.22 ± 0.019
		Week 4	0.21 ± 0.025	0.23 ± 0.019	0.20 ± 0.010	0.21 ± 0.010
QT _c (s ± SEM)	♂	Baseline	0.26 ± 0.007	0.26 ± 0.024	0.26 ± 0.022	0.28 ± 0.015
		Week 4	0.26 ± 0.002	0.26 ± 0.024	0.26 ± 0.019	0.28 ± 0.018
	♀	Baseline	0.26 ± 0.021	0.28 ± 0.015	0.27 ± 0.018	0.27 ± 0.021
		Week 4	0.26 ± 0.022	0.28 ± 0.00	0.25 ± 0.007	0.26 ± 0.009

Hematology:

HD ♂s(end of treatment)

↑ 42% in neutrophils, ↑200% in monocytes, ↑78% platelets, ↑48% WBC

HD ♂s (recovery)

↑57% WBC

Changes in neutrophils, monocytes, and platelets were reversible.

No significant changes noted in other groups

Clinical chemistry:

HD ♂s (end of treatment)

- ↓50% in IN PHOSPHORUS,
- ↓ 25% in ALBUMIN (statistically significant),
- ↑45% AST,
- ↑73% ALT

HD ♀s (end of treatment)-

↓ 30% in IN PHOSPHORUS

Findings were reversible. No significant changes noted in other groups

Urinalysis: Unremarkable

Organ weights:

Changes in the relative organ weights (organ:BW). Number in parentheses represent remarkable recovery data

	MD		HD	
	♂ (n=3)	♀ (n=3)	♂ (n=3)	♀ (n=3)
Adrenal	—	—	↑30%	—
Heart	—	—	↑8% (↑30%)	↑24%
Liver/ gallbladder	—	—	↑47% (↑20%)	↑20% (↑23%)
Ovary	NA	—	NA	↑63%
Spleen	—	—	↓20%	†↑80%
Testis	—	NA	↓25%	NA
Thymus	§↓16%	—	↓62%	↓16%
Thyroid/parathyroid	§↓20%	—	↓30% (↓29%)	— (↓40%)

NA: not applicable

§ Changes were dose-dependent in ♂s, starting from the LD (thymus: ↓6% at LD; thyroid/parathyroid: ↓15% at LD).

† Organ weight had large standard deviation (4.3 g ± 4.4 g).

End of treatment

Liver/gallbladder hypertrophy was noted at HD as evident by the 47% and 20% increase in the relative weights, in ♂s and ♀s, respectively. There appeared to be a tendency for cardiac hypertrophy, based on the slight ↑ in the relative weight of the heart in HD ♂s (↑8%) and HD ♀s (↑24%).

Dose dependant ↓ in organ weights occurred in the thymus and thyroid in ♂ animals, starting at the LD.

End of recovery

It should be noted that the recovery groups consisted of 1 ♂ and 1 ♀/ dose group. Therefore it would be difficult to make a final conclusion regarding reversibility of the findings. Based on the limited animal data, most changes appear to be reversible. Changes in thymus weight were reversible for all doses, whereas changes in the thyroid were reversible for the LD and MD, but not for HD.

Gross pathology:

Drug related macroscopic changes were noted in animals treated with 5 and 15 mg/kg. In males these observations included alopecia. One male monkey (15 mg/kg) had a raised skull cap with concomitant indentation of the dorsal surface of the brain. In females (5 and 15 mg/kg), aberrations were noted in the cecum and colon, and included distention and fluid/gas filled lumens. All changes detected were reversible and not detected in the recovery sacrifice groups.

		TERMINAL SACRIFICE				RECOVERY SACRIFICE			
		0 mg/kg	1 mg/kg	5 mg/kg	15 mg/kg	0 mg/kg	1 mg/kg	5 mg/kg	15 mg/kg
Brain					1 M				
Dorsal surface indented					1 M				
Gall Bladder									
Distended lumen-fluid		1 F		1 F	1 F				
Cecum	Raised area	1 M		1 F					
	Distended				1 F				
	Lumen, fluid				1 F				
	Lumen, gas				1 F				
Colon	Raised area	1 M		1 F					
	Lumen, fluid				1 F				
	Lumen, gas				1 F				
	Dark Area					1 M			
Ovary					1 F				
Cyst					1 F				
Bone					1 M				
Skull Cap raised					1 M				
Alopecia					1 M				

Histopathology:

Observations are summarized in the following table. 3 monkeys per group were examined for the terminal sacrifice, 1/group was measured in the recovery sacrifice group.

		TERMINAL SACRIFICE				RECOVERY SACRIFICE			
		0 mg/kg	1 mg/kg	5 mg/kg	15 mg/kg	0 mg/kg	1 mg/kg	5 mg/kg	15 mg/kg
Adrenal Cortex				1M					
Calcareous bodies				1M					
Bone					1M				
Focal osteosis					1M				
Brain					1 F				
Mononuclear cell infiltrate					1 F				
Lung			1F		1M				
Focal Pneumonitis			1F		1M				
Heart		1F	2M 2F	1M 2F	1M 2F			1M 1F	1F
Chronic inflammation		1F	2M 2F	1M 2F	1M 2F			1M 1F	1F
Spleen					2M				
Lymphoid depletion					2M				
Kidney		1M 1F	2M 2F	1M 2F	1M 2F	1M			1M 1F
Inflammation-chronic		1M 1F	2M 2F	1M 2F	1M 2F	1M			1M 1F
Liver	Inflammation-chronic	2M	2M 2F	1M 1F	1M 1F	1M	1M	1F	
	Focal Necrosis			1M					
Stomach-Fundic				2M		1M		1M 1F	
Cryptosporidia				2M		1M		1M 1F	
Stomach- Pyloric				1M		1M		1M	
Cryptosporidia				1M		1M		1M	

	TERMINAL SACRIFICE				RECOVERY SACRIFICE			
	0 mg/kg	1 mg/kg	5 mg/kg	15 mg/kg	0 mg/kg	1 mg/kg	5 mg/kg	15 mg/kg
Pancreas Decreased zymogen granules				1M	1F			
Mesenteric LN Increased pigmented macrophages	1M 2F		1M	1M	1M 1F	1M 1F	1F	1F
Thymus Depletion, lymphoid				3M 1F				
Tongue Degeneration, muscle							1M	
Ovary Cyst				1F				

Toxicokinetics:

BMS-354825							
Dose [mg/kg/day]	Study Day	C _{max} (ng/mL)		T _{max} ^a (h)		AUC ^b (ng•h/mL)	
		Male	Female	Male	Female	Male	Female
1	1	20	11	1.5	1	36	17
	26	12	8	2	1.5	34	16
5	1	91	93	1.5	1	206	221
	26	50	64	1.5	2	181	280
15	1	480	399	1	1.5	1162	1053
	26	154	374	1.5	1.5	774	976
Ratios							
1:5:15 Dose Ratio	1	1:4.6:24	1:8.5:36.3			1:5.7:32.3	1:13:61.9
	26	1:4.2:12.8	1:8:46.8			1:5.3:22.8	1:17.5:61

^a Median value

^b Calculated from time zero to the time of last measurable concentration. equal to 24 h. ranging between 8 to 24 h.

Table provided by the sponsor.

- Systemic exposure to BMS-354825 in monkeys was greater than dose-proportional at HD.
- Exposures were comparable between males and females.
- AUCs were similar between Days 1 and 26 indicating BMS-354825 did not accumulate with repeated intermittent dosing in monkey.

Summary of individual study findings:

BMS-354825 (dasatinib) was administered orally to cynomolgus monkeys at 1, 5, and 15 mg/kg (12, 60, and 180 mg/m²), 5-days-on 2-days-off, for a total of 4 weeks. Necropsies were done at the end of the treatment and after a 2-week recovery period.

All monkeys survived to the scheduled terminal and recovery sacrifices. Clinical signs of toxicity were seen mainly at MD and HD and included a reversible increased incidence of stool changes (liquid, non-formed, or no feces), and low food consumption. Low food consumption was also observed in the control groups. Additional clinical signs at HD included: vomiting, a reversible decrease in mean body weight gain (Weeks 1 - 4) and, in one male, hunched posture, and thin and dehydrated appearance.

Drug-related hematology changes were limited to: ↑ 42% in neutrophils, ↑200% in monocytes, ↑78% platelets, ↑48% WBC in HD ♂s, all of which appear to be secondary to inflammations seen in multiple organs and internal injury. ↑WBCs persisted to the end of the recovery period. Drug-related clinical chemistry changes included reversible ↑ in ALT (↑73%) and AST (↑45%) and Albumin (↓35%) in HD ♂s. These data together with the liver hypertrophy in both genders and histopathology findings (e.g focal necrosis in 1 ♂) suggest the potential for dasatinib to cause hepatotoxicity. Reversible hypo-phosphatemia was noted in HD ♂s (↓50%) and ♀s (↓30%). The reason for hypo-phosphatemia is not clear. Multiple factors can contribute to ↓ in serum phosphorus, e.g. ↓renal tubular re-absorption, GI toxicity, and parathyroid dysfunction. Since other electrolytes, Na, Cl, and Ca, were not affected, GI toxicity (e.g. diarrhea) may not be the cause of this finding. Based on the summary of the 10-day oral toxicity study in monkeys, renal tubular dysfunction might be contributing to the ↓serum phosphorus.

At necropsy, reversible or fluid-filled contents of the cecum and colon were noted. Together with the clinical observations of vomiting and abnormal feces, these findings indicate drug-induced GI toxicity.

Although EKG did show any sign of cardiac toxicity, the potential for drug-induced cardiotoxicity cannot be eliminated, because of the slight cardiac hypertrophy in HD ♂s (↑8%) and ♀s (↑24%) and cardiac findings in other animal toxicology studies.

Dose dependant ↓ in organ weights occurred in the thymus and thyroid in ♂ animals, starting at the LD. Since the recovery groups consisted of 1 ♂ and 1 ♀/ dose group, it would be difficult to make a final conclusion regarding reversibility of the findings. Based on the limited animal data, most changes appeared to be reversible. ↓Thyroid/parathyroid weight, ↑heart weight, ↑liver/gallbladder weights were still observed at the end of the recovery period.

Microscopically, reversible, lymphoid depletion was noted in the spleen of HD ♂s, and reversible, slight to moderately severe lymphoid depletion was noted in the thymus in HD ♂s and ♀s. Partially reversible chronic inflammation of the heart, kidney and liver were noted in all treatment groups, but with higher frequency in the animals receiving BMS 354825. Other microscopic findings included: adrenal mineralization, liver necrosis, cyst in ovary, ↓pancreatic zymogen, mononuclear cell infiltrate in brain.

TK

- Systemic exposure to BMS-354825 in monkeys was greater than dose-proportional at HD.

- Exposures were comparable between males and females.
- AUCs were similar between Days 1 and 26 indicating BMS-354825 did not accumulate with repeated intermittent dosing in monkey.

Overall the following toxicities were observed:

- GI toxicity: vomiting, diarrhea, gas/fluid-filled contents of the cecum and colon
- Lymphoid depletion: depletion in spleen and thymus; ↓thymic weight
- Liver: slightly ↑AST and ALT, (↓albumin), liver hypertrophy, focal necrosis (in 1 ♂)
- Heart: inflammation, hypertrophy
- Kidney: inflammation
- Hypo-phosphatemia

Study title: BMS-354825: Nine-Month Oral Toxicity Study in Cynomolgus Monkeys

Key study findings: Due to toxicities, doses were reduced at MD and HD and dosing interruptions of up to 30 days took place in several MD and HD animals.

The following toxicities were detected; most findings were based on the histopathology results since the clinical pathology was inconclusive:

- GI tract: vomiting, red/liquid feces, inflammation, ulceration/erosion, edema, flattening of superficial epithelium, rectal crypt abscess
- lymphoid organs: ↓lymphocytes, lymphocytic depletion of thymus and mesenteric lymph node
- kidney: cortical mineralization of tubules and glomeruli, fibrosis, inflammation, tubular ectasia/proteinosis, ↑BUN
- lung: hyperplasia/hypertrophy, hemorrhage, fibrosis, inflammation
- liver: hypertrophy, hepatocellular necrosis, ↑triglycerides
- Bile duct: hyperplasia
- Pancreas: inflammation
- Adrenal medulla: mineralization
- Male reproductive organs: immature prostate, seminal vesicle, and testis
- Female reproductive organ: mineralization and inflammation of uterus
- Multiple organs: vascular mineralization e.g. in heart, tongue, spleen, stomach, and pancreas
- ↑WBC, ↑neutrophils, and ↑reticulocytes at HD on week 15 appeared to be secondary to internal injury and inflammation

Report no.: DS03073

— Study no. 6108-409

Volume #, and page #: Item 5

Conducting laboratory and location:

Date of study initiation: 29 April 2003

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity:

Vehicle: 80 mM sodium citrate buffer

Test Article	Lot Number	Storage	Purity (%)
BMS-354825	RD71123-45	Room temperature, protected from light	— (as is)

Methods

Doses:

Initial dose: 1, 3, 10 mg/kg/day x 8
No dosing on Days 9-15

Modified dose (from D16): 1, 3, 6 mg/kg; 5-days on/ 2-days off

Modified dose (from D83): 1, 3, 4.5 mg/kg; 5-days on/ 2-days off (x 22 weeks for HD)

Modified dose (from D190): 1, 2.5, no HD animal; 5-days on/ 2-days off
(12, 24 mg/m², 5-days on/ 2-days off x 41 weeks for LD and MD)

Notes:

- Since dosing was suspended during Days 8/9 through 15, the duration of dosing was extended from 39 to 41 weeks to ensure 39 consecutive weeks of dosing.
- As a result of persistent toxicity at HD (10/6/4.5 mg/kg), this group was terminated on Day 150 (Week 22) and 2 ♂s and 3 ♀s were necropsied. The remaining two males and two females were placed on a 4-week recovery period and necropsied on Day 181 in Week 26.
- Scheduled necropsies for surviving animals in other groups (2-4/sex/group) were conducted at the end of the dosing period on Day 289 (Week 41).
- Week 26 is the end of recovery for HD animals; Week 45 is the end of recovery for other groups.

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Group	No. of Animals ^a		Dose Level ^b (mg/kg/day)	Dose Concentration ^b (mg/mL)
	Male	Female		
1 (Control) ^c	6	6	0	0
2 (Low)	6	6	1	0.2
3 (Intermediate)	6	6	2 ^d	0.4 ^d
4 (High) ^e	6	6	4.5 ^f	0.9 ^f

- a Animals designated for recovery sacrifice (the last two surviving animals/sex/group) underwent 4 weeks of recovery following the dose administration period. Due to toxicity observed, dosing was suspended from Day 8 (Group 4) or 9 (Groups 1, 2, and 3) until Day 15, when dosing was resumed at a modified dosing schedule of 5 days on (Monday through Friday) and 2 days off (Saturday and Sunday). Replacement animals (two Group 4 females) were dosed on schedule with the rest of the animals beginning on Day 22.
- b Animals were dosed at a volume of 5 mL/kg.
- c Group 1 animals were dosed with the control article only.
- d Due to toxicity observed, the intermediate-dose level was lowered from 3 to 2 mg/kg/day (beginning on Day 190).
- e Due to toxicity observed, the high-dose group was terminated early. During Week 22, up to four animals/sex in Group 4 were euthanatized. Two animals/sex in Group 4 were designated as recovery animals and placed on a 4-week recovery period beginning on Day 151.
- f Due to toxicity observed, the high-dose level was lowered from 10 to 6 mg/kg/day (beginning on Day 15) and from 6 to 4.5 mg/kg/day (beginning on Day 83).

Species/strain: monkey/ cynomolgus

Number/sex/group (main study): see the Table, 6/sex/group

Route, formulation, volume: oral (gavage or nasogastric), solution, 5 mL/kg

Satellite groups used for toxicokinetics or recovery:

Age: 4-8 years old at initiation of dosing

Weight: 2.8-4.5 kg (♂s)/ 2.2-3.0 kg (♀s)

Stability of Dosing Formulations

Formulations of 0.2, 0.6, and 2 mg/mL were stable for at least 17 days. Duplicate samples of the 2-mg/mL formulation that were analyzed 3 and 10 days after preparation had concentrations of BMS-354825 assayed at ~ 1 µg/mL (85% of the theoretical concentration), which were slightly below the acceptable range (90-110% of the theoretical concentration). However, the samples of the 2-mg/mL formulation analyzed 17 days after preparation were within the acceptable range. Stability analyses of samples from the 0.2- and 0.6-mg/mL formulations were within the acceptable range after 17 days.

Concentration Verification of Dosing Formulations

For all samples, the mean concentration of BMS-354825 was within the acceptable range (90-110% of the theoretical concentration). The results of vehicle control analysis confirmed the absence of test article.

Protocol deviations: included stopping the dose administration for several days (up to 30 days), mainly in MD and HD animals.

Observations and times:

Mortality: twice daily

Clinical signs: once daily for cage-side observation
Once weekly for detailed observations

Physical Examinations

Once prior to initiation of treatment and during Weeks 15, 28, 41, and 45.
During Week 22, examinations were also performed on HD animals and the MD ♀ that was euthanatized. These examinations included heart rate, respiration rate, and rectal body temperature measurements. Animals were anesthetized for the procedures.

Body weights: prior to treatment, on the day of treatment initiation, and weekly thereafter.

Food consumption: once daily

Ophthalmoscopy: Once prior to initiation of treatment; during Weeks 15, 22 (HD animals and the MD ♀ male that was euthanatized), 28, 41, and 45. All animals were examined by an indirect ophthalmoscope.

EKG: once prior to initiation of treatment and during Weeks 15, 22 (HD animals and the MD ♀ male that was euthanatized), 28, 41, and 45 for all animals. Routine measurements of electrocardiograms, including heart rate, were performed using 10 leads.

Hematology: prior to initiation and during Weeks 15, 22 (HD animals and the MD ♀ that was sacrificed; Animal No I09651), 26 (recovery animals in Group 4), 28, 41, and 45.

Platelet Aggregometry

During Weeks 15 (13 weeks after the restart of dosing), 22 (for HD animals and the MD animal euthanatized during Week 22), 26 (HD recovery animals), and 28, additional blood was collected for platelet aggregometry.

Clinical chemistry: prior to initiation and during Weeks 15, 22 (HD animals and the MD ♀ that was sacrificed; Animal No I09651), 26 (recovery animals in Group 4), 28, 41, and 45.

Urinalysis: prior to initiation and during Weeks 15, 22 (HD animals and the MD ♀ that was sacrificed; Animal No I09651), 26 (recovery animals in Group 4), 28, 41, and 45.

Gross pathology: at sacrifice. The necropsy included an examination of the external features of the carcass; external body orifices; the abdominal, thoracic, and cranial cavities; organs; and tissues.

Sacrifices were as follows:

- ***Terminal Sacrifice (HD):*** On Day 150 (Week 22), 2 ♂s and 3 ♀s. Referred to as interim sacrifice in the report. The remaining 2 animals/sex in Group 4 were placed on a 4-week recovery period.
- ***Recovery Sacrifice (HD):*** On Day 181 (Week 26). Referred to as interim recovery sacrifice in the report.
- ***Terminal Sacrifice (Control, LD, and MD):*** On Day 289 (Week 42), after 39 weeks of 5-days-on, 2-days-off treatment; 4 ♂s and 3 ♀s in the control group, 4 ♂s and 4 ♀s at LD, and 2 ♂s at MD.

- **Recovery Sacrifice (Control, LD, and MD):** On Day 317 (Week 46)

Organ weights: at scheduled necropsies:

adrenal (2)	pituitary gland
brain	prostate
heart	seminal vesicles (2)
kidney (2)	spleen
liver with gallbladder (drained)	testis (2)
ovary (2)	thyroid (2) with parathyroid

Histopathology: Adequate Battery: yes (x), no ()

Peer review: yes (x), no ()

The following organs/tissues from the animals necropsied at scheduled or unscheduled intervals were preserved in 10% neutral-buffered formalin unless otherwise noted.

adrenal (2)	ovary (2)
aorta	pancreas
brain	peripheral nerve (sciatic)
cecum	pituitary gland
cervix	prostate
colon	rectum
duodenum	salivary gland [mandibular (2)]
epididymis (2)	seminal vesicle (2)
esophagus	skeletal muscle (biceps femoris)
eye (2) with optic nerve; preserved in 5% glutaraldehyde (scheduled necropsies) ^a	spinal cord (cervical, thoracic, and lumbar)
femur with bone marrow (articular surface of the distal end)	rib with bone marrow
gallbladder	skin
heart	spleen
ileum	sternum with bone marrow
jejunum	stomach (cardia, fundus, and pylorus)
kidney (2)	testis (2)
lacrimal gland	thymus
lesions	thyroid (2) with parathyroid
liver	tongue
lung with mainstem bronchi	trachea
lymph node (mesenteric and mandibular)	urinary bladder
mammary gland	uterus
	vagina

^a After collection, the eyes were placed in 5% glutaraldehyde, refrigerated for at least 48 but not more than 72 hours, and then transferred to 10% neutral-buffered formalin.

From the paraffin block of brain tissue from Animal No I09751 (HD ♀), the following special stains were performed: periodic acid-Schiff, phosphotungstic acid-hematoxylin, bilirubin, iron, lipofuscin, and acid fast stains. From the formalin-fixed thalamus tissue of Animal No I09751, tissue was frozen and slides prepared with the following special stains: Oil red O and sudan black. Representative control special stains were also made from a normal monkey brain

Toxicokinetics: TK sampling was conducted on all animals on Day 1 and during Weeks 15, 28, and 41, approximately 1, 2, 4, 8, 12, and 24 hours following dose administration

Results

Mortality: 11 (6 at MD and 5 at HD), primarily due to GI toxicities

- MD: 2 ♂s on Days 188 and 261; 4 ♀s on Days 150, 188, 277, or 279.

- HD (10 mg/kg; prior to the initial dose reduction): 2 ♀s on Day 14. These 2 ♀s were replaced on study with two new females at an initial dose of 6 mg/kg beginning on Day 22.
- HD (after dose reduction): 2 ♂s on Days 24 and 73 and 1 ♀ on Day 133.

Major Findings in Unscheduled sacrifices	
Clinical observations	<p>MD and HD:</p> <ul style="list-style-type: none"> • Abnormal feces (discolored/red, liquid, mucoid, nonformed feces), vomitus, hunched posture, low or no food consumption, and decreases in individual body weights. <p>HD:</p> <ul style="list-style-type: none"> • Intermittent ataxia in one male, persistent ataxia and tremors prior to euthanasia in 1 ♀, and decreased frequency of menstrual cycling in females.
Clinical pathology	<p>MD and HD:</p> <ul style="list-style-type: none"> • ↓RBC (50-90% of pretest), ↓lymphocytes (20-30%), ↓hemoglobin (50-90%), hematocrit (↓50-80%), • ↓albumin (60-80%), ↓Na (80-90%), ↓K (60%), and ↓Cl (70-80%) and ↑ total leukocytes (1.3-2.3-fold increase of pretest), ↑neutrophils (1.3-6.6-fold), ↑fibrinogen (1.2-1.7-fold), ↑BUN (3.1-9.2-fold), and ↑creatinine (1.4-13.8-fold).
Gross Pathology	<p>MD and HD:</p> <ul style="list-style-type: none"> • GI tract: red foci in the large intestine and/or stomach. <p>HD:</p> <ul style="list-style-type: none"> • GI tract: erosion/ulceration in the stomach (1 ♀), enlarged, gas-distended (1 ♂); and red fluid contents in the stomach and small intestine (1 ♂).
Histopathology	<p>MD and HD:</p> <p>GI Tract:</p> <ul style="list-style-type: none"> • villous blunting/fusion • Stomach: minimal-moderate erosion/ulceration; minimal-moderate inflammation. • Small intestine: minimal to moderate villous blunting and/or fusion. Moderate erosion/ulceration and slight/minimal acute to Subacute inflammation. • Large intestine: minimal to moderately severe erosion/ulceration of the cecum, colon, and/or rectum; minimal to moderately severe acute to subacute inflammation; slight to moderate superficial epithelial flattening in the large intestine. • Rectum: ulceration, hemorrhage <p>Additional findings at MD and/or HD:</p> <ul style="list-style-type: none"> • A MD ♀ (3 mg/kg/day) had slight vascular inflammation in the heart and lacrimal gland. • Lymphocytic depletion in thymus and/or spleen • Findings in the kidney (cortical mineralization of tubules and glomeruli, fibrosis, and tubular ectasia/proteinosis) and testis (degeneration).

SEX:	-----MALE-----	-----FEMALE-----
GROUP:	-1- -2- -3- -4-	-1- -2- -3- -4-
NUMBER:	0 0 2 2	1 0 4 2

MARROW, STERNUM (SE)	NUMBER EXAMINED:	0	0	2	2	1	0	4	2
	NOT REMARKABLE:	0	0	1	2	1	0	4	1
--DECREASED ERYTHROID CELLS		0	0	1	0	0	0	0	1
KIDNEY (KD)	NUMBER EXAMINED:	0	0	2	2	1	0	4	2
	NOT REMARKABLE:	0	0	0	0	0	0	0	0
--MINERALIZATION, CORTICAL (AFFECTS TUBULES OR GLOMERULI, OFTEN ACCOMPANIED BY MINIMAL GRANULAMATOUS INFLAMMATION AND/OR FIBROSIS.)		0	0	0	1	0	0	2	1
--MINERALIZATION, MEDULLARY		0	0	0	0	1	0	3	0
--INFILTRATE, LYMPHOHISTIOCYTIC		0	0	2	2	0	0	3	1
--EOSINOPHILIA, TUBULAR		0	0	0	2	0	0	1	2
--EKTASIA/PROTEINOSIS, TUBULAR		0	0	1	2	1	0	2	2
--HEMORRHAGE, INTRATUBULAR		0	0	0	0	0	0	1	0
--INFLAMMATION, GRANULAMATOUS		0	0	0	0	0	0	1	0
--VACUOLATION, TUBULAR EPITHELIUM		0	0	0	0	0	0	1	0
--PIGMENT, TUBULAR EPITHELIUM		0	0	1	0	0	0	0	0
--GLOMERULOSCLEROSIS		0	0	0	0	0	0	0	1
HEART (HT)	NUMBER EXAMINED:	0	0	2	2	1	0	4	2
	NOT REMARKABLE:	0	0	2	0	0	0	0	1
--INFILTRATE, LYMPHOHISTIOCYTIC		0	0	0	2	1	0	4	0
--INFLAMMATION, VASCULAR		0	0	0	0	0	0	1	0
--DEGENERATION/NECROSIS, INDIVIDUAL CARDIOMYOCYTES		0	0	0	0	1	0	1	1
SPLEEN (SP)	NUMBER EXAMINED:	0	0	2	2	1	0	4	2
	NOT REMARKABLE:	0	0	2	1	0	0	4	0
--DEPLETION, LYMPHOCYTIC		0	0	0	1	1	0	0	2
--FIBROSIS, CAPSULAR		0	0	0	1	1	0	0	1
THYMUS (TH)	NUMBER EXAMINED:	0	0	2	2	1	0	4	2
	NOT REMARKABLE:	0	0	1	0	0	0	2	0
--DEPLETION, LYMPHOCYTIC		0	0	1	2	1	0	2	2
--CYST		0	0	1	1	0	0	1	0
LN, MESENTERIC (MS)	NUMBER EXAMINED:	0	0	2	2	1	0	4	2
	NOT REMARKABLE:	0	0	0	1	0	0	4	1
--DEPLETION, LYMPHOCYTIC		0	0	2	1	1	0	0	1
ADRENAL, CORTEX (AC)	NUMBER EXAMINED:	0	0	2	2	1	0	4	2
	NOT REMARKABLE:	0	0	1	2	1	0	3	1
--NECROSIS, INDIVIDUAL CELL		0	0	0	0	0	0	0	1
--HYPERTROPHY, FOCAL/MULTIFOCAL		0	0	0	0	0	0	1	0
--MINERALIZATION		0	0	0	0	0	0	1	0
LACRIMAL GL, INT (LG)	NUMBER EXAMINED:	0	0	2	2	1	0	4	1
	NOT REMARKABLE:	0	0	0	0	0	0	0	0
--INFILTRATE, LYMPHOHISTIOCYTIC		0	0	1	2	1	0	3	1
--INFLAMMATION, VASCULAR		0	0	0	0	0	0	1	0
--ONE EXAMINED		0	0	1	0	0	0	0	0
TESTIS (TE)	NUMBER EXAMINED:	0	0	2	2	0	0	0	0
	NOT REMARKABLE:	0	0	2	0	0	0	0	0
--ATROPHY/DEGENERATION		0	0	0	2	0	0	0	0

Clinical signs:

The following signs were seen in all HD animals, most MD animals, and infrequently at LD, with an onset usually during Week 1 or 2:

- Abnormal feces (red/ liquid, mucoid, non-formed, and/or decreased)
- low or no food consumption
- vomitus

Additional clinical sign at HD and MD:

- Hunched posture

Body weights:

- No drug-related effect at LD or MD
- HD: ↓ 3-13% (♂s) and ↓4-18% (♀s); during Weeks 2-22. Reversible upon recovery.

Food consumption: ↓ food consumption in all HD animals, most MD animals, and infrequently at LD, with an onset usually during Week 1 or 2.

Ophthalmoscopy: no effect

EKG: No apparent effect on the EKG, based on the individual animal data (summary data was not presented). There appear to be no effect on the heart rate or the respiratory rate. No effect was observed for body temperature.

Hematology:

Week 15 data: changes were unremarkable at LD, MD, and in HD ♀s; data for HD ♂s are presented below

	HD ♀s (Week 15)
Reticulocytes (Abs)	↑15%
Platelets	*↓30%
WBC	*↑65%
Neutrophils (Abs)	↑3-fold

Week 22 (terminal sacrifice for HD) and Week 26 (recovery sacrifice for HD) data are inconclusive, because no hematology data is available for control animals for these two weeks. Data for Weeks 22 and 26 were compared to the control data for Week 28.

	HD (Week 22) Terminal Sacrifice		HD (Week 26) Recovery Sacrifice	
	♂	♀	♂	♀
Reticulocytes (Abs)	↑14%	↑10%	↑70%	↑100%
WBC	↑30%	↓20%	↑70%	↑20%
Neutrophils (Abs)	↑100%	—	↑100%	—
Monocytes (Abs)	↑100%	↑66%	↑100%	↑30%
Platelets	↓13%	↑10%	↓17%	↓9%
aPTT	↑20%	—	↑20%	—
Fibrinogen	↑25%	↑11%	—	↓18%

WBC: White blood cell; Neut (Abs): Absolute neutrophil; Lymph (Abs): Absolute lymphocyte; Mono (Abs): Absolute monocyte; aPTT: Activated partial thromboplastin time; —: No effect/ no remarkable effect.

Week 41, end of treatment data (Week 45, end of recovery) for the MD
Changes, if any, were minimal for LD animals and are not reported below.

Parameter	MD	
	♂	♀
WBC	—	↓50% (↓50%)
Neut (Abs)	↑100% (↑20%)	↓60% (↓60%)
Lymph (Abs)	↓10% (↓40%)	↓30% (↓40%)

—: effect not remarkable/ no effect

No data available on HD, due to mortalities and early termination.

Note: most findings at the end of the recovery period are due to changes in the control parameters.

Clinical chemistry:

No clear test article-related effect. Changes were non-dose-dependent and non-remarkable.

Week 15 data:

- BUN: ↑17% in HD ♂s
- Globulin: ↓14% in HD ♂s

Week 22 and 26 data (available for HD only). In the absence of corresponding control, results are compared to Control Week 28:

- BUN: ↑25% and ↑45% at Weeks 22 and 26, respectively

Week 22 and 26 data (available for HD only).

	HD		HD	
	Week 22: end of treatment		Week 26: end of recovery	
	♂	♀	♂	♀
BUN	↑25%	—	↑45%	—
Globulin	↓14%	—	—	—

BUN: blood urea nitrogen

—: effect not remarkable/ no effect.

Week 41 data (Week 45 recovery data); no data available for HD due to early termination:

- Triglycerides: ↑80% in MD ♀s (Week 41) and ↑15% in MD ♀s (Week 45: end of recovery)

Urinalysis: no clear test article-related effect

Gross pathology:

Red foci in large intestine: 1 HD ♀, Week 22; 1MD ♂, Week 46 (recovery).

Organ weights:

Relative (organ: BW) changes in the organ weights at the end of the treatment period.

	LD		MD		HD	
	♂	♀	♂	§♀	♂	♀
Testis	—	NA	—		↑19%	NA
Pituitary	—	—	↓25%		↓25%	↓30%
Adrenal	—	—	—		↑17%	↑5%
†Liver/gallbladder	↑14%	—	↑9%		*↑48%	↑9%
Ovary	NA	↓30%	NA		NA	↓25%

* Statistically significant.

§ Data not available for MD ♀s.

† Statistically significant ↑ in absolute liver weight was noted at all dose levels. Changes in the Liver: brain relative weights were statistically significant at all dose levels.

—: effect not remarkable/ no effect; NA: not applicable.

Notes:

- HD data are for Week 22 and LD and MD data are for 41 (the above numbers for HD compare Week 22 to the control on Week 41).
- Recovery data showed small, non-conclusive changes. No recovery data was available for controls on Week 26 as a comparison for the HD group.

Histopathology:

Major drug-related findings for the HD group:

	Week 22 (treatment)		Week 26 (recovery)	
	♂ (n=2)	♀ (n=2)	♂ (n=2)	♀ (n=2)
Lung				
Inflammation (acute)	1	0	0	0
Cystic space with peripheral fibrosis	0	0	0	1
Kidney				
Mineralization (cortical)	0	2	0	1
Fibrosis	1	0	1	0
Ectasia/proteinosis (tubular)	0	2	0	0
Liver				
Necrosis (hepatocellular)	0	0	1	0
Aorta				
Hyperplasia/ hypertrophy	0	0	0	1
Adrenal cortex				
Hypertrophy (focal/multifocal)	0	1	1	1
Stomach; glandular				
Edema/ hemorrhage	0	1	0	0
Inflammation (granulomatous)	1	0	0	1
Cecum				
Inflammation (acute, Subacute, or granulomatous)	1	1	0	0
Colon				
Vascular inflammation	1	1	0	0
Rectum				
Erosion/ulceration	1	0	0	0
Inflammation (acute/subacute)	2	0	0	0
Crypt abscess	0	1	0	0
Lymph node; mandibular				
Infiltrate (neutrophilic or eosinophilic)	0	2	2	1
Testis				
Atrophy/degeneration	0	NA	1	NA

Note: because no corresponding control was available, results were compared to Week 41 controls. Findings that may be associated with dasatinib treatment are listed above.

End of treatment data for the LD and MD groups (Week 41):

Note: no data available for MD ♀s.

- Lung: hyperplasia/hypertrophy; hemorrhage; fibrosis; inflammation.
- Kidney: cortical mineralization (affecting tubules or glomeruli, accompanied by inflammation or fibrosis); fibrosis, tubular ectasia/proteinosis; tubular epithelial cytomegaly.
- Liver and bile duct: hepatocellular necrosis, bile duct hyperplasia
- Heart: vascular mineralization; neutrophilic infiltrate
- Tongue: vascular mineralization
- Spleen: vascular mineralization
- Thymus: lymphocytic depletion

- Lymph node; mesenteric: lymphocytic depletion
- Lymph node; mandibular: neutrophilic and eosinophilic infiltrate
- Adrenal medulla: mineralization
- Stomach; glandular: inflammation; vascular mineralization
- Pancreas: inflammation; vascular mineralization; pigmentation
- Cecum: inflammation; edema; flattening of superficial epithelium; vascular mineralization
- Colon: inflammation; submucosal pigmentation; vascular mineralization
- Rectum: erosion/ulceration; inflammation; crypt abscess
- Uterus: mineralization; inflammation:
- Prostate: immature
- Seminal vesicle: immature
- Testis: immature

		SEX: -----MALE----- FEMALE----						
		GROUP: -1- -2- -3-			-1- -2- -3-			
		NUMBER:	4	4	2	3	4	0
LUNG (LU)	NUMBER EXAMINED:	4	4	2	3	4	0	
	NOT REMARKABLE:	1	2	0	1	1	0	
--HYPERPLASIA/HYPERTROPHY, ALVEOLAR/BRONCHIOLAR EPITHELIUM		0	0	1	0	0	0	
--INFILTRATE, MACROPHAGE, ALVEOLAR		3	1	2	1	2	0	
--INFLAMMATION, ACUTE		0	2	0	0	0	0	
--INFLAMMATION, SUBACUTE		0	0	1	1	1	0	
--HEMORRHAGE		0	0	2	0	0	0	
--FIBROSIS		0	0	0	0	2	0	
KIDNEY (KD)	NUMBER EXAMINED:	4	4	2	3	4	0	
	NOT REMARKABLE:	0	0	0	0	0	0	
--MINERALIZATION, CORTICAL (AFFECTS TUBULES OR GLOMERULI. OFTEN ACCOMPANIED BY MINIMAL GRANULOCYTIC INFLAMMATION AND/OR FIBROSIS.)		0	1	0	0	1	0	
--MINERALIZATION, MEDULLARY		1	1	0	0	2	0	
--INFILTRATE, LYMPHOHISTIOCYTIC		4	4	2	3	4	0	
--BASOPHILIA, TUBULAR		1	0	1	0	0	0	
--FIBROSIS		0	2	1	0	0	0	
--ECTASIA/PROTRUSION, TUBULAR		0	1	0	0	0	0	
--INFLAMMATION, ACUTE TO SUBACUTE		0	0	0	1	0	0	
--CYTOMEGALY, FOCAL, TUBULAR EPITHELIUM		0	1	0	0	0	0	
--HEMORRHAGE, INTRATUBULAR		0	0	0	1	0	0	
LIVER (LI)	NUMBER EXAMINED:	4	4	2	3	4	0	
	NOT REMARKABLE:	0	0	0	0	1	0	
--VACUOLATION, HEPATOCELLULAR		1	1	0	1	0	0	
--INFILTRATE, LYMPHOHISTIOCYTIC		4	4	1	3	3	0	
--NECROSIS, INDIVIDUAL HEPATOCYTES		0	1	0	0	0	0	
--PARASITISM		0	1	0	0	0	0	
--HYPERPLASIA, BILE DUCT, FOCAL		0	0	1	0	0	0	
HEART (HT)	NUMBER EXAMINED:	4	4	2	3	4	0	
	NOT REMARKABLE:	2	0	2	1	1	0	
--INFILTRATE, LYMPHOHISTIOCYTIC		2	3	0	2	3	0	
--MINERALIZATION, VASCULAR		0	1	0	0	0	0	
--INFILTRATE, NEUTROPHILIC		0	0	0	0	1	0	

TONGUE (TO)	NUMBER EXAMINED:	4	4	2	3	4	0
	NOT REMARKABLE:	2	3	1	1	2	0
--CYST, PROTOZOAL		1	0	0	2	1	0
--INFILTRATE, LYMPHOHISTIOCYTIC		1	0	1	0	1	0
--MINERALIZATION, VASCULAR		0	1	0	0	0	0
SPLEEN (SP)	NUMBER EXAMINED:	4	4	2	3	4	0
	NOT REMARKABLE:	4	3	2	2	4	0
--MINERALIZATION, VASCULAR		0	1	0	0	0	0
--INFILTRATE, MACROPHAGE, VACUCLATED		0	0	0	1	0	0
THYMUS (TH)	NUMBER EXAMINED:	4	4	2	3	4	0
	NOT REMARKABLE:	3	1	0	1	4	0
--DEPLETION, LYMPHOCYTIC		1	2	2	1	0	0
--CYST		0	2	1	2	0	0
LN, MESENTERIC (MS)	NUMBER EXAMINED:	4	4	2	3	4	0
	NOT REMARKABLE:	4	4	1	2	4	0
--DEPLETION, LYMPHOCYTIC		0	0	1	1	0	0
ADRENAL, CORTEX (AC)	NUMBER EXAMINED:	4	4	2	3	4	0
	NOT REMARKABLE:	2	2	1	3	2	0
--HYPERTROPHY, FOCAL/MULTIFOCAL		2	2	1	0	2	0
ADRENAL, MEDULLA (MA)	NUMBER EXAMINED:	4	4	2	3	4	0
	NOT REMARKABLE:	3	2	2	3	3	0
--INFILTRATE, LYMPHOHISTIOCYTIC		0	1	0	0	1	0
--ONE EXAMINED		1	0	0	0	0	0
--MINERALIZATION		0	1	0	0	1	0
STOMACH, GL (ST)	NUMBER EXAMINED:	4	4	2	3	4	0
	NOT REMARKABLE:	4	1	2	2	2	0
--INFLAMMATION, GRANULOMATOUS		0	1	0	0	0	0
--PARASITISM		0	1	0	1	1	0
--MINERALIZATION, VASCULAR		0	1	0	0	0	0
--INFLAMMATION, SUBACUTE, SEROSAL		0	0	0	0	1	0
PANCREAS (PA)	NUMBER EXAMINED:	4	4	2	3	4	0
	NOT REMARKABLE:	2	1	2	2	3	0
--INFILTRATE, LYMPHOHISTIOCYTIC		0	1	0	0	0	0
--HEMORRHAGE, PERI-ISLET, FOCAL TO MULTIFOCAL		1	1	0	0	0	0
--INFLAMMATION, GRANULOMATOUS		1	0	0	0	0	0
--MINERALIZATION, VASCULAR		0	1	0	0	0	0
--ECTOPIC SPLEEN		0	1	0	0	0	0
--INFLAMMATION, VASCULAR		0	0	0	1	0	0
--INFLAMMATION, SUBACUTE		0	0	0	0	1	0
--PIGMENT		0	0	0	0	1	0
CECUM (CE)	NUMBER EXAMINED:	4	4	2	3	4	0
	NOT REMARKABLE:	4	2	1	2	1	0
--INFLAMMATION, ACUTE/SUBACUTE		0	0	0	1	1	0
--PIGMENT, SUBMUCOSAL		0	0	0	0	1	0
--PARASITISM		0	1	1	0	0	0
--INFLAMMATION, GRANULOMATOUS		0	2	0	0	0	0
--MINERALIZATION		0	1	0	0	0	0
--FLATTENING, SUPERFICIAL EPITHELIUM		0	0	0	0	2	0
--EDEMA, SUBMUCOSAL		0	0	0	0	1	0
COLON (CO)	NUMBER EXAMINED:	4	4	2	3	4	0
	NOT REMARKABLE:	3	1	0	2	1	0
--EROSION/ULCERATION		0	0	0	1	0	0
--INFLAMMATION, ACUTE/SUBACUTE		0	1	0	1	1	0
--PARASITISM		0	2	2	0	0	0
--INFLAMMATION, VASCULAR		1	0	0	0	0	0
--MINERALIZATION, VASCULAR		0	1	0	0	0	0
--PIGMENT, SUBMUCOSAL		0	1	0	0	1	0
--INFLAMMATION, GRANULOMATOUS		0	0	0	0	1	0
RECTUM (RE)	NUMBER EXAMINED:	4	4	2	3	4	0
	NOT REMARKABLE:	4	3	0	3	4	0
--EROSION/ULCERATION		0	1	0	0	0	0
--INFLAMMATION, ACUTE/SUBACUTE		0	1	0	0	0	0
--CRYPT ABSCESS		0	0	1	0	0	0
--PARASITISM		0	1	1	0	0	0

LN, MANDIBULAR (MN)	NUMBER EXAMINED:	4	4	2	3	4	0
	NOT REMARKABLE:	3	4	1	2	0	0
--INFILTRATE, NEUTROPHILIC		0	0	1	0	1	0
--HYPERPLASIA, REACTIVE		1	0	0	1	2	0
--INFILTRATE, EOSINOPHILIC		0	0	0	0	1	0
UTERUS (UT)	NUMBER EXAMINED:	0	0	0	3	4	0
	NOT REMARKABLE:	0	0	0	2	3	0
--ADENOMYOSIS		0	0	0	1	0	0
--INFLAMMATION, GRANULOMATOUS		0	0	0	0	1	0
--MINERALIZATION		0	0	0	0	1	0
PROSTATE (PR)	NUMBER EXAMINED:	4	4	2	0	0	0
	NOT REMARKABLE:	0	1	1	0	0	0
--INFILTRATE, LYMPHOHISTIOCYTIC		4	2	1	0	0	0
--IMMATURE		0	1	0	0	0	0
SEMINAL VESICLE (SV)	NUMBER EXAMINED:	4	4	2	0	0	0
	NOT REMARKABLE:	2	2	1	0	0	0
--MINERALIZATION		2	1	1	0	0	0
--IMMATURE		0	1	0	0	0	0
TESTIS (TE)	NUMBER EXAMINED:	4	4	2	0	0	0
	NOT REMARKABLE:	4	3	2	0	0	0
--IMMATURE		0	1	0	0	0	0
--INFILTRATE, LYMPHOHISTIOCYTIC		0	1	0	0	0	0

Week 45: recovery data:

	SEX: -----MALE-----	-----FEMALE----				
	GROUP: -1-	-2-	-3-	-1-	-2-	-3-
	NUMBER:	2	3	2	2	2
LUNG (LU)	NUMBER EXAMINED:	2	2	2	2	2
	NOT REMARKABLE:	0	1	0	0	1
--HYPERPLASIA/HYPERTROPHY, ALVEOLAR/BRONCHIOLAR EPITHELIUM		0	0	0	0	1
KIDNEY (KD)	NUMBER EXAMINED:	2	2	2	2	2
	NOT REMARKABLE:	0	0	0	0	0
--MINERALIZATION, CORTICAL (AFFECTS TUBULES OR GLOMERULI, OFTEN ACCOMPANIED BY MINIMAL		0	0	0	0	1
--BASOPHILIA, TUBULAR		0	0	1	0	1
--ECTASIA/PROTEINOSIS, TUBULAR		0	0	1	0	0
--INFLAMMATION, ACUTE TO SUBACUTE		0	0	0	0	1
LIVER (LI)	NUMBER EXAMINED:	2	2	2	2	2
	NOT REMARKABLE:	1	0	0	0	0
--FIBROSIS		0	0	1	0	0
--B-ADENOMA, HEPATOCELLULAR		0	1	0	0	0
--NECROSIS, COAGULATIVE, FOCAL		0	0	0	0	1
AORTA (AO)	NUMBER EXAMINED:	2	2	2	2	2
	NOT REMARKABLE:	2	2	2	2	1
--HYPERPLASIA/HYPERTROPHY, INTIMAL		0	0	0	0	1
STOMACH, GL (ST)	NUMBER EXAMINED:	2	2	2	2	2
	NOT REMARKABLE:	1	1	2	1	0
--EDEMA/HEMORRHAGE		0	1	0	0	0
--INFLAMMATION, GRANULOMATOUS		0	0	0	0	1
PANCREAS (PA)	NUMBER EXAMINED:	2	2	2	2	2
	NOT REMARKABLE:	1	1	2	1	0
--HEMORRHAGE, PERI-ISLET, FOCAL TO MULTIFOCAL		1	0	0	0	1
--INFLAMMATION, VASCULAR		0	0	0	0	1
--HYPERPLASIA, DUCTULAR, FOCAL		0	1	0	0	0

CECUM (CE)	NUMBER EXAMINED:	2	2	2	2	2	2
	NOT REMARKABLE:	2	1	1	1	1	1
--INFLAMMATION, ACUTE/SUBACUTE		0	1	1	0	0	0
--PIGMENT, SUBMUCOSAL		0	0	1	0	0	0
--PARASITISM		0	0	1	1	1	0
--HEMORRHAGE		0	0	1	0	0	0
--INFLAMMATION, VASCULAR		0	0	0	0	0	1
JEJUNUM (JE)	NUMBER EXAMINED:	2	2	2	2	2	2
	NOT REMARKABLE:	2	2	2	2	2	1
--INFLAMMATION, VASCULAR		0	0	0	0	0	1
ILEUM (IL)	NUMBER EXAMINED:	2	2	2	2	2	2
	NOT REMARKABLE:	2	2	2	2	1	1
--INFLAMMATION, ACUTE/SUBACUTE		0	0	0	0	1	0
--INFLAMMATION, VASCULAR		0	0	0	0	0	1
COLON (CO)	NUMBER EXAMINED:	2	2	2	2	2	2
	NOT REMARKABLE:	0	1	1	1	1	1
.....		-	-	-	-	-	-
--INFLAMMATION, VASCULAR		0	0	0	0	0	1
--INFLAMMATION, GRANULOMATOUS		0	1	0	0	0	0
RECTUM (RE)	NUMBER EXAMINED:	2	2	2	2	2	2
	NOT REMARKABLE:	2	1	2	0	1	1
--HEMORRHAGE		0	0	0	1	0	0
--INFLAMMATION, VASCULAR		0	0	0	0	0	1
LN, MANDIBULAR (MN)	NUMBER EXAMINED:	2	2	2	2	2	2
	NOT REMARKABLE:	1	1	1	2	1	0
--INFILTRATE, NEUTROPHILIC		0	0	1	0	1	1
TESTIS (TE)	NUMBER EXAMINED:	2	2	2	0	0	0
	NOT REMARKABLE:	2	1	2	0	0	0
--MINERALIZATION		0	1	0	0	0	0
LN, AXILLARY (AX)	NUMBER EXAMINED:	0	0	0	0	1	0
	NOT REMARKABLE:	0	0	0	0	0	0
--HYPERPLASIA, REACTIVE		0	0	0	0	1	0

Toxicokinetics:

Dose (mg/kg)	Study Interval	C _{max} (ng/mL)		AUC (ng•hr/mL) ^a	
		Male	Female	Male	Female
1	Day 1	16.4	11.8	38.2	26.3
1	Week 15	13.0	17.5	36.5	38.6
1	Week 28	11.8	19.8	27.1	36.0
1	Week 41	28.7	31.3	56.2	54.1
3	Day 1	50.2	55.6	148.7	130.5
3	Week 15	22.7	47.4	107.3	118.2
2	Week 28	21.0	29.4	85.7	79.1
2	Week 41	55.5	52.4	146.3	93.1
10	Day 1	291.1	244.7	949.0	755.2
4.5	Week 15	115.7	68.9	315.9	206.4
4.5	Week 28	ND	ND	ND	ND
4.5	Week 41	ND	ND	ND	ND

ND = Not determined; due to toxicity, the high-dose group was terminated during Week 26.

a Calculated from time zero to the time of last measurable concentration, ranging between 8 to 24 hours.

Toxicokinetic Ratios

Dose Ratio	Study Interval	C _{max} Ratios		AUC Ratios	
		Male	Female	Male	Female
1:3:10	Day 1	1: 3.1: 17.8	1: 4.7: 20.7	1: 3.9: 24.8	1: 5.0: 28.7
1:3:4.5	Week 15	1: 1.7: 8.9	1: 2.7: 3.9	1: 2.9: 8.7	1: 3.1: 5.3
1:2	Week 28	1: 1.8	1: 1.5	1: 3.2	1: 2.2
1:2	Week 41	1: 1.9	1: 1.7	1: 2.6	1: 1.7

- Systemic exposure to BMS-354825 on Day 1 increased in a greater-than-dose proportional manner between 1 and 10 mg/kg.
- However, for Weeks 15, 28, and 41, the systemic exposure increased in a generally dose-proportional manner.
- Systemic exposure to BMS-354825 was generally similar between females and males.
- There was no obvious accumulation or reduction in the exposure of BMS-354825 over the 41 weeks of dosing.
- As expected, exposures were generally lower at later times in the HD and MD groups as the dose was reduced.

Summary of the study:

BMS-354825 was administered by gavage (oral or nasogastric) at initial daily doses of 1, 3, or 10 mg/kg. The control group received the vehicle, 80 mM sodium citrate buffer. Doses of 1, 3, and 10 mg/kg were selected for this study based on the results of 10-day and 1-month oral toxicity studies in monkeys administered BMS-354825 on a 5-days-on, 2-days-off repeating schedule. Dose reductions and interruptions occurred several times during the study. Dose interruptions of approximately 30 days were reported. Dose reductions were done for MD and HD. The MD was reduced to 2.5 mg/kg and the HD was reduced to 6 mg/kg then to 4.5 mg/kg. Dose schedule was also changed from continuous dosing to 5-days-on 2-days-off early in the study.

Due to extreme toxicities, dosing at HD terminated during Week 22 (after ~4.5 months of dosing). Recovery sacrifice for this group took place on Week 26. Because no control data was available for either Week 22 or Week 26, when possible, HD results were compared to the controls on Weeks close to that for the HD.

Mortalities at MD and HD appeared to be primarily due to the GI toxicity (MD: 2/6 ♂s and 4/6 ♀s; HD: 2/6 ♂s and 3/6 ♀s). Lymphoid depletion, renal toxicity (↑creatinine, ↑BUN, cortical mineralization of tubules and glomeruli, fibrosis, and tubular ectasia/proteinosis), and vascular inflammation and mineralization were also recorded.

Major Findings in Unscheduled sacrifices	
Clinical observations	MD and HD: <ul style="list-style-type: none"> • Abnormal feces (discolored/red, liquid, mucoid, nonformed feces), vomitus, hunched posture, low or no food consumption, and decreases in individual body weights. HD: <ul style="list-style-type: none"> • Intermittent ataxia in one male, persistent ataxia and tremors prior to euthanasia in 1 ♀, and decreased frequency of menstrual cycling in females.
Clinical pathology	MD and HD:

	<ul style="list-style-type: none"> • ↓RBC (50-90% of pretest), ↓lymphocytes (20-30%), ↓hemoglobin (50-90%), hematocrit (↓50-80%), • ↓albumin (60-80%), ↓Na (80-90%), ↓K (60%), and ↓Cl (70-80%) and ↑ total leukocytes (1.3-2.3-fold increase of pretest), ↑neutrophils (1.3-6.6-fold), ↑fibrinogen (1.2-1.7-fold), ↑BUN (3.1-9.2-fold), and ↑creatinine (1.4-13.8-fold).
Gross Pathology	<p>MD and HD:</p> <ul style="list-style-type: none"> • GI tract: red foci in the large intestine and/or stomach. <p>HD:</p> <ul style="list-style-type: none"> • GI tract: erosion/ulceration in the stomach (1 ♀), enlarged, gas-distended (1 ♂); and red fluid contents in the stomach and small intestine (1 ♂).
Histopathology	<p>MD and HD:</p> <p>GI Tract:</p> <ul style="list-style-type: none"> • villous blunting/fusion • Stomach: minimal-moderate erosion/ulceration; minimal-moderate inflammation. • Small intestine: minimal to moderate villous blunting and/or fusion. Moderate erosion/ulceration and slight/minimal acute to Subacute inflammation. • Large intestine: minimal to moderately severe erosion/ulceration of the cecum, colon, and/or rectum; minimal to moderately severe acute to subacute inflammation; slight to moderate superficial epithelial flattening in the large intestine. • Rectum: ulceration, hemorrhage <p>Additional findings at MD and/or HD:</p> <ul style="list-style-type: none"> • A MD ♀ (3 mg/kg/day) had slight vascular inflammation in the heart and lacrimal gland. • Lymphocytic depletion in thymus and/or spleen • Findings in the kidney and testis.

In animals that survived to the scheduled necropsy, the primary toxicity appeared to be in the GI tract. GI toxicity was observed at all dose levels, but was most evident at MD and HD. Those included: erosion/ulceration, hemorrhage, inflammation, superficial epithelial flattening, in addition to liquid/red feces and vomiting.

Clinical signs of toxicity seen in all HD animals, most MD animals, and infrequently at LD, with an onset usually during Week 1 or 2, consisted of abnormal feces (red/ liquid, mucoid, non-formed, and/or decreased), low or no food consumption, and vomitus. Additional clinical sign at HD and MD was hunched posture.

Reduced BW of 3-18% seen at HD throughout the study was reversible.

In general hematology and serum chemistry results were inconclusive: no corresponding control was available for HD animals for either end of treatment or end of recovery period. In addition, dosing interruptions in MD and HD animals, could have affected the magnitude of changes in the clinical pathology parameters.

Urinalysis did not show remarkable/clear signs of toxicity. Week 15 hematology parameters showed no remarkable changes for LD and MD animals. HD animals had thrombocytopenia. ↑WBC, ↑neutrophils, and ↑reticulocytes at HD on week 15 appeared to be secondary to internal injury and inflammation. Because no Week 22 clinical pathology control data was available, the reviewer compared these data to the Week 28

control data. Hematology parameters on Week 22 (HD) included ↑reticulocytes, ↑neutrophils (♂s only), ↑monocytes, ↑fibrinogen, and ↑aPTT (♂s only). End of treatment (Week 41) hematology showed ↓lymphocytes in MD ♂s and ♀s. BUN was increased in HD animals on Week 22, as compared to Week 28 control data and there was an 80% increase in triglycerides in MD ♀s at Week 41.

There was no apparent effect on the EKG, based on the individual animal data (summary data was not presented). There appear to be no effect on the heart rate or the respiratory rate. No effect was observed for body temperature.

Microscopic findings which appeared to be drug-related were observed in the:

- GI tract: inflammation, ulceration/erosion, edema, flattening of superficial epithelium, rectal crypt abscess
- lymphoid organs: lymphocytic depletion of thymus and mesenteric lymph node
- kidney: cortical mineralization of tubules and glomeruli, fibrosis, inflammation, tubular ectasia/proteinosis
- lung: hyperplasia/hypertrophy, hemorrhage, fibrosis, inflammation
- liver: hepatocellular necrosis
- Bile duct: hyperplasia
- Pancreas: inflammation
- Adrenal medulla: mineralization
- Male reproductive organs: immature prostate, seminal vesicle, and testis
- Female reproductive organ: mineralization and inflammation of uterus
- Multiple organs: vascular mineralization e.g. in heart, tongue, spleen, stomach, and pancreas

At all doses, an increased incidence and/or severity in mineralization of the kidney was observed at unscheduled necropsies and at the scheduled interim and terminal necropsies. Mineralization of the kidney at the intermediate and high doses was generally accompanied by granulomatous inflammation and/or fibrosis. At the recovery necropsies, minimal mineralization of the kidney was observed at similar incidences in controls and BMS-354825-treated groups. Therefore, this finding was considered a drug-related exacerbation of a background lesion. Vascular mineralization and pigmentation were seen in multiple organs.

In summary the following toxicities were detected; most findings were based on the histopathology results since the clinical pathology was inconclusive:

- GI tract: vomiting, red/liquid feces, inflammation, ulceration/erosion, edema, flattening of superficial epithelium, rectal crypt abscess
- lymphoid organs: ↓lymphocytes, lymphocytic depletion of thymus and mesenteric lymph node
- kidney: cortical mineralization of tubules and glomeruli, fibrosis, inflammation, tubular ectasia/proteinosis, ↑BUN
- lung: hyperplasia/hypertrophy, hemorrhage, fibrosis, inflammation
- liver: hypertrophy, hepatocellular necrosis, ↑triglycerides
- Bile duct: hyperplasia
- Pancreas: inflammation
- Adrenal medulla: mineralization

- Male reproductive organs: immature prostate, seminal vesicle, and testis
- Female reproductive organ: mineralization and inflammation of uterus
- Multiple organs: vascular mineralization e.g. in heart, tongue, spleen, stomach, and pancreas

Summary of the repeat-dose toxicology studies conducted in monkeys

Repeated dosing of dasatinib in cynomolgus monkeys for 10 days, 1-month, or 9 months resulted in severe toxicities to the GI tract (vomiting, red/liquid feces, inflammation/ulceration/hemorrhage of the GI tract, abnormalities in villi) and to the lymphocytes and lymphoid organs (\downarrow lymphocytes/lymphoid depletion), regardless of the duration of the study. Renal toxicities was mainly evident by histopathology examination and included degeneration of cortical tubular epithelial cells; dilatation of cortical tubules in the 10-day study as well as cortical mineralization of tubules and glomeruli, fibrosis, inflammation, tubular ectasia/proteinosis in the 9-month study. It should be noted that there was no strong serum chemistry changes indicative of renal toxicities. BUN was not always increased and dehydration of the animals could have contributed to this finding; no changes were observed in the serum creatinine. Hypo-phosphatemia observed in the 10-day study may have been secondary to the renal damage.

Although not strong, hepatotoxicity manifested in the 1-month and 9-month toxicology studies: liver hypertrophy, small incidence of hepatocellular necrosis, \uparrow triglycerides, \downarrow albumin. There were slight increases in the hepatic enzymes; however, changes were not toxicologically significant.

Organ/tissues that were affected in the 9-month study but not in the shorter term repeat-dose studies included the following: bile duct (hyperplasia), lung (hyperplasia/hepertrophy, fibrosis, hemorrhage, and inflammation), adrenal medulla (mineralization), reproductive organs (♂ : immature prostate/ seminal vesicle/ testis; ♀ : mineralization and inflammation of uterus), pancreas (inflammation). Of note, multiple organs presented with vascular mineralization in the 9-month study, e.g. in heart, tongue, spleen, stomach, and pancreas.

10-day oral toxicology (study not reviewed):

Doses administered:

1, 10, and 15 mg/kg (12, 120, and 180 mg/m²); 5-days-on 2-days-off x 2 cycles.

Dose (mg/kg)	Study Day	C _{MAX} (μ M)		AUC (0-24 hr)	
		M	F	M	F
1	1	0.03	0.03	0.17	0.06
10	1	0.88	0.73	2.71	1.69
15	1	1.16	0.74	3.39	2.68

Animals survived to the scheduled necropsy on Day 13. The following toxicities were noted:

- GI tract: vomiting, red/liquid feces, ulceration, hemorrhage, edema, inflammation, enterocyte vacuolation and villous fusion.
- Lymphocytic system: lymphoid depletion
- Kidney: degeneration of cortical tubular epithelial cells; dilatation of cortical tubules
- ↑WBC, neutrophils, and monocytes appear to be secondary to the internal injury/inflammation

1-Month oral toxicology study

Doses administered:

1, 5, and 15 mg/kg (12, 60, and 180 mg/m²); 5-days-on 2-days-off x 4 cycles (4 weeks)

BMS-354825							
Dose [mg/kg/day]	Study Day	Cmax (ng/mL)		Tmax ^a (h)		AUCT ^b (ng•h/mL)	
		Male	Female	Male	Female	Male	Female
1	1	20	11	1.5	1	36	17
	26	12	8	2	1.5	34	16
5	1	91	93	1.5	1	206	221
	26	50	64	1.5	2	181	280
15	1	480	399	1	1.5	1162	1053
	26	154	374	1.5	1.5	774	976
Ratios							
1:5:15 Dose Ratio	1	1:4.6:24	1:8.5:36.3			1:5.7:32.3	1:13.6:1.9
	26	1:4.2:12.8	1:8:46.8			1:5.3:22.8	1:17.5:61

^a Median value

^b Calculated from time zero to the time of last measurable concentration, equal to 24 h, ranging between 8 to 24 h.

There were no unscheduled deaths during the study. The following organs/tissues/parameters were affected;

- GI tract: vomiting, diarrhea, gas/fluid-filled contents of the cecum and colon
- Lymphocytic system: lymphoid depletion in spleen and thymus; ↓thymic weight
- Liver: slightly ↑AST and ALT, (↓albumin), liver hypertrophy, focal necrosis (in 1 ♂)
- Heart: inflammation, hypertrophy
- Kidney: inflammation
- Phosphorus: hypo-phosphatemia
- ↑WBC, neutrophils, and monocytes appear to be secondary to the inflammation seen in multiple organs and possible internal injuries.

9-Month oral toxicology study

Doses administered:

Initial dose: 1, 3, 10 mg/kg/day x 8
No dosing on Days 9-15

Modified dose (from D16): 1, 3, 6 mg/kg; 5-days on/ 2-days off
 Modified dose (from D83): 1, 3, 4.5 mg/kg; 5-days on/ 2-days off (x 22 weeks for HD)
 Modified dose (from D190): 1, 2.5, no HD animal; 5-days on/ 2-days off (12, 24 mg/m², 5-days on/ 2-days off x 41 weeks for LD and MD)

Dose (mg/kg)	Study Interval	C _{max} (ng/mL)		AUC (ng•hr /mL) ^a	
		Male	Female	Male	Female
1	Day 1	16.4	11.8	38.2	26.3
1	Week 15	13.0	17.5	36.5	38.6
1	Week 28	11.8	19.8	27.1	36.0
1	Week 41	28.7	31.3	56.2	54.1
3	Day 1	50.2	55.6	148.7	130.5
3	Week 15	22.7	47.4	107.3	118.2
2	Week 28	21.0	29.4	85.7	79.1
2	Week 41	55.5	52.4	146.3	93.1
10	Day 1	291.1	244.7	949.0	755.2
4.5	Week 15	115.7	68.9	315.9	206.4
4.5	Week 28	ND	ND	ND	ND
4.5	Week 41	ND	ND	ND	ND

ND = Not determined; due to toxicity, the high-dose group was terminated during Week 26.

a Calculated from time zero to the time of last measurable concentration, ranging between 8 to 24 hours.

Due to toxicities, doses were reduced at MD and HD and dosing interruptions of up to 30 days took place in several MD and HD animals.

The following toxicities were detected; most findings were based on the histopathology results since the clinical pathology was inconclusive:

- GI tract: vomiting, red/liquid feces, inflammation, ulceration/erosion, edema, flattening of superficial epithelium, rectal crypt abscess
- lymphoid organs: ↓lymphocytes, lymphocytic depletion of thymus and mesenteric lymph node
- kidney: cortical mineralization of tubules and glomeruli, fibrosis, inflammation, tubular ectasia/proteinosis, ↑BUN
- liver: hypertrophy, hepatocellular necrosis, ↑triglycerides
- lung: hyperplasia/hypertrophy, hemorrhage, fibrosis, inflammation
- Bile duct: hyperplasia
- Pancreas: inflammation
- Adrenal medulla: mineralization
- Male reproductive organs: immature prostate, seminal vesicle, and testis
- Female reproductive organ: mineralization and inflammation of uterus
- Multiple organs: vascular mineralization e.g. in heart, tongue, spleen, stomach, and pancreas
- ↑WBC, ↑neutrophils, and ↑reticulocytes appeared to be secondary to internal injury and inflammation. No clear serum chemistry or hematology changes were noted (due to the lack of appropriate control animals and interruptions in dosing).

2.6.6.4 Genetic toxicology

Study title: Spiral Ames Reverse-Mutation Study in Salmonella

Key findings: The sponsor concluded that dasatinib was not mutagenic in bacterial strains TA98 and TA100. Since this was an exploratory study, presented without full protocol or data, a definite conclusion cannot be drawn.

Study no: DS01124 (#920012131)

Volume #, and page #: Item 5

Conducting laboratory and location: BMS, Syracuse, NY

Date of study initiation: July 7, 2001

GLP compliance: no

Drug, lot #, radiolabel, and % purity: not provided

Formulation/vehicle: Dimethyl sulfoxide (DMSO)

Test agent stability: not provided.

Methods:

Species/ Strains: *Salmonella typhimurium* tester strains TA98, TA100

Basis of dose selection: This was an exploratory study utilizing 21 - 5000µg/plate.

Metabolic activation system: rat liver S9.

Controls:

Vehicle: DMSO

Negative controls: Vehicle control

Positive controls: not provided

Exposure condition: 48 hours incubation at 37°C

Analysis:

No. of replicates: Triplicate for dasatinib and negative controls
Duplicate for positive controls

Counting method: not provided

Criteria for positive results:

A positive response was indicated by a two-fold or greater dose related elevation of mean histidine positive revertant counts, with respect to spontaneous (negative control) counts

Summary of individual study findings:

Study validity: Not enough information to evaluate.

Test Article	TA98 +S9		TA98 -S9		TA100 +S9		TA100 -S9	
	Fold	Result	Fold	Result	Fold	Result	Fold	Result
BMS-354825	1.7	Neg	1.5	Neg	2.1*	Neg	1.9	Neg

Fold: Largest fold increase in mean revertant counts compared to negative-control revertant counts

Neg: Negative

* Isolated elevation was not dose related.

Study outcomes:

- 2.1 fold increase in revertant counts with respect to negative control counts was observed with BMS-354825 in the plate at 21 µg/plate. This finding was not dose-dependent and was observed at the lowest concentration tested, hence, it was not considered to be test article-related.
- Summary indicates these findings but data was not presented:
 - Minimal to marked cytotoxicity to both strains was noted in the presence and absence of S-9 at concentrations ≥ 880 µg/plate, as measured by decreased bacterial lawn density and/or histidine⁺ revertant colonies.
 - Significant increases in histidine⁺ revertants were observed in the cultures treated with positive control articles.

Conclusions:

Based on 1) the summary provided for this exploratory Ames test and 2) the criteria for positive results, dasatinib was not mutagenic in TA98 and TA100 bacterial strains, up to the highest concentration (5000 µg/plate) tested, in the absence or presence of S9 metabolic activation system. In the absence of the data, a definite conclusion cannot be made.

Study title: Exploratory Ames Reverse-Mutation Study in Salmonella

Key findings: Under the conditions tested, BMS 354825 appeared to be negative (non-mutagenic) in the bacterial reverse mutation assay. This was an exploratory study.

Study no: DS02066
Volume #, and page #: Item 5
Conducting laboratory and location: BMS; Syracuse, NY
Date of study initiation: April 2002
GLP compliance: No
Drug, lot #, radiolabel, and % purity: not provided
Formulation/vehicle: Dimethyl sulfoxide (DMSO)
Test agent stability: not provided

Methods:

Species/Strains: *S. typhimurium* TA98, TA100
Dose selection criteria: This was an exploratory study. An initial assay, up to the highest recommended concentration (5000 µg/plate) was conducted. Because of the uncertainty in results of the TA100 strain, the study was repeated (repeat assay), using TA100 only.

Initial Assay Date: April 10, 2002:**Sample Size:** Test article, n = 2; Positive control, n = 2; Negative control, n = 5**Concentrations:** 5, 16, 50, 160, 500, 1600, and 5000 µg/plate**Repeat Assay Date: April 23, 2002:****Sample Size:** Test article, n = 3; Positive control, n = 2; Negative control, n = 5**Concentrations:** 10, 50, 100, 200, 300, 400, and 500 µg/plate**Volume:** 100 µl/plate**Metabolic activation system:** rat liver S9.**Controls:**

Vehicle: DMSO)

Negative controls: Vehicle control

Positive controls: 2-Aminoanthracene, 2 Nitrofluorene, and Sodium Azide

Exposure conditions: 48 hours incubation at 37°C**Analysis:****No. of replicates:** n=2 or 3 for test article (initial assay and repeat assay, respectively), n=2 for positive control, n=5 negative control.**Counting method:** not provided**Criteria for positive results:** A positive response was indicated by a two-fold or greater dose related elevation of mean histidine positive revertant counts, with respect to spontaneous (negative control) counts**Summary of individual study findings:****Study validity:**

The sponsor presented data that met the following criteria.

- Positive control values (mean positive control value exhibited \geq three-fold increase over the mean vehicle control for each tester strain).
- Toxicity levels

Not all criteria required for a valid test were provided, e.g. the following information was not presented:

- Confirmation of the presence of the *rfa* wall mutation by demonstrating sensitivity to crystal violet and confirmation of R-factor plasmid by verifying resistance to ampicillin in tester strain cultures of TA98 and TA100
- Spontaneous revertant background frequency based on historical control data
- Tester strain titers

Study outcomes:**Initial assay:**

- In the initial study, precipitate formation was noted following the addition of 1600 and 5000 µg concentrations, the 2 highest concentrations tested.

- Marked reduction to annihilation of the bacterial background lawn was evident at concentrations $\geq 500\mu\text{g}/\text{plate}$ in both strains, with and without metabolic activation. Due to excessive cytotoxicity at concentrations $\geq 1600\mu\text{g}/\text{plate}$, revertants were not scored.
- A statistically significant increase in mean revertants in the TA100 strain, with and without metabolic activation, was noted when $500\mu\text{g}/\text{plate}$ was utilized. However, it was stated that counts were not reliable due to a problem with this isolate of the strain TA100, hence, repeat assay was conducted with this strain.

Test Article	Concentration ($\mu\text{g}/\text{plate}$)	TA98 ¹	TA98 ²	TA100 ¹	TA100 ²
		Mean \pm SD ³			
DMSO	100 $\mu\text{l}/\text{plate}$	32 \pm 10	24 \pm 6	142 \pm 7	117 \pm 9
BMS-354825	5	32	21	142	96
	16	24	25	134	117
	50	31	32	144	116
	160	32	22	149	106
	500	14	9	307**	201**
	1600	*	0	*	*
	5000	0	0	*	0
2-Aminoanthracene	2.5	640	-	847	-
2-Nitrofluorene	2	-	829	-	-
Sodium Azide	2	-	-	-	848

¹In the presence of metabolic activation

²In the absence of metabolic activation

³Standard Deviation

-Not tested

*Could not score due to excessive cytotoxicity

**Counts not reliable due to a problem with this isolate of strain TA100 and/or excessive cytotoxicity

Repeat assay:

- In the repeat assay, normal growth was observed for the tester strain (TA100) at concentrations of up to $300\mu\text{g}/\text{plate}$. Reduction in bacterial background lawn occurred in a dose dependant manner and was greater in the absence of metabolic activation.
- Dasatinib was not mutagenic in TA100 under the conditions of this study.

Test Article	Concentration ($\mu\text{g}/\text{plate}$)	TA100 ¹	TA100 ²
		Mean \pm SD ³	Mean \pm SD ³
DMSO	100 $\mu\text{l}/\text{plate}$	109 \pm 6	110 \pm 11
BMS-354825	10	113	114
	50	123	107
	100	141	104
	200	118	104
	300	120	92
	400	79	77
	500	53	*
2-Aminoanthracene	2.5	1900	-
Sodium Azide	2	-	820

Conclusions:

Under the conditions of this study, BMS 354825 did not cause positive increases in mean number of revertants per plate in tester strains TA98 or TA100, in the presence or absence of Aroclor™ induced rat liver (S9) activation system. It should be noted that this was an exploratory study and all criteria for a valid test were not presented.

Study title: Reverse Mutation Study in *Salmonella typhimurium* and *Escherichia coli*

Key findings: BMS354825 was negative (non-mutagenic) in the bacterial reverse mutation assay under the conditions assayed

Study no: DS02193

Volume #, and page #: Item 5

Conducting laboratory and location: BMS; Syracuse, NY

Date of study initiation: October 23, 2002

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, radiolabel, and % purity: BMS-354825

Lot/Batch # C007A-354825-01, purity → (free base)

Note: this study was conducted before the Certificate of Analysis was available.

Therefore, dose concentrations were corrected using an estimated purity of →

Formulation/vehicle: Dimethyl sulfoxide

Methods:

Species/ strains: *S. typhimurium* tester strains TA98, TA100, TA1535, and TA1537, and *Escherichia coli* tester strain WP2uvrA.

Dose selection criteria:

Basis of dose selection: Dose selection was based on the result of two preliminary assays.

Range finding studies: In the first assay, 7 dose levels (10, 25, 50, 100, 200, 300 and 400 µg/plate) of the test article were plated, with a 48 hour culture of each strain in both the presence and absence of rat liver S9 activation. No precipitate was observed in this assay. A second range-finding assay evaluated BMS354825 in the E.Coli strain, at 4 additional concentrations (800, 1600, 3200, and 5000 µg/plate) with the same study design. Cytotoxicity was detected at concentrations ≥300 µg/plate in all strains with the exception of WP2 *uvrA*. Toxicity was defined by decreased lawn growth and reductions and/or decreased in mean revertant count. Toxicity was evident in the WP2 *uvrA* strain at concentration ≥ 3200µg/plate. Precipitate precluded analysis of the 5000µg/plate group.

Test agent stability: The stability of BMS-354825 in the vehicle (DMSO) was accepted based on the concentration analyses conducted for the highest and the lowest concentrations prepared, i.e. 16 mg/mL stock solution of BMS-354825 in

DMSO and the 0.125 mg/mL solution (prepared by diluting the stock solution in DMSO)

Metabolic activation system: Aroclor 1254-induced rat liver S9.

Controls:

Vehicle: Dimethyl sulfoxide (DMSO)

Negative controls: Vehicle control

Positive controls: See table below:

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
TA98	+	2-aminoanthracene	2.5
	-	2-nitrofluorene	2
TA100	+	2-aminoanthracene	2.5
	-	Sodium azide	2.0
TA1535	+	2-aminoanthracene	2.5
	-	Sodium azide	2.0
TA1537	+	2-aminoanthracene	2.5
	-	9 aminoacridine	100
WP2uvrA	+	2-aminoanthracene	10
	-	Methyl methane sulfonate	2.5

Note: According to the OECD Guideline for Bacterial Reverse Mutation Test: 2-aminoanthracene should not be used as the sole indicator of the efficacy of the S9-mix. If 2-aminoanthracene is used, each batch of S9 should also be characterized with a mutagen that requires metabolic activation by microsomal enzymes, e.g., benzo(a)pyrene, dimethylbenzanthracene. The sponsor stated that the supplier confirmed the ability of the S9 to activate both benzo(a)pyrene and 2-aminoanthracene to intermediates mutagenic to test strain TA100.

Exposure conditions:

Incubation and sampling times: The plates were incubated for approximately 48 hours at 37°C.

Doses used in definitive study:

- 12.5, 25, 50, 100, 200, and 400 µg/plate in *S. typhimurium* strains
- 50, 100, 200, 400, 800, and 1600 µg/plate in the *E. coli* strain

Study design: Plate incorporation method

Analysis:

No. of replicates: Duplicates for the 2 range-finding assays
Triplicates for the definitive study

Counting method: Revertant colonies for a given tester strain and activation condition were counted manually or by — automated colony counter

Criteria for positive results:

- **Strains TA1535, and TA1537:** Data sets were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than three times the mean vehicle control value and had to be accompanied by a dose response to increasing concentrations of the test article.

- **Strains TA98 and TA100 and WP2 *uvrA*:** Data sets were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than two times the mean vehicle control value and had to be accompanied by a dose response to increasing concentrations of the test article.
- Increases in revertant counts for all strains must be concentration-dependant
- A positive response in any one tester strain either with or without metabolic activation is sufficient to designate the test article as a bacterial mutagen.

Summary of individual study findings:

Study validity: Criteria for a valid test consisted of the following:

- *rfa* Wall Mutation: Confirmation of the presence of the *rfa* wall mutation by demonstrating sensitivity to crystal violet for all Salmonella strains. Resistance to crystal violet in the *E. coli* strain to demonstrate the absence of the *rfa* mutation.
- R-factor plasmid: Confirmation of the presence of the R-factor plasmid pKM101 by demonstrating resistance to ampicillin in tester strains TA98 and TA100. The *S. typhimurium* strains TA1535 and TA1537, and the *E. coli* WP2 *uvrA* must exhibit sensitivity to ampicillin to demonstrate the absence of the pKM101 plasmid.
- Range of spontaneous revertants: Spontaneous revertant background frequency based on historical control data. The following was provided as the range of spontaneous revertants:

The established range of spontaneous revertants per plate is as follows:

TA98	10-65
TA100	50-200
TA1535	2-35
TA1537	1-25
WP2 <i>uvrA</i>	10-60

The mean number of revertants /plate for the negative control must fall within the stated range.

- Positive control values: mean positive control value must exhibit \geq three-fold increase over the mean vehicle control for each tester strain.
- Confirmation/ toxicity: plates containing the high concentration of the test article with and without S9 must exhibit no greater than 2 colony forming units per plate.

Study outcomes:

- Cytotoxicity was observed in each of the strains at the highest concentrations tested, with and without metabolic activation. No precipitate was observed; toxicity was observed at ≤ 1000 μg per plate.
- The positive controls induced satisfactory mutagenic responses.
- The mean vehicle control (revertants per plate) values fell in the range of historical data for all tester strains in the presence or absence of S9 activation.

- Under the conditions of this study, BMS354825 did not cause positive increases in mean number of revertants per plate in any tester strains in the presence or absence of Aroclor™ induced rat liver (S9) (see tables below)

Mean Revertant Counts: Definitive (Full) Assay

In the Presence of S9 Metabolic Activation¹

Test Article	(µg/plate)	TA98	TA100	TA1535	TA1537	WP2 <i>uvrA</i>
BMS-354825 (in DMSO)	0	17 ± 9	133 ± 5	8 ± 2	4 ± 2	12 ± 3
	12.5	18 ± 5	141 ± 1	9 ± 3	6 ± 1	-
	25	13 ± 4	136 ± 7	10 ± 1	6 ± 3	-
	50	14 ± 4	131 ± 9	12 ± 5	4 ± 1	15 ± 1
	100	17 ± 5	122 ± 15	11 ± 6	7 ± 5	17 ± 5
	200	13 ± 2	117 ± 32	10 ± 5	8 ± 2	13 ± 3
	400	15 ± 2	96 ± 7	10 ± 2	4 ± 2	14 ± 2
	800	-	-	-	-	13 ± 2
1600	-	-	-	-	12 ± 3	
2-AA	2.5	1124	1516	194	46	-
2-AA	10	-	-	-	-	314

In the Absence of S9 Metabolic Activation¹

Test Article	(µg/plate)	TA98	TA100	TA1535	TA1537	WP2 <i>uvrA</i>
BMS-354825 (in DMSO)	0	11 ± 2	120 ± 14	8 ± 1	4 ± 3	9 ± 3
	12.5	14 ± 2	108 ± 4	7 ± 4	2 ± 3	-
	25	13 ± 2	110 ± 23	5 ± 2	7 ± 1	-
	50	13 ± 1	118 ± 10	5 ± 2	6 ± 2	12 ± 3
	100	14 ± 6	99 ± 7	10 ± 1	4 ± 4	13 ± 2
	200	15 ± 2	107 ± 10	7 ± 2	6 ± 2	11 ± 1
	400	6 ± 3	72 ± 9	5 ± 3	1 ± 1	11 ± 4
	800	-	-	-	-	10 ± 5
1600	-	-	-	-	10 ± 4	
2-NF	2	1029	-	-	-	-
Na Az	2	-	991	803	-	-
9-AA	100	-	-	-	768	-
MMS	2.5 µl/plate	-	-	-	-	388

¹Mean ± Standard Deviation

2-AA, 2-animoanthracene; 2-NF, 2-nitrofluorene; Na Az, sodium azide;

9-AA, 9-aminoacridine; MMS, methyl methane-sulfonate

- Not tested

Conclusions:

Dasatinib was not mutagenic in the bacterial reverse mutation assays under the conditions assayed for *S. typhimurium* strains TA98, TA100, TA1535, TA1537, and for *E. coli* strain WP2 *uvrA*.

Study title: Cytogenetics study in Chinese Hamster Ovary (CHO) cells

Key findings: BMS-354825 was clastogenic in CHO cells, in the 4 hr-treatment in the presence and absence of S9 metabolic activation mix and in the 20-hr treatment in the absence of S9.

Study no.: DS03025

Volume #, and page #: Item 5

Conducting laboratory and location:

Date of study initiation:

Jan 22, 2003

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: BMS-354825, Batch # C007A-354825-01. —

Concentration analyses indicated that concentrations were within 10% of target.

Methods

Strains/species/cell line: Chinese Hamster Ovary (CHO-K1) cells

This cell line has an average cell cycle time of 10-14 hours with a modal chromosome number of 20.

Concentrations used in definitive study:

Treatment Condition	Treatment Time	Recovery Time	Concentrations (µg/mL)
Non-activated	4 hr	16 hr	2.5, 5, 10, 20, 40, 60, 80
	20 hr	0 hr	2.5, 5, 10, 20, 40, 60, 80
S9 activated	4 hr	16 hr	2.5, 5, 10, 20, 40, 60, 80.

Based on the findings of the concurrent toxicity test, the dose levels selected for microscopic analysis were as follows:

Non-activated 4-hr treatment group: 5, 20, and 60 µg/mL
 S9-activated 4-hr treatment group: 5, 10, and 40 µg/mL
 Non-activated 20-hr treatment group: 2.5, 5, and 10 µg/mL.

Substantial toxicity (i.e., at least 50% cell growth inhibition relative to the solvent control) was observed at concentrations ≥ 60 µg/mL in the non-activated 4 hour exposure group and at concentrations ≥ 40 µg/mL in the activated 4 hour exposure group and at concentrations ≥ 20 µg/mL in the non-activated 20 hour exposure group.

Basis of dose selection: A preliminary toxicity test was performed to establish the dose range for the chromosome aberration assay.

- In the preliminary toxicity assay, the maximum dose tested was 2500 µg/mL.
- Visible precipitate was observed in treatment medium at concentrations ≥ 250 µg/mL at the beginning of the treatment period.
- Concentrations ≤ 75 µg/mL were soluble in treatment medium.

- Selection of concentrations for the chromosome aberration assay was based on cell growth inhibition relative to the solvent control. Substantial toxicity (i.e, at least 50% cell growth inhibition, relative to the solvent control) was observed at concentrations $\geq 75 \mu\text{g/mL}$ in the non-activated 4 and 20 hour exposure groups and in the S9 activated 4 hour exposure group.

Vehicle: DMSO

Negative controls: solvent (DMSO) alone

Positive controls:

- Mitomycin C dissolved in water at a concentration within 0.05-0.3 $\mu\text{g/ml}$ as the positive control in the non-activated study.
- Cyclophosphamide dissolved in water at a concentration within 10-50 $\mu\text{g/ml}$ as the positive control in the S9-activated study.

Incubation and sampling times: incubation at 37°C in a humidified atmosphere of 5% CO₂ in air. CHO cells were exposed to solvent alone or to different concentrations of the test article for 4 hours in both the presence and absence of S9 activation (harvest after an additional 16-hr incubation in the absence of the test article), and for 20 hours continuously in the absence of S9 activation.

Metabolic activation system: Aroclor 1254-induced rat liver S9

Study design:

- Duplicate cultures of CHO cells were exposed to the test article as well as positive and solvent controls.
- A concurrent toxicity test was conducted in both the non-activated and the S9 activated test systems. After cell harvest an aliquot of the cell suspension was removed from each culture and counted using a Coulter counter.
- Cell viability was determined by trypan blue dye exclusion. The cell counts and percent viability were used to determine cell growth inhibition relative to the solvent control.
- The presence of test article precipitate was assessed using the unaided eye.
- Selection of concentrations for microscopic analysis was based on cell growth inhibition (the lowest dose with at least 50% reduction in cell growth and two lower doses) in the non-activated and S9 activated 4 hour exposure groups, and on mitotic inhibition (the lowest dose with at least 50% reduction in mitotic index relative to the solvent control and two lower doses) in the non-activated 20 hour exposure group.

Results

Study validity:

- Duplicate cultures of CHO cells were exposed to the test article as well as positive and solvent controls.
- The frequency of cells with structural chromosome aberrations in the solvent control must be within the range of the historical solvent control.
- The percentage of cells with chromosome aberrations in the positive control must be statistically increased ($p < 0.05$, Fisher's exact test) relative to the solvent control.
- The historical control data for years 1999-2001 are presented below.

Historical control values (1999-2001): structural aberrations

NON-ACTIVATED TEST SYSTEM

Historical Values	Solvent (%)	Positive Control ² (%)
Mean	1.3	21.2
\pm SD ¹	1.4	12.1
Range	0.0-5.5	6.5-87.0

S9-ACTIVATED TEST SYSTEM

Historical Values	Solvent (%)	Positive Control ³ (%)
Mean	1.6	33.0
\pm SD ¹	1.5	18.1
Range	0.0-6.5	7.0-84.0

¹ SD = standard deviation.

² Positive control for non-activated studies, Mitomycin C (MMC, 0.08-0.2 μ g/mL).

³ Positive control for S9-activated studies, cyclophosphamide (CP, 10-60 μ g/mL).

Historical control values (1999-2001): combined numerical aberrations (polyploidy and endo-reduplicated cells)

NON-ACTIVATED TEST SYSTEM

Historical Values	Solvent (%)	Positive Control ² (%)
Mean	2.1	2.8
±SD ¹	1.4	1.6
Range	0.0-7.5	0.0-8.0

S9-ACTIVATED TEST SYSTEM

Historical Values	Solvent (%)	Positive Control ³ (%)
Mean	2.8	2.6
±SD ¹	1.9	1.5
Range	0.0-11.0	0.0-6.0

¹ SD = standard deviation.

² Positive control for non-activated studies, Mitomycin C (MMC, 0.08-0.2 µg/mL).

³ Positive control for S9-activated studies, cyclophosphamide (CP, 10-60 µg/mL).

Study outcome:

- The percentage of cells with structural aberrations in the test article-treated groups was significantly increased above that of the solvent control at concentrations 20 and 60 µg/mL in the non-activated 4 hour exposure group ($p \leq 0.01$, Fisher's exact test). The Cochran-Armitage test was also positive for a dose response ($p < 0.05$). However, the percentage of cells with structural aberrations in the test article groups at 20 µg/mL (5.0%) was within the historical solvent control range of 0.0% to 5.5%.
- The percentage of cells with structural aberrations in the test article-treated groups was significantly increased above that of the solvent control at 40 µg/mL in the S9 activated 4 hour exposure group ($p \leq 0.01$, Fisher's exact test). The Cochran-Armitage test was also positive for a dose response ($p < 0.05$).
- The percentage of cells with structural aberrations in the test article-treated groups was significantly increased above that of the solvent control at 5 and 10 µg/mL in the non-activated 20 hour exposure group ($p \leq 0.05$ at 5 µg/mL and $p \leq 0.01$ at 10 µg/mL, Fisher's exact test). The Cochran-Armitage test was also positive for a dose response ($p < 0.05$). However, the percentage of cells with structural aberrations in the test article groups at 5 µg/mL (2.5%) was within the historical solvent control range of 0.0% to 5.5%.
- The percentage of cells with numerical aberrations in the test article-treated groups was not significantly increased above that of the solvent control at any concentration in any of the three exposure groups ($p > 0.05$, Fisher's exact test).

Treatment/ Recovery Time (hours)	Harvest Time (hours)	S9	Toxicity* at highest dose scored (µg/mL)	Mitotic Index Reduction**	LED ¹ for Structural Aberrations ²	LED ¹ for Numerical Aberrations ²
4 / 16	20	-	61 at 60	39	60	None
20 / 0	20	-	19 at 10	62	10	None
4 / 16	20	+	55 at 40	16	40	None

* cell growth inhibition

** relative to solvent control at high dose evaluated for chromosome aberrations

¹LED = lowest effective dose

² µg/mL

Treatment (µg/mL)	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored	Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations Numerical (%)	Structural (%)
DMSO	-	4	7.0	200	0.000	±0.000	3.0	0.0
BMS-354825								
5	-	4	8.4	200	0.000	±0.000	2.0	0.0
20	-	4	7.5	200	0.060	±0.295	3.5	5.0**
60	-	4	4.3	100	0.430	±1.465	2.0‡	21.0**
MMC	-	4	5.5	100	0.300	±0.595	3.5‡	24.0**
0.2								
DMSO	+	4	7.5	200	0.000	±0.000	1.5	0.0
BMS-354825								
5	+	4	7.9	200	0.020	±0.172	3.0	1.5
10	+	4	6.8	200	0.015	±0.122	3.5	1.5
40	+	4	6.3	200	0.170	±0.577	2.5	12.0**
CP	+	4	6.7	100	0.470	±0.937	3.5‡	27.0**
10								
DMSO	-	20	6.5	200	0.000	±0.000	2.5	0.0
BMS-354825								
2.5	-	20	5.9	200	0.000	±0.000	2.5	0.0
5	-	20	6.6	200	0.070	±0.720	3.5	2.5*
10	-	20	2.5	100	2.640	±4.315	4.0‡	31.0**
MMC	-	20	6.8	100	0.390	±0.863	2.5‡	24.0**
0.1								

Treatment: Cells from all treatment conditions were harvested 20 hours after the initiation of the treatments.

Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.

Percent Aberrant Cells: *, p≤0.05; **, p≤0.01; using Fisher's exact test.

‡ Numerical aberrations are out of 200 cells scored.

Conclusions:

BMS-354825 was clastogenic in CHO cells, in the absence or presence of S-9 metabolic activation: BMS-354825 was positive for the induction of structural chromosome aberrations in the activated and non-activated test systems in CHO cells.

The maximum clastogenic responses were at the highest concentrations evaluated, 60 µg/ml in the 4-hr treatment without S9 (21% aberrations vs. 0% for the vehicle control), 40 µg/ml in the 4-hr treatment with S9 (12% aberrations vs. 0%), and 10 µg/ml in the 20 hr-treatment without S9 (31% vs. 0%). There increase in the percent structural aberrations was dose-related for the –S9 system (4 hr or 20 hr incubations). Cytotoxicity was noted at each of the highest concentrations evaluated with cell growth inhibition of 61% in the 4-hr treatment without S9 at 60 µg/ml, 55% in the 4-hr treatment with S9 at 40 µg/ml, and a reduction in the mitotic index of approximately 62% in the 20 hr-treatment without S9.

Study title: Oral Micronucleus Study in Rats

Key findings: The test article, BMS354825, was evaluated as negative in the rat bone marrow micronucleus assay under the conditions of this assay.

Study no: DS02177
Volume #, and page #: Volume 14 page 12
Conducting laboratory and location: BMS; Syracuse, NY
Date of study initiation: September 23, 2002
GLP compliance: Yes
QA reports: yes (x) no ()
Drug, lot #, radiolabel, and % purity: BMS-354825-01, Lot # C007A-354825-01, purity = — (freebase)
Formulation/vehicle: 80 mM Citrate buffer, pH3.0-3.4

Methods:

Strains/species/cell line: Male and female rats of the Sprague-Dawley strain

Dose selection criteria:

Basis of dose selection: The selection of doses was based on a dose range finding study which utilized daily observations of toxic signs and/or mortality following 20, 40, 60, and 80 mg/kg/day (n=1/dose/sex); PO daily x 3

Range finding studies: The studies assessed mortality, clinical toxicology, and bone marrow toxicity in 1/sex/dose. Two males died at 60 and 80 mg/kg.

Test agent stability: provided, no significant deviation detected.

Controls:

Vehicle: 80 mM Citrate buffer, pH3.0-3.4

Negative controls: vehicle control

Positive controls: Cyclophosphamide C diluted with sterile water (7 mg/kg)

Exposure conditions:

Sampling times: Bone marrow cells, collected 24 hours following the last dose

Doses in definitive study: 10, 20, and 40 mg/kg BMS 354825 (DX3); vehicle control, Cyclophosphamide, in males/females.

Experimental Design

Group Number	Daily Dose		Concentration	Number of Animals
	BMS-354825 (mg/kg)	Volume (ml/kg)	BMS-354825 (mg/ml)	
1	0 ^a	8	0	5 M, 5F
2	10	2	5	5 M, 5F
3	20	4	5	5 M, 5F
4	40	8	5	5 M, 5F
5	cyclophosphamide (7 mg/kg)	10	0.7	5 M, 5F

^a The vehicle (negative) control was 80mM citrate buffer.

Study design: The assay was performed in two phases. The first phase, designed to assess toxicity of the test article and set dose levels for the definitive study, consisted of a toxicity study. The second phase, the definitive micronucleus study, was designed to evaluate the potential of BMS-354825 to increase the incidence of micronucleated polychromatic erythrocytes in bone marrow of male and female rats.

Analysis:

No. of replicates: Not applicable.

Counting method: The bone marrow from femurs was flushed, and marrow cells were pelleted, re-suspended, and smeared onto glass slides. The micronucleus frequency was determined by analyzing the number of micronucleated polychromatic erythrocytes (PCEs) from at least 1000 PCEs per animal by two different evaluators (2000 total). The percent PCE values for this study were calculated based upon a minimum of 1000 erythrocytes (NCE and PCE in bone marrow)

Animal Observations: Animals were examined immediately after dosing and immediately prior to sacrifice

Criteria for positive results: For a test article to be evaluated positive, it must cause a statistically significant increase in micronucleated polychromatic erythrocytes.

Summary of individual study findings:

Study validity:

- The vehicle control group had less than 0.5% micronucleated PCEs and the group mean was within the historical range and the positive control,

cyclophosphamide induced a significant increase in PCEs, and was within historical control data values.

- The high dose produced clinical signs of toxicity
- A minimum of 4 males and females per dose level survived test article treatment for bone-marrow sampling and microscopic analysis.
- In bone-marrow smears, 2000 scorable PCE per animal were available

Study outcome:

- In the dose range finding assay, two males died (60 and 80 mg/kg). Clinical signs included decreased activity, chromodacryorrhea/chromorhinorrhea, dehydration, hunched and pale body, feces (absent, dark, loose, or tarry) rough haircoat, rales, salivation, soiling, and lacrimation. Marrow toxicity was indicated by 60% reduction in PCEs at ≥ 60 mg/kg and a 40% reduction at 40 mg/kg. Therefore 40 mg/kg was chosen for the top dose in the definitive study.
- In the definitive study, clinical signs of toxicity were observed at 40mg/kg in male and female rats and included chromorhinorrhea, dark and liquid feces and hair coat soiling. Additionally, chromodacryorrhea and rough haircoat was observed in females.
- Dose dependent decreases in PCEs provided evidence of bone marrow toxicity, however there was not a significant increase in the MN-PCE ratio following BMS 354825 administration (see table below).

Micronucleus data summary table of the definitive study

Article	Dose (mg/kg)	Sex	No. Rats Evaluated	Mean % PCE (\pm SD)	Mean % MN-PCE (\pm SD)
vehicle ^a	0	M	5	55 \pm 3.8	0.18 \pm 0.04
BMS-354825	10	M	5	56 \pm 4.1	0.16 \pm 0.11
BMS-354825	20	M	5	47 \pm 3.8	0.25 \pm 0.11
BMS-354825	40	M	5	31 \pm 4.7	0.22 \pm 0.04
cyclophosphamide	7	M	5	54 \pm 5.8	2.94 \pm 1.75**
vehicle ^a	0	F	5	58 \pm 6.5	0.25 \pm 0.14
BMS-354825	10	F	5	56 \pm 5.3	0.20 \pm 0.05
BMS-354825	20	F	5	44 \pm 4.6	0.14 \pm 0.07
BMS-354825	40	F	5	31 \pm 6.5	0.28 \pm 0.08
cyclophosphamide	7	F	5	50 \pm 4.1	1.97 \pm 0.38**

^a The vehicle (negative) control article was 80 mM citrate buffer.

** Statistically significant at $P < 0.01$ (Student's 't' test).

Conclusion:

BMS-354825 did not cause chromosomal damage in the rat bone marrow micronucleus test, under the conditions of the assays.

Genetic toxicology: overall conclusions

BMS-354825 was clastogenic to CHO cells, in the absence or presence of S9 metabolic activation: BMS-354825 was positive for the induction of structural chromosome aberrations in CHO cells.

Dasatinib was not mutagenic in the bacterial reverse mutation assays (Ames Test) under the conditions assayed for *S. typhimurium* strains TA98, TA100, TA1535, TA1537, and for *E. coli* strain WP2 *uvrA*. BMS-354825 did not cause chromosomal damage in the rat bone marrow micronucleus test, under the conditions of the study.

2.6.6.5 Carcinogenicity

No study conducted

2.6.6.6 Reproductive and developmental toxicology**Fertility and early embryonic development**

Not evaluated

Prenatal and postnatal development

Not evaluated

Embryofetal development

Study title: Oral study of embryo-fetal development in rats

Key study findings: BMS-354825 caused embryo-lethality and fetal abnormalities including malformations at doses that did not produce maternal toxicity and is therefore considered a developmental toxicant in rats. The lowest observed adverse effect level was 2.5 mg/kg/day or 15 mg/m²/day, with maternal AUC of 105 ng/hr/mL (0.3 x the human AUC in females at the recommended dose of 70 mg BID)

Study no.: DN04078

Volume #, and page #: Item 5

Conducting laboratory and location: Bristol-Myers Squibb
Department of Reproductive Toxicology
New Brunswick, NJ

Date of study initiation: 1st dose given on Aug 31, 2004

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: BMS-354825, Batch # C004B-354825-03, pure

Vehicle: 80 mM sodium citrate buffer

Methods

Doses: 2.5, 5, 10, or 20 mg/ kg/ day x GD 6-15 (sacrificed on GD 20)
15, 30, 60, or 120 mg/m²/day

Group Number	Daily Dose		Concentration	Number of Female Rats
	BMS-354825 (mg/kg/day)	Volume (mL/kg)	BMS-354825 (mg/mL)	
1	0	5	0	22
2	2.5	5	0.5	22+10 ^a
3	5	5	1	22+10 ^a
4	10	5	2	22+10 ^a
5	20	5	4	22+10 ^a

^a Ten rats were evaluated for maternal toxicokinetic endpoints beginning on Day 15 of gestation.

Test article was made as 0.5 mg/mL, 1 mg/mL, 2 mg/mL or 4 mg/mL stock solutions and diluted in 80 mM sodium citrate buffer.

Stock solutions prepared at least once weekly.

All diluted solutions were used within the established stability period (10 days under refrigeration for stock solutions of 0.5, 1.0, and 2 mg/mL and 7 days under refrigeration for stock solution of 4 mg/mL).

Analysis of dosing formulations showed formulations to be within 4% of the target concentrations.

Day of confirmed mating= Day 0 of gestation

Note: Table submitted by the sponsor.

Species/strain: rats/ CD@ (SD)IGS

Number/sex/group: see the Table

Route, formulation, volume: Oral gavage, solution, 5 mL/kg

Satellite groups used for toxicokinetics: see the Table

Weight of animals: mean BW of 214.7 on DG0 for all main groups

Weight range of 200 to 225 g

Age of animals: All rats were approximately 9 weeks at the time of mating

Observations:

Maternal

Mortality: twice daily

Clinical signs/ abortion/ premature delivery: daily

Body weight: DG0 (by the vendor), upon receipt, daily (DG 6-20)

Food consumption: daily

Necropsy/Organ weight/C-sectioning: on DG20

Dams examined for gross lesions of the abdominal and thoracic cavities

The intact uterus (gravid and non-gravid, including ovaries) was weighed

Corpora lutea and implantation sites were counted

Evaluated: the placement of implantation sites, early and late resorptions, live and dead fetuses

Each placenta was examined grossly for alterations in size, shape, or color.

Fetal

Fetuses were individually weighed and examined for gross external alterations. Approximately one-half of the fetuses in each litter were examined for soft tissue alterations by fresh visceral dissection. The remaining fetuses in each litter were evaluated for skeletal alterations.

Toxicokinetics (maternal)

Blood samples (0.5 mL each) collected from 5 rats/group/time-point at approximately 1, 2, 4, 6, 8, and 24 hr post-dose. After blood collection, TK rats were euthanized and examined for pregnancy status (no further evaluation)

Results

Mortality (dams): 7 out of 22 dams at HD (2 found dead on Days 12 or 13 of gestation and 5 euthanatized in moribund condition on Days 13 to 15 of gestation) preceded by agonal signs of soft/loose feces, piloerection, brown/clear periocular substance, and decreased motor activity.

	HD (N=22) 20 mg/kg 120 mg/m2	Gross Pathology Findings
Mortality		
Found dead	2	Enlarged adrenals
Sacrificed moribund	5	Enlarged and/or dark red adrenals Gas-filled and/or red fluid-filled intestines Dark/red lobes of lungs Red substance adhering to kidney Red substance in uterus

Clinical signs (dams):

HD:

Clinical observations of ungroomed coat, fecal/ urine-stained coat, brown/ clear perinasal or perioral substance, and reduced/ absent feces during Gestation Days 7 to 18.

LMD, HMD, and HD:

Red/brown/black perivaginal substance; appeared to be secondary to the resorption of conceptuses.

Body weight (dams): ↓BW gain at all doses, when compared to that for the control group

Gestation Days	Changes in BW gain compared to control
GD 6-12	↓79% (HD)
GD 12-16	↓20% (LD) ↓45% (LMD) ↓89% (HMD) *BW loss of 1 g at HD (compared to 32 g gain in controls)
GD 16-20 (post-dose)	↓12% (LD) ↓56% (LMD) ↓95% (HMD) *BW loss of 0.3 g at HD (compared to 61 g gain in controls)

* BW losses at HD appear to be mainly due to the resorption of conceptuses.

Food consumption (dams):

Gestation Days	Changes food consumption compared to control
GD 6-16 (dosing period)	↓9% (HMD) ↓24% (HD)
GD 16-20 (post-dose)	↓14% (HMD) ↓9% (HD)

Gross pathology: gross pathology findings were only seen in unscheduled sacrifices (see the Table under “Mortality”).

Toxicokinetics:

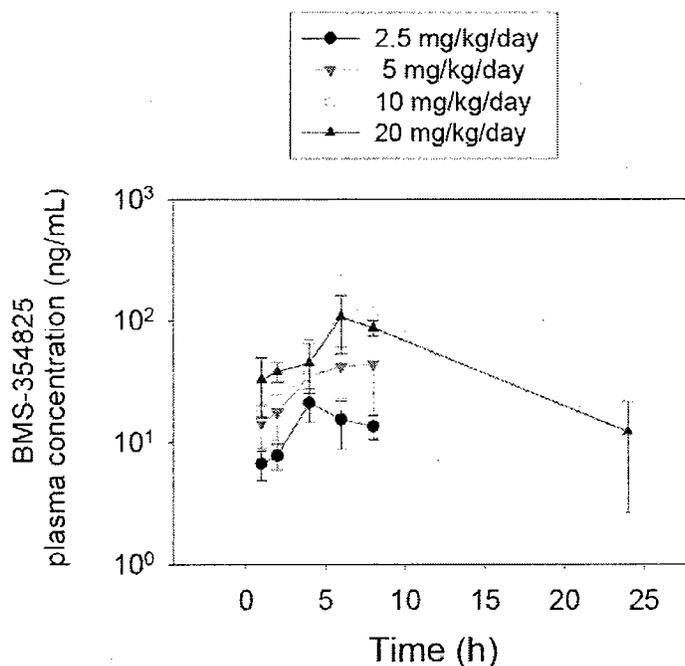
Dose (mg/kg/day)	C _{max} (ng/mL)	AUC _t (ng/mL·h) ^a	T _{max} (h)
2.5	21.1	105 ^b	4
5	43.7	239 ^b	8
10	128.2	1490	6
20	107.2	1270	6
Dose Ratio	Dose proportionality		
1:2:4:8	1:2:6:5	1:2:14:12	

^a Calculated from time zero to 24 h unless otherwise noted.

^b Calculated from time zero to 8 h.

Note: Table submitted by the sponsor.

Mean Plasma Concentrations in pregnant Rats



Note: Graph submitted by the sponsor.

Embryo-Fetal Findings

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

Post-implantation loss/ embryo-lethality:

Post-implantation loss was calculated as [(dead +resorbed conceptuses/ implantations) x 100]

- No surviving fetuses at HMD and HD
- Drug-related effects were seen in all dose groups

Dose Groups	Resorption of conceptuses per litter
C	4%
LD	*17%
LMD	*77%
HMD	*100%
HD	*100%

* Statistically significant

Dose Groups	Dams with no viable conceptuses
C N=22	0 (0%)
LD N=21	0 (0%)
LMD N=22	1 (4.5%)
HMD N=22	*22 (100%)
HD N=15	*15 (100%)

N represents the number of pregnant rats that survived to Day 20.

* Statistically significant.

Resorptions:

Dose Groups	Resorptions (Early + Late)	Early Resorptions	Late Resorptions	Dams with any Resorptions
C N=22	12	12	0	9 (41%)
LD N=21	52	51	1	15 (71%)
LMD N=22	*249	*237	12	22 (100%)
HMD N=22	*307	*306	1	22 (100%)
HD N=15	*215	*215	0	15 (100%)

N represents the number of pregnant rats that survived to Day 20.

* Statistically significant.

Litter size:

- Drug-related effects in all dose groups

Dose Groups	Mean litter size (Live + Dead)	Mean litter size (Live)	Mean litter size (Dead)
C N=22	13.9	13.9	0
LD N=21	11.2	11.2	0
LMD N=22	*3.5	*3.5	0
HMD N=22	*0.0	*0	0
HD N=15	*0.0	*0	0

N represents the number of pregnant rats that survived to Day 20.

* Statistically significant.

Fetal Observations (malformations, variations, etc.): No data for HMD and HD fetuses due to mortality.

Fetal abnormalities at LD and LMD included bent scapula or humerus and reduced ossification of the sternbrae and thoracic vertebral centra (irregularly/dumbbell shaped and/or reduced ossification site counts).

Additional fetal abnormalities at LMD (5 mg/kg) included the following: Fluid-filled thoracic and abdominal cavities, edema (body), microhepatia (small liver), misshapen clavicles, bent radius and femur, wavy or nodulated ribs, and reduced ossification of the thoracic, lumbar, and sacral vertebrae (hypoplastic, not ossified, and/or incompletely ossified centra) and forepaw phalanges (reduced ossification site counts).

A slight elevation in the fetal and litter incidence of cervical ribs occurred at the LD but was not considered to be drug-related because the value for the litter incidence (10.5%) was within range of those observed historically in controls at the test facility (0 to 10.5%). All other fetal malformations and variations noted during the study were considered unrelated to BMS-354825 because the observations were incidental and/or not significantly increased as compared with controls.

Note: Based on the Test Facility Historical Control Data, in 16 rat teratology studies conducted at this test facility from 1999 to 2004, evaluating 331 control litters and 2,146 fetuses, cervical ribs occurred in 6 fetuses from 6 litters (litter incidences ranged from 0 to 10.5%).

Summary of Fetal Alterations

	Control 0	LD 2.5 mg/kg	LMD 5 mg/kg
Fetuses evaluated	306	235	76
Live fetuses	306	235	76
Litters evaluated	22	21	21
Alterations (malformations + variations)			
Fetuses with any alterations	15%	14%	*38%
Litters containing fetuses with any alterations	68%	71%	81%
Percent fetuses per litter with alterations	15%	13%	*42%
†Variations			
Fetuses with any variations	15%	12%	*33%
Litters containing fetuses with any variations	68%	67%	81%
Percent fetuses per litter with variations	15%	12%	*37%
‡Malformations			
Fetuses with any malformations	0.3%	2%	*26%
Litters containing fetuses with any malformations	4.5%	19%	*67%
Percent fetuses per litter with malformations	0.4%	2%	*32%

All values were calculated on the basis of live fetuses in each group.

No data for HMD and HD (no live fetuses).

* Statistically significant changes

† Refers to common observations in this species and strain and reversible delays or accelerations in development

‡ Refers to irreversible changes occurring at low incidence in this species and strain.

SUMMARY OF FETAL GROSS EXTERNAL AND PLACENTAL OBSERVATIONS

GROUP		1	2	3	4	5
TREATMENT		Control	BMS-354825	BMS-354825	BMS-354825	BMS-354825
DAILY DOSE (mg/kg/day)		0	2.5	5	10	20
FETUSES EVALUATED:	N	306	235	76	0	0
Live Fetuses	N	306	235	76	-	-
Dead Fetuses	N	0	0	0	-	-
LITTERS EVALUATED	N	22	21	21	0	0
<u>BODY: Edema (V)</u>						
Fetal Incidence	N(%)	0	0	3 (3.9)**	-	-
Litter Incidence	N(%)	0	0	3 (14.3)	-	-

ALL PLACENTAE APPEARED NORMAL

NOTE: All values were calculated on the basis of live fetuses in each group.
(V) = Variation

Statistical Analysis: Fisher's exact test was used for proportion data.
** Significantly different from the control at P≤0.01.

SUMMARY OF FETAL VISCERAL OBSERVATIONS

GROUP		1	2	3	4	5
TREATMENT		Control	BMS-354825	BMS-354825	BMS-354825	BMS-354825
DAILY DOSE (mg/kg/day)		0	2.5	5	10	20
FETUSES EVALUATED:	N	152	117	36	0	0
Live Fetuses	N	152	117	36	-	-
Dead Fetuses	N	0	0	0	-	-
LITTERS EVALUATED	N	22	21	18	0	0
<u>THORACIC CAVITY: Fluid-filled (M)</u>						
Fetal Incidence	N(%)	0	0	2 (5.5) ^{a*}	-	-
Litter Incidence	N(%)	0	0	2 (11.1)	-	-
<u>ABDOMINAL CAVITY: Fluid-filled (M)</u>						
Fetal Incidence	N(%)	0	0	2 (5.5) ^{a*}	-	-
Litter Incidence	N(%)	0	0	2 (11.1)	-	-
<u>LIVER: Small (microhepatia) (M)</u>						
Fetal Incidence	N(%)	0	0	2 (5.5) ^{a*}	-	-
Litter Incidence	N(%)	0	0	2 (11.1)	-	-
<u>STOMACH: Outer surface and inner lining, white areas present (M)</u>						
Fetal Incidence	N(%)	0	2 (1.7)	0	-	-
Litter Incidence	N(%)	0	2 (9.5)	0	-	-

NOTE: All values were calculated on the basis of live fetuses in each group.
(M) = Malformation

Statistical Analysis: Fisher's exact test was used for proportion data.
* Significantly different from the control at P≤0.05.

a - Observed in fetuses 3F0051-R7 and 3F0052-R3.

SUMMARY OF FETAL SKELETAL OBSERVATIONS

GROUP		1	2	3	4	5
TREATMENT		Control	BMS-354825	BMS-354825	BMS-354825	BMS-354825
DAILY DOSE (mg/kg/day)		0	2.5	5	10	20
FETUSES EVALUATED:	N	154	118	40	0	0
Live Fetuses	N	154	118	40	-	-
Dead Fetuses	N	0	0	0	-	-
LITTERS EVALUATED	N	22	21	19	0	0
<u>SKULL: Supraoccipital and/or interparietal, hypoplastic (TOTAL) (V)</u>						
Fetal Incidence	N(%)	2 (1.3)	0	0	-	-
Litter Incidence	N(%)	2 (9.1)	0	0	-	-
<u>SKULL: Ectopic ossification site present on right side of supraoccipital (V)</u>						
Fetal Incidence	N(%)	0	1 (0.8)	0	-	-
Litter Incidence	N(%)	0	1 (4.8)	0	-	-
<u>HYOID: Body, incomplete ossification or not ossified (TOTAL) (V)</u>						
Fetal Incidence	N(%)	19 (12.3)	1 (0.8)**	0*	-	-
Litter Incidence	N(%)	7 (31.8)	1 (4.8)*	0**	-	-
<u>CLAVICLES: Misshapen (M)</u>						
Fetal Incidence	N(%)	0	0	3 (7.5)**	-	-
Litter Incidence	N(%)	0	0	2 (10.5)	-	-
<u>SCAPULAS: Bent (M)</u>						
Fetal Incidence	N(%)	0	2 (1.7)	16 (40.0)**	-	-
Litter Incidence	N(%)	0	2 (9.5)	13 (68.4)**	-	-
<u>STERNEBRAE: Dumbbell- or irregularly-shaped (TOTAL) (V)</u>						
Fetal Incidence	N(%)	4 (2.6)	8 (6.8)	8 (20.0)**	-	-
Litter Incidence	N(%)	4 (18.2)	6 (28.6)	7 (36.8)	-	-
<u>STERNEBRAE: Asymmetric or unilateral ossification (TOTAL) (V)</u>						
Fetal Incidence	N(%)	1 (0.6)	0	1 (2.5)	-	-
Litter Incidence	N(%)	1 (4.5)	0	1 (5.3)	-	-
<u>STERNEBRAE: Bifid (V)</u>						
Fetal Incidence	N(%)	2 (1.3)	0	2 (5.0)	-	-
Litter Incidence	N(%)	2 (9.1)	0	2 (10.5)	-	-
<u>RIBS: Wavy (V)</u>						
Fetal Incidence	N(%)	0	1 (0.8)	13 (32.5)**	-	-
Litter Incidence	N(%)	0	1 (4.8)	11 (57.9)**	-	-
<u>RIBS: Nodulated (V)</u>						
Fetal Incidence	N(%)	0	1 (0.8)	10 (25.0)**	-	-
Litter Incidence	N(%)	0	1 (4.8)	8 (42.1)**	-	-

NOTE: All values were calculated on the basis of live fetuses in each group.
(V) = Variation (M) = Malformation

Statistical Analysis: Fisher's exact test was used for proportion data.

* Significantly different from the control at P≤0.05.

** Significantly different from the control at P≤0.01.

GROUP		1	2	3	4	5
TREATMENT		Control	BMS-354825	BMS-354825	BMS-354825	BMS-354825
DAILY DOSE (mg/kg/day)		0	2.5	5	10	20
FETUSES EVALUATED:	N	154	118	40	0	0
Live Fetuses	N	154	118	40	-	-
Dead Fetuses	N	0	0	0	-	-
LITTERS EVALUATED	N	22	21	19	0	0
<u>RIBS: 7th cervical (V)</u>						
Fetal Incidence	N(%)	0	1 (0.6)	2 (5.0)*	-	-
Litter Incidence	N(%)	0	1 (4.8)	2 (10.5)	-	-
<u>VERTEBRAE: Cervical, arches, bifid (V)</u>						
Fetal Incidence	N(%)	1 (0.6)	1 (0.6)	2 (5.0)	-	-
Litter Incidence	N(%)	1 (4.5)	1 (4.8)	2 (10.5)	-	-
<u>VERTEBRAE: Thoracic, arches, bifid (V)</u>						
Fetal Incidence	N(%)	1 (0.6)	0	0	-	-
Litter Incidence	N(%)	1 (4.5)	0	0	-	-
<u>VERTEBRAE: Thoracic, centra, hypoplastic (V)</u>						
Fetal Incidence	N(%)	0	0	7 (17.5)**	-	-
Litter Incidence	N(%)	0	0	6 (31.6)**	-	-
<u>VERTEBRAE: Thoracic, centra, bifid (V)</u>						
Fetal Incidence	N(%)	3 (1.9)	0	0	-	-
Litter Incidence	N(%)	2 (9.1)	0	0	-	-
<u>VERTEBRAE: Thoracic, centra, not ossified and/or incomplete ossification (TOTAL) (V)</u>						
Fetal Incidence	N(%)	1 (0.6)	0	5 (12.5)**	-	-
Litter Incidence	N(%)	1 (4.5)	0	5 (26.3)	-	-
<u>VERTEBRAE: Thoracic, centra, dumbbell- or irregularly-shaped (TOTAL) (V)</u>						
Fetal Incidence	N(%)	5 (3.2)	14 (11.9)**	1 (2.5)	-	-
Litter Incidence	N(%)	5 (22.7)	6 (28.6)	1 (5.3)	-	-
<u>VERTEBRAE: Lumbar, centra, hypoplastic (V)</u>						
Fetal Incidence	N(%)	0	0	6 (15.0)**	-	-
Litter Incidence	N(%)	0	0	6 (31.6)**	-	-
<u>VERTEBRAE: Sacral, centra, hypoplastic (V)</u>						
Fetal Incidence	N(%)	0	0	4 (10.0)**	-	-
Litter Incidence	N(%)	0	0	4 (21.1)*	-	-
<u>VERTEBRAE: Caudal, not ossified (V)</u>						
Fetal Incidence	N(%)	24 (15.6)	9 (7.6)	9 (29.0)	-	-
Litter Incidence	N(%)	9 (40.9)	5 (23.8)	7 (36.8)	-	-

NOTE: All values were calculated on the basis of live fetuses in each group.
(V) = Variation (M) = Malformation

Statistical Analysis: Fisher's exact test was used for proportion data.
* Significantly different from the control at P<0.05.
** Significantly different from the control at P<0.01.

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GROUP		1	2	3	4	5
TREATMENT		Control	BMS-354825	BMS-354825	BMS-354825	BMS-354825
DAILY DOSE (mg/kg/day)		0	2.5	5	10	20
FETUSES EVALUATED:	N	154	118	40	0	0
Live Fetuses	N	154	118	40	-	-
Dead Fetuses	N	0	0	0	-	-
LITTERS EVALUATED	N	22	21	19	0	0
<u>PELVIS: Ilia, misshapen (M)</u>						
Fetal Incidence	N(%)	1 (0.6)	0	0	-	-
Litter Incidence	N(%)	1 (4.5)	0	0	-	-
<u>PELVIS: Pubes and/or ischia, not ossified or incomplete ossification (TOTAL) (V)</u>						
Fetal Incidence	N(%)	3 (1.9)	0	1 (2.5)	-	-
Litter Incidence	N(%)	3 (13.6)	0	1 (5.3)	-	-
<u>FORELIMBS: Humeri, bent (M)</u>						
Fetal Incidence	N(%)	0	2 (1.7)	10 (25.0)**	-	-
Litter Incidence	N(%)	0	2 (9.5)	8 (42.1)**	-	-
<u>FORELIMBS: Radii, bent (M)</u>						
Fetal Incidence	N(%)	0	1 (0.8)	3 (7.5)**	-	-
Litter Incidence	N(%)	0	1 (4.8)	3 (15.8)	-	-
<u>HINDLIMBS: Femur, bent (M)</u>						
Fetal Incidence	N(%)	0	0	4 (10.0)**	-	-
Litter Incidence	N(%)	0	0	4 (21.0)*	-	-

NOTE: All values were calculated on the basis of live fetuses in each group.
(V) = Variation (M) = Malformation

Statistical Analysis: Fisher's exact test was used for proportion data.

* Significantly different from the control at P≤0.05.

** Significantly different from the control at P≤0.01.

Note: Tables submitted by the sponsor.

Summary:

Dasatinib was administered to pregnant rats by gavage on Gestation Days 6-15, at doses of 2.5, 5, 10, or 20 mg/kg/day (15, 30, 60, or 120 mg/m², respectively). Rats were sacrificed on GD20. TK was evaluated on GD15 on satellite animals. According to the sponsor, dose selection was based on the results of a previous 2-week oral toxicity study in rats with BMS-3548252 in which doses of 1, 15, and 30 mg/kg/day (6, 90, and 180 mg/m²) were evaluated. At 15 and 30 mg/kg/day, BMS-354825 caused generally dose-dependent clinical signs [chromorrhinorrhea, soiled/rough haircoat, bloated/swollen abdomen (females only at 15 mg/kg/day), dehydration, and soft stool], tissue lymphoid depletion, hematopoietic toxicity, and enteropathy and edema of the intestinal tract. Severe toxicity including substantial reductions in body-weight gain (approximately 56% less than controls in females; body-weight losses in males) and lethality occurred in all rats at 30 mg/kg/day after 8 to 12 days of dosing. On the basis of these data, a high dose of 20 mg/kg/day (120 mg/m²) was selected for evaluation in this study. This dose was expected to produce maternal toxicity over the 10-day dosing period.

Maternal findings:

- 7 (out of 22) unscheduled deaths occurred in the HD group during the study, on GD 12-15. Deaths were preceded by clinical signs of ↓motor activity, soft/loose

feces, piloerection, and periocular substances. Gross pathology in these animals revealed enlarged and/or dark adrenals, dark/red lobes of lungs, red substance adhering to kidneys, and red substance in uterus.

- Clinical signs at LMD, HMD, and HD consisted of red/brown perivaginal substance that was speculated to be secondary to the resorption of conceptuses. Additional clinical signs at HD included: perinasal or perioral substance and ↓ or absent feces during GD 7-18.
- ↓BW gain was seen in all doses groups (LD through HD). BW loss occurred at HD.
- Gross pathology findings were noted in the unscheduled deaths only.
- The sponsor stated that the lowest-observed adverse effect level (LOAEL) in dams was the HMD (10 mg/kg/day or 60 mg/m²/day). Since maternal toxicities were not fully evaluated, this statement cannot be verified.

Maternal TK:

- dose proportional increase in C_{max} and AUC when going from LD to LMD
- More than dose proportional increase in exposure when increasing the dose from LMD to HMD: 1-fold/ 100% increase in the dose (doubling the dose), resulted in 2-fold increase in the C_{max} and 6-fold increase in AUC.
- Slight reduction in exposure when increasing the dose from HMD to HD: 1-fold/ 100% increase in the dose (doubling the dose), resulted in 16% reduction in the C_{max} and 15% reduction in the AUC. This might indicate saturation of absorption at higher doses.

Embryo-fetal findings

- All placentas appeared normal.
- There were no surviving fetuses at HMD and HD, therefore no fetal data (including fetal abnormalities) are available for these 2 groups.
- Embryo-lethality was observed starting at the LD (based on the resorptions and the mean litter size)
- Dose-dependent ↑resorptions were observed at all doses, starting from the LD group, which reached the 100% in the HMD and HD groups.
- Dose-dependent ↓litter size was observed, starting at the LD. The mean litter size was 14 in the control, 11 at LD, 4 at LMD and 0 at HMD and HD.
- Fetal abnormalities were observed starting at the lowest dose of 2.5 mg/kg or 15 mg/m². Increased abnormalities at the LD were mainly due to ↑malformations (irreversible changes occurring at low incidences in this species). Increased abnormalities at the LMD were due to both ↑variations and ↑malformations. 2% of the fetuses at LD and 26% at LMD had malformations, compared to the 0.3% in the control.
- The LD, with an AUC=105 ng.hr/mL is roughly 0.3-fold the AUC in females in the human trials at the recommended dose of 70 mg BID (140 mg/day). Therefore fetal abnormalities occurred at sub-therapeutic exposures.
- Dasatinib-induced toxicities in the dams was mostly evident at HD (120 mg/m²), whereas drug-related changes in the fetuses was evident starting at the LD (15

mg/m²). Therefore, teratogenic effects of Dasatinib occur at doses that may not be toxic to the dams.

- Drug-related abnormalities in the fetuses at LD and LMD (no surviving fetuses at HMD and HD) included malformations of the scapula or humerus (bent) and reduced ossification of the sternbrae and thoracic vertebral centra (irregularly/dumbbell shaped and/or reduced ossification site counts). Additional fetal abnormalities at the LMD included fluid-filled thoracic and abdominal cavities, edema (body), microhepatia (small liver), misshapen clavicles, bent radius and femur, wavy or nodulated ribs, and reduced ossification of the thoracic, lumbar, and sacral vertebrae (hypoplastic, not ossified, and/or incompletely ossified centra) and forepaw phalanges (reduced ossification site counts).
- A NOAEL for embryo-fetal toxicity was not identified in this study.

Study title: Oral study of embryo-fetal development in rabbits

Key study findings: BMS-354825 altered fetal development at doses that did not cause maternal toxicity. Fetal alterations occurred starting at the LD (0.5 mg/kg/day or 6 mg/m²/day), at an AUC 0.1 x that seen in humans (females) at the recommended dose of 70 mg BID (140 mg/day).

Study no.: DN04080

Volume #, and page #: Item 5

Conducting laboratory and location: Bristol-Myers Squibb
Department of Reproductive Toxicology
New Brunswick, New Jersey USA

Date of study initiation:

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: BMS-354825 Batch # C004B-354825-03, as the free base was used; purity of —

Stock solution: 30 mg/mL in 80 mM citric acid (were diluted to appropriate concentrations with 80 mM citrate buffer and used within the established stability period, defined to be 7 days)

Methods

Doses: 0, 0.5, 2, and 6 mg/kg x GD 7-19 (sacrificed on GD 29)
0, 6, 24, and 72 mg/m²

Group Number	Daily Dose		Concentration BMS-354825 (mg/mL)	Number of Female Rabbits
	BMS-354825 (mg/kg)	Volume (mL/kg)		
1 (Control)	0 (80 mM sodium citrate)	1.5	0	22
2	0.5	1.5	0.33	27 ^a
3	2	1.5	1.3	27 ^a
4	6	1.5	4	27 ^a

^a 5 rabbits were evaluated for maternal toxicokinetic endpoints beginning on Day 19 of Gestation.

Based of the analysis of dosing formulations, solutions were within 9% of the target concentrations.

Note: Table submitted by the sponsor.

Species/strain: Rabbit/ New Zealand White Hra:(NZW) SPF

Number/sex/group: 22 ♀s/group

Route, formulation, volume: oral gavage, solution, 1.5 mL/kg

Satellite groups used for toxicokinetics: 5 ♀s/ Groups 2-4

Body weight: 3.5 kg on GD 0 (main animals)

Age: approximately 5.5 to 6 months at the time of mating

Observations:

Maternal

Mortality: twice daily

Clinical signs/ abortion/ premature delivery: daily

Body weight: DG0 (by the vendor), upon receipt, daily (DG 7-29)

Food consumption: daily

Necropsy/Organ weight/C-sectioning: on DG29

Does examined for gross lesions of the abdominal and thoracic cavities

The intact uterus (gravid and non-gravid, including ovaries) was weighed

Corpora lutea and implantation sites were counted

Evaluated: the placement of implantation sites, early and late resorptions, live and dead fetuses

Each placenta was examined grossly for alterations in size, shape, or color.

Fetal

Fetuses were individually weighed and examined for gross external alterations. Fetuses were examined for gender, soft tissue alterations, and skeletal alterations.

Toxicokinetics (maternal)

Blood sampling for TK evaluations was done on GD 19. Rabbits designed for TK evaluations were euthanized on GD 20. All TK rabbits survived to scheduled necropsy, were pregnant and were included in the analysis.

Blood samples (1 mL each) were collected from 5 rabbits/group/timepoint from Groups 2-4, at approximately 0.5, 1, 2, 4, 8, and 24 hr postdose.

Results

Mortality (does): None

Clinical signs (does): No drug-related effect

Body weight (does): No drug-related effect

Food consumption (does): No drug-related effect

Gross pathology (does): No drug-related effect

Toxicokinetics:

Dose (mg/kg/day)	C _{max} (ng/mL)	AUC _t (ng·hr/mL)
0.5	14	44
2	63	248
6	227	834

Note: Table provided by the sponsor.

Embryo-fetal findings

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

All changes in cesarean-sectioning and litter parameters (corpora lutea, implantations, litter sizes, live or dead fetuses, early and late resorptions, and fetal sex ratios) were considered unrelated to BMS-354825 because the differences were non-dose-dependent and/or were comparable to controls. Some of the parameters are shown in Table below.

	Control	LD	MD	HD
Rabbits evaluated	N=22	N=22	N=22	N=22
Rabbits pregnant	20 (90%)	22 (100%)	20 (91%)	21 (96%)
Pregnant rabbit surviving to GD 29	*19	22	20	21
Does with no viable conceptuses	0	0	0	0
Implantations	9.3	8.7	9.9	9.3
Pre-implantation loss	4.7%	6.3%	2.8%	6.5%
Post-implantation loss	4.0%	1.2%	3.5%	6.0%
Does with any resorptions	6 (32%)	2 (9%)	4 (20%)	6 (29%)
Resorptions (early + late)	8	2	7	11
Litter size (live + †dead)	8.9	8.6	9.6	8.8
Live fetuses/ litter	8.9	8.6	9.6	8.8
% dead or resorbed conceptuses/ litter	4.0%	1.2%	3.5%	6.0%
% live male fetuses/litter	58.7%	51.6%	50.1%	56.2%
Fetal body weight (g)/ litter	42.26	41.89	41.73	42.20

* Excludes one doe which had a single conceptus litter.

† No dead fetus.

Offspring (malformations, variations, etc.):

All placentas appeared normal.

LD, MD, and HD (variations with ↑incidence):

- Delays in ossification of the fetal lumbar vertebrae (bifid arches)
- Pelvis (incomplete ossification or not ossified pubes)
- Hyoid body (incomplete ossification or not ossified): The sponsor did not consider this event drug-related, stating that “1) the incidence did not present a dose-dependent pattern; 2) the litter incidence at 0 and 6 mg/kg/day were identical; and 3) values for each of the treated groups (litter incidences of 9.1, 30.0, and 14.3% at 0.5, 2, and 6 mg/kg/day, respectively) were within range of those observed historically in controls at the test facility (litter incidences ranged from 4.5 to 30%)”.

Note: Based on the test Facility Historical Control Data (per sponsor), in 14 studies conducted at this test facility from 1999 to 2004, in which 2,414 fetuses from 292 control New Zealand White Hra: (NZW) SPF litters were evaluated, incomplete or unossified hyoid (body) occurred in 62 fetuses from 37 litters (litter incidence ranged from 4.5 to 30%).

HD (variations with ↑incidence):

- Irregular ossification of the hyoid (angulated)
- 7th cervical ribs

Summary of Fetal Alterations

	Control0	LD	MD	HD
Fetuses evaluated	170	190	191	185
Live fetuses	170	190	191	185
Litters evaluated	20	22	20	21
Alterations (malformations + variations)				
Fetuses with any alterations	32 (19%)	58 (31%)	62 (33%)	67 (36%)
Litters containing fetuses with any alterations	16 (80%)	16 (73%)	18 (90%)	17 (81%)
Percent fetuses per litter with alterations	23	28	33	33
†Variations				
Fetuses with any variations	31 (18%)	58 (31%)	62 (33%)	66 (36%)
Litters containing fetuses with any variations	16 (80%)	16 (73%)	18 (90%)	17 (81%)
Percent fetuses per litter with variations	22%	28%	32.7%	32.3%
‡Malformations				
Fetuses with any malformations	3 (2%)	2 (1%)	0	1 (0.5%)
Litters containing fetuses with any malformations	2 (10%)	1 (4.5%)	0	1 (4.8%)
Percent fetuses per litter with malformations	2	1	0	0.4

All values were calculated on the basis of live fetuses in each group.

No data for HMD and HD (no live fetuses).

* Statistically significant changes

† Refers to common observations in this species and strain and reversible delays or accelerations in development

‡ Refers to irreversible changes occurring at low incidence in this species and strain.

SUMMARY OF FETAL GROSS EXTERNAL AND PLACENTAL OBSERVATIONS

GROUP		1	2	3	4
TREATMENT		Control	BMS-354825	BMS-354825	BMS-354825
DAILY DOSE (mg/kg/day)		0	0.5	2	6
FETUSES EVALUATED:	N	170	190	191	185
Live Fetuses	N	170	190	191	185
Dead Fetuses	N	0	0	0	0
LITTERS EVALUATED	N	20	22	20	21

ALL FETUSES AND PLACENTAE APPEARED NORMAL

SUMMARY OF FETAL VISCERAL OBSERVATIONS

GROUP		1	2	3	4
TREATMENT		Control	BMS-354825	BMS-354825	BMS-354825
DAILY DOSE (mg/kg/day)		0	0.5	2	6
FETUSES EVALUATED:	N	170	190	191	185
Live Fetuses	N	170	190	191	185
Dead Fetuses	N	0	0	0	0
LITTERS EVALUATED	N	20	22	20	21
<u>EYES: Circumcornea, hemorrhagic (V)</u>					
Fetal Incidence	N(%)	1(0.6)	0	0	0
Litter Incidence	N(%)	1(5.0)	0	0	0
<u>URETERS: Retrocaval (V)</u>					
Fetal Incidence	N(%)	0	3(1.6)	0	3(1.6)
Litter Incidence	N(%)	0	2(9.1)	0	2(9.5)
<u>KIDNEYS: Severe dilation (M)</u>					
Fetal Incidence	N(%)	0	0	0	1(0.5)
Litter Incidence	N(%)	0	0	0	1(4.8)
<u>GALLBLADDER: Small (V)</u>					
Fetal Incidence	N(%)	0	0	0	1(0.5)
Litter Incidence	N(%)	0	0	0	1(4.8)
<u>HEART: Misshapen; aorta, enlarged; pulmonary artery, stenosis; interventricular septum, absent (M)</u>					
Fetal Incidence	N(%)	1(0.6)	0	0	0
Litter Incidence	N(%)	1(5.0)	0	0	0

NOTE: All values were calculated on the basis of live fetuses in each group.
(M) = Malformation (V) = Variation

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SUMMARY OF FETAL SKELETAL OBSERVATIONS

GROUP TREATMENT DAILY DOSE (mg/kg/day)		1 Control	2 BMS-354825 0.5	3 BMS-354825 2	4 BMS-354825 6
FETUSES EVALUATED:	N	170	190	191	185
Live Fetuses	N	170	190	191	185
Dead Fetuses	N	0	0	0	0
LITTERS EVALUATED	N	20	22	20	21
<u>SKULL: Nasals, supernumerary bone(s) (V)</u>					
Fetal Incidence	N(%)	1 (0.6)	0	4 (2.1)	1 (0.5)
Litter Incidence	N(%)	1 (5.0)	0	4 (20.0)	1 (4.8)
<u>SKULL: Nasals, hypoplastic (V)</u>					
Fetal Incidence	N(%)	0	0	3 (1.6)	0
Litter Incidence	N(%)	0	0	2 (10.0)	0
<u>SKULL: Frontals, supernumerary bone(s) (V)</u>					
Fetal Incidence	N(%)	4 (2.4)	4 (2.1)	5 (2.6)	1 (0.5)
Litter Incidence	N(%)	4 (20.0)	2 (9.1)	4 (20.0)	1 (4.8)
<u>SKULL: Anterior fontanel, supernumerary bone(s) (V)</u>					
Fetal Incidence	N(%)	0	0	2 (1.0)	0
Litter Incidence	N(%)	0	0	2 (10.0)	0
<u>SKULL: Anterior fontanel, enlarged (V)</u>					
Fetal Incidence	N(%)	1 (0.6)	0	0	0
Litter Incidence	N(%)	1 (5.0)	0	0	0
<u>SKULL: Interparietal, incomplete ossification (V)</u>					
Fetal Incidence	N(%)	0	0	0	1 (0.5)
Litter Incidence	N(%)	0	0	0	1 (4.8)
<u>HYOID: Body, incomplete or not ossified (V)</u>					
Fetal Incidence	N(%)	5 (2.9)	9 (4.7)	18 (9.4)*	10 (5.4)
Litter Incidence	N(%)	3 (15.0)	2 (9.1)	6 (30.0)	3 (14.3)
<u>HYOID: Alae, not ossified (V)</u>					
Fetal Incidence	N(%)	0	1 (0.5)	0	0
Litter Incidence	N(%)	0	1 (4.5)	0	0
<u>HYOID: Alae, angulated (V)</u>					
Fetal Incidence	N(%)	5 (2.9)	4 (2.1)	4 (2.1)	9 (4.9)
Litter Incidence	N(%)	3 (15.0)	3 (13.6)	2 (10.0)	5 (23.8)
<u>STERNEBRAE: Hyperplastic (V)</u>					
Fetal Incidence	N(%)	1 (0.6)	0	5 (2.6)	0
Litter Incidence	N(%)	1 (5.0)	0	4 (20.0)	0
<u>STERNEBRAE: Asymmetric (V)</u>					
Fetal Incidence	N(%)	0	0	2 (1.0)	1 (0.5)
Litter Incidence	N(%)	0	0	2 (10.0)	1 (4.8)

(V) = Variation

Statistical Analysis: Analysis of Variance with Dunnett's procedure was used for continuous data.
Fisher's exact test was used for proportion data.

* Significantly different from the control at P=0.05.

STERNEBRAE: Dumbbell or irregularly-shaped (V)

Fetal Incidence	N(%)	7 (4.1)	10 (5.3)	11 (5.3)	9 (4.9)
Litter Incidence	N(%)	6 (39.0)	6 (27.3)	10 (50.0)	4 (19.0)

STERNEBRAE: Bifid (V)

Fetal Incidence	N(%)	2 (1.2)	4 (2.1)	6 (3.1)	2 (1.1)
Litter Incidence	N(%)	2 (10.0)	4 (18.2)	5 (25.0)	2 (9.5)

RIBS: 7th cervical (V)

Fetal Incidence	N(%)	1 (0.6)	1 (0.5)	1 (0.5)	20 (10.8)**
Litter Incidence	N(%)	1 (5.0) ^a	1 (4.5)	1 (5.0)	9 (42.9)**

RIBS: Fused (M)

Fetal Incidence	N(%)	2 (1.2)	1 (0.5) ^b	0	0
Litter Incidence	N(%)	1 (5.0) ^a	1 (4.5)	0	0

RIBS: Bifurcated (M)

Fetal Incidence	N(%)	1 (0.6)	0	0	0
Litter Incidence	N(%)	1 (5.0) ^a	0	0	0

RIBS: Discontinuous (V)

Fetal Incidence	N(%)	0	0	0	1 (0.5)
Litter Incidence	N(%)	0	0	0	1 (4.8)

VERTEBRAE: Cervical, arches, misaligned (M)

Fetal Incidence	N(%)	1 (0.6)	0	0	0
Litter Incidence	N(%)	1 (5.0) ^a	0	0	0

VERTEBRAE: Cervical, hemivertebrae (M)

Fetal Incidence	N(%)	1 (0.6)	0	0	0
Litter Incidence	N(%)	1 (5.0) ^a	0	0	0

VERTEBRAE: Cervical, centra, unilateral ossification (V)

Fetal Incidence	N(%)	2 (1.2)	0	0	0
Litter Incidence	N(%)	1 (5.0) ^a	0	0	0

(M) = Malformation

(V) = Variation

Statistical Analysis: Analysis of Variance with Dunnett's procedure was used for continuous data.
Fisher's exact test was used for proportion data.

** Significantly different from the control at P=0.01.

a - Observation is present in litter 1F0004 among fetuses R2, R3, and R7.
b - Observation is present in fetus 2F0044 R6.

VERTEBRAE: Cervical, centra, misaligned (M)

Fetal Incidence	N(%)	1 (0.6)	0	0	0
Litter Incidence	N(%)	1 (5.0) ^a	0	0	0

VERTEBRAE: Thoracic, arches, fused (M)

Fetal Incidence	N(%)	0	1 (0.5) ^b	0	0
Litter Incidence	N(%)	0	1 (4.5)	0	0

VERTEBRAE: Thoracic, hemivertebrae (M)

Fetal Incidence	N(%)	2 (1.2)	2 (1.1) ^{b,c}	0	0
Litter Incidence	N(%)	1 (5.0) ^a	1 (4.5)	0	0

VERTEBRAE: Thoracic, arches, misaligned (M)

Fetal Incidence	N(%)	1 (0.6)	0	0	0
Litter Incidence	N(%)	1 (5.0) ^a	0	0	0

VERTEBRAE: Thoracic, centra, fused (M)

Fetal Incidence	N(%)	0	1 (0.5) ^b	0	0
Litter Incidence	N(%)	0	1 (4.5)	0	0

VERTEBRAE: Thoracic, centra, misaligned (M)

Fetal Incidence	N(%)	0	1 (0.5) ^b	0	0
Litter Incidence	N(%)	0	1 (4.5)	0	0

VERTEBRAE: Thoracic, centra, dumbbell or irregularly-shaped (V)

Fetal Incidence	N(%)	3 (1.8)	2 (1.1) ^{b,c}	1 (0.5)	0
Litter Incidence	N(%)	1 (5.0) ^a	1 (4.5)	1 (5.0)	0

VERTEBRAE: Thoracic, centra, bifid (V)

Fetal Incidence	N(%)	1 (0.6)	1 (0.5) ^c	0	0
Litter Incidence	N(%)	1 (5.0) ^a	1 (4.5)	0	0

(M) = Malformation (V) = Variation

Statistical Analysis: Analysis of Variance with Dunnett's procedure was used for continuous data. Fisher's exact test was used for proportion data.

a - Observation is present in litter 1F0004 among fetuses R3, R6, and R7.

b - Observation is present in fetus 2F0044 R3.

c - Observation is present in fetus 2F0044 R6.

GROUP TREATMENT DAILY DOSE (mg/kg/day)		1 Control	2 BMS-354825 0.5	3 BMS-354825 2	4 BMS-354825 6
FETUSES EVALUATED:		170	190	191	185
Live Fetuses	N	170	190	191	185
Dead Fetuses	N	0	0	0	0
LITTERS EVALUATED	N	20	22	20	21
<u>VERTEBRAE: Lumbar, arches, bifid (V)</u>					
Fetal Incidence	N(%)	1 (0.6)	26 (13.7) ^{**}	12 (6.3) ^{**}	29 (15.7) ^{**}
Litter Incidence	N(%)	1 (5.0)	9 (40.9) ^{**}	7 (35.0) [*]	10 (47.6) ^{**}
<u>PELVIS: Pubes, incomplete or not ossified (V)</u>					
Fetal Incidence	N(%)	0	8 (4.2) ^{**}	4 (2.1)	5 (2.7)
Litter Incidence	N(%)	0	3 (13.6)	2 (10.0)	4 (19.0)

(V) = Variation

Statistical Analysis: Analysis of Variance with Dunnett's procedure was used for continuous data.
Fisher's exact test was used for proportion data.

* Significantly different from the control at P=0.05.

** Significantly different from the control at P=0.01.

Note: Tables provided by the sponsor.

Summary

Dasatinib was administered orally by gavage to pregnant rabbits once daily on Gestation Days 7-19, at doses of 0.5, 2, or 6 mg/kg/day (6, 24, and 72 mg/m², respectively). Animals were sacrificed on GD 29. Toxicokinetic evaluations were done on GD 19 on satellite animals.

Dose selection was based on the results of a range-finding study in which BMS-354825 was administered orally by gavage to presumed-pregnant rabbits (7/group) once daily on Days 7 through 19 of Gestation at doses of 0, 1, 3, 6, and 10 mg/kg/day (0, 12, 45, 90, and 120 mg/m²).² The does and fetuses were euthanatized and evaluated on Day 29 of Gestation. There were no drug-related changes in either the does or the fetuses at 1 and 3 mg/kg/day. At 6 and 10 mg/kg/day, BMS-354825 caused reductions in body-weight gain or body-weight loss (approximately 50% less weight gain than controls with a gain of 150 g in controls during Gestation Days 7 to 20) and decreased food consumption. At 10 mg/kg/day, BMS-354825 caused embryoletality (approximately 69% of conceptuses/litter were resorbed, compared with 3% in controls). Based on these findings, a high dose of 6 mg/kg/day (90 mg/m²) was selected for evaluation in this study. This dose was expected to produce maternal toxicity over the 13-day dosing period without causing excessive embryo-lethality, which could compromise the objectives of the study.

Maternal findings:

- There were no drug-related changes in the does at any dose tested.

Maternal TK:

- Increases in the exposures were slightly more than dose proportional when increasing the dose from LD to MD or from MD to HD.

Embryo-fetal findings:

- All placentas appeared normal
- No drug-related changes in corpora lutea, implantations, litter size, live/dead fetuses, early/late resorptions, or fetal sex ratios
- Fetal alterations were observed starting from the LD (0.5 mg/kg/day or 6 mg/m²/day; AUC= 44 ng.hr/mL). Alterations were mainly due to increased incidence of variations (common observations in this species and strain and reversible delays or accelerations in development).
- The following skeletal variations were noted at LD, MD, and HD, with higher incidence than the control: delays in ossification of the fetal lumbar vertebrae (bifid arches), pelvis (incomplete ossification or not ossified pubes), and possibly hyoid body (incomplete ossification or not ossified).
- The following skeletal variations were observed at HD, with higher incidence than the control animals: irregular ossification of the hyoid (angulated), 7th cervical ribs
- Fetal abnormalities occurred at doses that did not cause maternal toxicities.
- Fetal alterations occurred starting at the LD, at an AUC 0.1 x that seen in females in the clinical trials, at the recommended dose of 70 mg BID (140 mg/day). Therefore teratogenic effects of Dasatinib occurred at sub-therapeutic exposures.
- A NOAEL for embryo-fetal toxicity was not identified in this study.

Summary and conclusions of the reproductive toxicity studies in rats and rabbits

No animal study was conducted with Dasatinib to evaluate the fertility/early embryonic or prenatal/postnatal developments.

Dasatinib was evaluated for embryo-fetal toxicities in rats and rabbits. Dosing in both species was from implantation (presumed GD 6 in rats and GD 7 in rabbits) to the end of organogenesis (presumed GD15 in rats and GD 19 in rabbits). Rats were sacrificed on GD 20 and rabbits on GD 29.

Dasatinib was teratogenic in both species tested. Embryo-fetal effects, i.e. lethality (rats) and/or abnormalities (rats and rabbits), started to manifest at doses that did not cause maternal toxicities. In addition, in both studies embryo-fetal lethality and/or abnormalities started to manifest at sub-therapeutic exposures.

Embryo-fetal toxicities included the following:

Rats:

- Embryo-lethality, starting at the LD (2.5 mg/kg or 15 mg/m²): ↑resorptions and ↓mean litter size
- Fetal abnormalities starting at the LD: malformations of the scapula or humerus (bent) and reduced ossification of the sternbrae and thoracic vertebral centra (irregularly/dumbbell shaped and/or reduced ossification site counts). Additional fetal abnormalities at the LMD (5 mg/kg or 30 mg/m²) included fluid-filled thoracic and abdominal cavities, edema (body),

microhepatia (small liver), misshapen clavicles, bent radius and femur, wavy or nodulated ribs, and reduced ossification of the thoracic, lumbar, and sacral vertebrae (hypoplastic, not ossified, and/or incompletely ossified centra) and forepaw phalanges (reduced ossification site counts).

Rabbits:

- Fetal abnormalities starting at the LD: delays in ossification of the fetal lumbar vertebrae (bifid arches), pelvis (incomplete ossification or not ossified pubes), and possibly hyoid body (incomplete ossification or not ossified). Additional observations at the HD consisted of irregular ossification of the hyoid (angulated) and presence of 7th cervical ribs.

In rats, the lowest dose (2.5 mg/kg/day or 15 mg/m2/day) resulted in embryo-fetal toxicities. This dose had maternal AUC of 105 ng/hr/mL (0.3 x the human AUC in females at the recommended dose of 70 mg BID). In rabbits the lowest dose (0.5 mg/kg/day or 6 mg/m2/day) caused embryo-fetal toxicities. This dose had a maternal AUC of 44 ng.hr/mL (0.1 x the human AUC in females at the recommended dose of 70 mg BID).

Mean systemic exposures (AUC₀₋₂₄) to dasatinib in the embryo-fetal development studies in rats and rabbits are summarized and compared to the AUC in humans at the recommended oral clinical dose of 70 mg BID in Table below.

Table 6.2 Dasatinib Exposures in Rats and Rabbits vs Humans

Species	Study	Dose (mg/kg)	Mean AUC (ng-hr/mL, 0-24 hour)	Multiple of Human Exposure Based on AUC 70 mg BID
Human	Continuous daily dose	70 mg BID	308 ^a (Daily AUC, ng-hr/mL)	-
			Females	Females
Rat	Daily oral. Gestation Days 6-15	2.5 5 10 20	105 239 1491 1278	0.3 0.8 4.8 4.1
Rabbit	Daily oral. Gestation Days 7-19	0.5 2 6	44 248 834	0.1 0.8 2.7

^a Daily AUC based on twice the geometric mean AUC(0-12hr) of 154 ng-hr/mL.

Note: Table provided by the sponsor.

2.6.6.7 Local tolerance

No studies.

2.6.6.8 Special toxicology studies**Study title: Report on analysis of immunosuppressive potential of BMS 354825**

Key findings: BMS354825 exhibits immunosuppressive potential as measured by when administered by murine lymphocyte response and in the murine neonatal heart to ear transplant model at doses of 60 and 75 mg/m², respectively in mice.

Study no: 930003471

Volume #, and page #: Item 5

Conducting laboratory and location: BMS; Lawrenceville, NJ

Date of study initiation: Not provided

GLP compliance: no

Drug, lot #, radiolabel, and % purity: not provided

Formulation/vehicle: not provided

Rationale for this study:

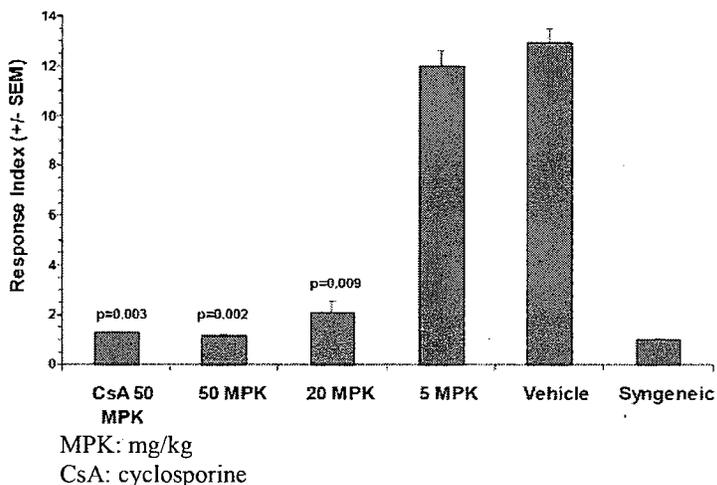
Immunosuppressive agents are used to prevent graft rejection following solid organ transplantation. The mechanism by which some of these immunosuppressive compounds act is via inhibition of T cell activation and proliferation. Some of the immunosuppressive agents mediate their inhibitory activity by blocking the function of critical intracellular signaling molecules of T cells which are activated following T cell receptor engagement by antigen presenting cells. LCK (belonging to the family of SRC tyrosine kinases) is thought to be one of these T cell signaling molecules. Because BMS-354825 inhibits the activity of a number of SRC family members including LCK, its potential to exert immunosuppressive effects on T cell functions was tested.

Methods: Murine Lymphocyte Response (MLR; a murine model of T cell proliferation) and murine neonatal heart to ear transplant (a murine model of graft rejection).

Results:

- Adoptive transfer of allogeneic CFSE labeled spleen and LN cells from C57BL/6 mice to lethally irradiated C3H/HeJ mice results in significant expansion of the CD4⁺ T cells as measured by flow cytometric analysis. Administration of BMS-354825 once daily (QD) by oral gavage to the recipient mice inhibited this T-cell proliferation in a dose dependent manner (see Figure below). Administration of BMS354825 inhibited T-cell proliferation in a dose dependent manner at doses of 20 and 50 mg/kg/day (60 and 150 mg/m²) but not at 5 mg/kg/day (15 mg/m²).

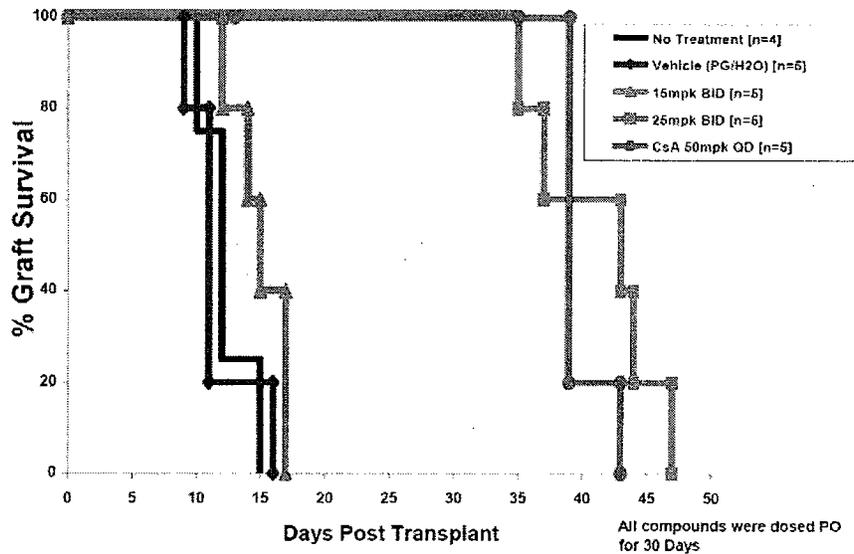
Effect of BMS-354825 on T cell Proliferation In Vivo: In Vivo MLR



- To further evaluate the immunosuppression induced by BMS-354825, a non-vascularized murine heart transplant experiment was conducted. In this model (Figure below), the no treatment and vehicle treated groups rejected the donor heart, within about 2 weeks post-transplant. The median survival time (MST) of the untreated grafts was 12 days and the MST of the vehicle group was 11 days. Oral administration of cyclosporine (CsA) at 50 mg/kg QD for 30 days maintained graft survival. Withdrawal of CsA after 30 days of dosing led to graft rejection in 9 days (MST=39 Days). Administration of BMS-354825 twice daily (BID) by oral gavage to the recipient mice at a dose of 25 mg/kg also inhibited graft rejection similar to CsA. When BMS-354825 was dosed at 15 mg/kg/BID, little extension of graft survival was observed (MST=14 days). Therefore, oral administration of BMS-354825 had immunosuppressive effects in this murine model of graft rejection, under the conditions tested.

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Effect of BMS-354825 Administration on Non-vascularized Heart Graft Rejection Rates



- To evaluate the effect of a “drug holiday” (5 day-on/2day-off schedule) on the immunosuppression by BMS-354825, a similar non-vascularized heart transplant experiment was performed. In this experiment, BMS-354825 was dosed at 25 mg/kg BID either continuously or on a 5 day-on/2 day-off schedule. The addition of a drug holiday abrogated the immunosuppressive effect of BMS-354825, as the mice dosed Monday through Friday had no significant prolongation of graft survival.

2.6.7 TOXICOLOGY TABULATED SUMMARY

The following summary Tables were provided by the sponsor.

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Table 2.6.7.13A Reproductive and Developmental Toxicity – Effects on Embryo-Fetal Development

Report Title: Oral Study of Embryo-Fetal Development in Rats	Test Article: Dasatinib (BMS-354825)
Design similar to ICH 4.1.3? Yes	Study Number: DN04078
Species/Strain: Rat (CD-1)(SD)(GS BR)	Document Control Number: 930011508
Initial Age: ~9 to 10 weeks at mating	Location in Dossier: dn04078.pdf
Date of First Dose: 31-Aug-2004	GLP Compliance: Yes
Special Features: None	Vehicle/Formulation: 80 mM sodium citrate buffer
No Observed Adverse-Effect Level:	
F₀ Females: 5 mg/kg/day	
F₁ Litters: < 2.5 mg/kg/day	

Daily Dose (mg/kg)	0 (Control)	2.5	5	10	20
Dams:					
Toxicokinetics					
AUC (ng·hr/mL)	♦	105	239	1490	1270
Cmax (ng/mL)	♦	21.1	43.7	128.2	107.2
No. Pregnant/No. Assigned to Study - N/N (%)	22/22 (100)	21/22 (95.5)	22/22 (100)	22/22 (100)	22/22 (100)
No. Died or Sacrificed Moribund	0	0	0	0	7 ^a
No. Aborted or with Total Resorption of Litter ^b	0	0	1	22**	15**
Clinical Observations	--	--	--	--	... ^c
Necropsy Observations	--	--	--	--	... ^d
Body Weight (% ^e) Gestation Day 13	301.7 grams	-1%	-1% _a	-6% ^g **	-12% ^h ** ^f

Abbreviations: AUC = Area under the concentration-time curve; BMS = Bristol-Myers Squibb; Cmax = Maximum observed concentration; GLP = Good Laboratory Practices; hr = Hour; kg = Kilogram; mg = Milligram; mL = Milliliter; mM = Millimolar; N or No. = Number; ng = Nanogram; -- = No noteworthy findings; ♦ = Not performed; ^a Mild; ^b Moderate; ^c Marked; * P ≤ 0.05, ** P ≤ 0.01

Table 2.6.7.13A Reproductive and Developmental Toxicity – Effects on Embryo-Fetal Development

Document Control Number: 930011508 (Continued)	Test Article: Dasatinib (BMS-354825)				
	Study No.: DN04078				
Daily Dose (mg/kg)	0 (Control)	2.5	5	10	20
Dams: (Continued)					
Body Weight (% ^e) Gestation Day 20	371.4 grams	-3%	-11% ^g **	-22% ^h **	-20% ^g ** ^f
Body-Weight Change (% ^e) Gestation Days 6 to 12	35.0 grams	+6%	+23%	+11%	+79% ^g ** ^h
Body-Weight Change (% ^e) Gestation Days 12 to 16	31.5 grams	-20% ^g *	-45% ^g **	-89% ^g **	loss of 1 gram ^g ** ^f
Body-Weight Change (% ^e) Gestation Days 16 to 20	60.5 grams	-12% ^g *	-36% ^g **	-95% ^g **	loss of 0.3 grams ^g ** ^f
Food Consumption (% ^e) Gestation Days 6 to 16	26.7 grams/day	+2%	0%	-9% ^g **	-24% ^g ** ^f
Food Consumption (% ^e) Gestation Days 16 to 20	29.8 grams/day	-1%	-4%	-14% ^g **	-9% ^g ** ^f
Mean No. Corpora Lutea	16.0	14.3*	15.5	15.0	16.6*
Mean No. Implantations	14.5	13.7	14.8	14.0	14.3
Litters:					
No. Evaluated	22	21	22	22	15
Mean No. Live Fetuses	13.9	11.2	3.5**	0**	0**
Mean No. Resorptions	0.5	2.5	11.3	14.0	14.3
No. of Litters with Dead Fetuses	0	0	0	0	0
Mean % Preimplantation Loss ⁱ	9.1	4.1	4.7	5.9	12.9
Mean % Postimplantation Loss ^j	3.8	17.4**	76.6**	100.0**	100.0**

Abbreviations: AUC = Area under the concentration-time curve; BMS = Bristol-Myers Squibb; Cmax = Maximum observed concentration; GLP = Good Laboratory Practices; hr = Hour; kg = Kilogram; mg = Milligram; mL = Milliliter; mM = Millimolar; N or No. = Number; ng = Nanogram; -- = No noteworthy findings; ♦ = Not performed; ^a Mild; ^b Moderate; ^c Marked; * P ≤ 0.05, ** P ≤ 0.01

Table 2.6.7.13A Reproductive and Developmental Toxicity – Effects on Embryo-Fetal Development

Document Control Number: 930011508 (Continued)

Test Article: Dasatinib (BMS-354825)

Study No. DN04078

Daily Dose (mg/kg)	0 (Control)	2.5	5	10	20
Litters: (Continued)					
Mean Fetal Body Weight/Litter (grams)	3.66	3.84	3.54	b	b
Fetal Sex Ratios (% male fetuses)	52.7	47.3	40.0	b	b
Summary of Gross External, Visceral, & Skeletal Anomalies:					
Total Affected Fetuses / Total Fetuses Evaluated - N/N (%)	46/306 (15.0)	32/235 (13.6)	29/76 (38.2)**	b	b
Total Affected Litters / Total Litters Evaluated - N/N (%)	15/22 (68.2)	15/21 (71.4)	17/21 (81.0)	b	b
Percent Affected Fetuses / Litter - (Mean %)	15.1	13.1	42.4**	b	b
Fetal Gross External Anomalies:					
No. Fetuses Examined / No. Litters Examined	306/22	235/21	76/21	b	b
Body: Edema					
Fetal Incidence N (%)	0	0	3 (3.9)**	b	b
Litter Incidence N (%)	0	0	3 (14.3)	b	b
Fetal Visceral Anomalies:					
No. Fetuses Examined / No. Litters Examined	152/22	117/21	36/18	b	b
Thoracic and Abdominal Cavities: Fluid-filled					
Fetal Incidence N (%)	0	0	2 (5.5)*	b	b
Litter Incidence N (%)	0	0	2 (11.1)	b	b
Liver: Small (microhepatia)					

Abbreviations: AUC = Area under the concentration-time curve; BMS = Bristol-Myers Squibb; Cmax = Maximum observed concentration; GLP = Good Laboratory Practices; hr = Hour; kg = Kilogram; mg = Milligram; mL = Milliliter; mM = Millimolar; N or No. = Number; ng = Nanogram; -- = No noteworthy findings; • = Not performed, + Mild, + Moderate, + = Marked, * P ≤ 0.05, ** P ≤ 0.01

Table 2.6.7.13A Reproductive and Developmental Toxicity – Effects on Embryo-Fetal Development

Document Control Number: 930011508 (Continued)

Test Article: Dasatinib (BMS-354825)

Study No. DN04078

Daily Dose (mg/kg)	0 (Control)	2.5	5	10	20
Fetal Visceral Anomalies: (Continued)					
Fetal Incidence N (%)	0	0	2 (5.5)*	b	b
Litter Incidence N (%)	0	0	2 (11.1)	b	b
Stomach: Outer surface and inner lining: white areas present					
Fetal Incidence N (%)	0	2 (1.7)	0	b	b
Litter Incidence N (%)	0	2 (9.5)	0	b	b
Fetal Skeletal Anomalies:					
No. Fetuses Examined / No. Litters Examined	154/22	118/21	40/19	b	b
Skull: Ectopic ossification site present on right side of supraoccipital					
Fetal Incidence N (%)	0	1 (0.8)	0	b	b
Litter Incidence N (%)	0	1 (4.8)	0	b	b
Hyoid: Body, incomplete ossification or not ossified					
Fetal Incidence N (%)	19 (12.3)	1 (0.8)**	0*	b	b
Litter Incidence N (%)	7 (31.8)	1 (4.8)*	0**	b	b
Clavicles: Misshapen					
Fetal Incidence N (%)	0	0	3 (7.5)**	b	b
Litter Incidence N (%)	0	0	2 (10.5)	b	b

Abbreviations: AUC = Area under the concentration-time curve; BMS = Bristol-Myers Squibb; Cmax = Maximum observed concentration; GLP = Good Laboratory Practices; hr = Hour; kg = Kilogram; mg = Milligram; mL = Milliliter; mM = Millimolar; N or No. = Number; ng = Nanogram; -- = No noteworthy findings; • = Not performed, + Mild, + Moderate, + = Marked, * P ≤ 0.05, ** P ≤ 0.01

Table 2.6.7.13A Reproductive and Developmental Toxicity – Effects on Embryo-Fetal Development

Document Control Number: 930011508 (Continued)

Test Article: Dasatinib (BMS-354825)

Study No. DN04078

Daily Dose (mg/kg)		0 (Control)	2.5	5	10	20
Fetal Skeletal Anomalies: ^k (Continued):						
Scapulas: Bent						
Fetal Incidence	N (%)	0	2 (1.7)	16 (40.0)**	b	b
Litter Incidence	N (%)	0	2 (9.5)	13 (68.4)**	b	b
Sternebrae: Dumbbell- or irregularly shaped						
Fetal Incidence	N (%)	4 (2.6)	8 (6.8)	8 (20.0)**	b	b
Litter Incidence	N (%)	4 (18.2)	6 (28.6)	7 (36.8)	b	b
Sternebrae: Asymmetric or unilateral ossification						
Fetal Incidence	N (%)	1 (0.6)	0	1 (2.5)	b	b
Litter Incidence	N (%)	1 (4.5)	0	1 (5.3)	b	b
Sternebrae: Bifid						
Fetal Incidence	N (%)	2 (1.3)	0	2 (5.0)	b	b
Litter Incidence	N (%)	2 (9.1)	0	2 (10.5)	b	b
Ribs: Wavy						
Fetal Incidence	N (%)	0	1 (0.8)	13 (32.5)**	b	b
Litter Incidence	N (%)	0	1 (4.8)	11 (57.9)**	b	b

Abbreviations: AUC = Area under the concentration-time curve; BMS = Bristol-Myers Squibb; Cmax = Maximum observed concentration; GLP = Good Laboratory Practices; hr = Hour; kg = Kilogram; mg = Milligram; mL = Milliliter; mM = Millimolar; N or No. = Number; ng = Nanogram; -- = No noteworthy findings; ♦ = Not performed; - Mild; - Moderate; - - - Marked; * P ≤ 0.05, ** P ≤ 0.01

Table 2.6.7.13A Reproductive and Developmental Toxicity – Effects on Embryo-Fetal Development

Document Control Number: 930011508 (Continued)

Test Article: Dasatinib (BMS-354825)

Study No. DN04078

Daily Dose (mg/kg)		0 (Control)	2.5	5	10	20
Fetal Skeletal Anomalies: ^k (Continued):						
Ribs: Nodulated						
Fetal Incidence	N (%)	0	1 (0.8)	10 (25.0)**	b	b
Litter Incidence	N (%)	0	1 (4.8)	8 (42.1)**	b	b
Ribs: 7th cervical						
Fetal Incidence	N (%)	0	1 (0.8)	2 (5.0) [†]	b	b
Litter Incidence	N (%)	0	1 (4.8)	2 (10.5)	b	b
Vertebrae: Cervical, arches, bifid						
Fetal Incidence	N (%)	1 (0.6)	1 (0.8)	2 (5.0)	b	b
Litter Incidence	N (%)	1 (4.5)	1 (4.8)	2 (10.5)	b	b
Vertebrae: Thoracic, centra, hypoplastic						
Fetal Incidence	N (%)	0	0	7 (17.5)**	b	b
Litter Incidence	N (%)	0	0	6 (31.6)**	b	b
Vertebrae: Thoracic, centra, not ossified and/or incomplete ossification						
Fetal Incidence	N (%)	1 (0.6)	0	5 (12.5)**	b	b
Litter Incidence	N (%)	1 (4.5)	0	5 (26.3)	b	b

Abbreviations: AUC = Area under the concentration-time curve; BMS = Bristol-Myers Squibb; Cmax = Maximum observed concentration; GLP = Good Laboratory Practices; hr = Hour; kg = Kilogram; mg = Milligram; mL = Milliliter; mM = Millimolar; N or No. = Number; ng = Nanogram; -- = No noteworthy findings; ♦ = Not performed; - Mild; - Moderate; - - - Marked; * P ≤ 0.05, ** P ≤ 0.01

Table 2.6.7.13A Reproductive and Developmental Toxicity – Effects on Embryo-Fetal Development

Document Control Number: 930011508 (Continued)

Test Article: Dasatinib (BMS-354825)

Study No. DN04078

Daily Dose (mg/kg)		0 (Control)	2.5	5	10	20
Fetal Skeletal Anomalies: ^k (Continued):						
Vertebrae: Thoracic, centra, dumbbell- or irregularly-shaped						
Fetal Incidence	N (%)	5 (3.2)	14 (11.9)**	1 (2.5)	b	b
Litter Incidence	N (%)	5 (22.7)	6 (28.6)	1 (5.3)	b	b
Vertebrae: Lumbar, centra, hypoplastic						
Fetal Incidence	N (%)	0	0	6 (15.0)**	b	b
Litter Incidence	N (%)	0	0	6 (31.6)**	b	b
Vertebrae: Sacral, centra, hypoplastic						
Fetal Incidence	N (%)	0	0	4 (10.0)**	b	b
Litter Incidence	N (%)	0	0	4 (21.1)*	b	b
Vertebrae: Caudal, not ossified						
Fetal Incidence	N (%)	24 (15.6)	9 (7.6)	8 (20.0)	b	b
Litter Incidence	N (%)	9 (40.9)	5 (23.8)	7 (36.8)	b	b
Pelvis: Pubes and/or ischia, not ossified or incomplete ossification						
Fetal Incidence	N (%)	3 (1.9)	0	1 (2.5)	b	b
Litter Incidence	N (%)	3 (13.6)	0	1 (5.3)	b	b

Abbreviations: AUC = Area under the concentration-time curve; BMS = Bristol-Myers Squibb; C_{max} = Maximum observed concentration; GLP = Good Laboratory Practices; hr = Hour; kg = Kilogram; mg = Milligram; mL = Milliliter; mM = Millimolar; N or No. = Number; ng = Nanogram; -- = No noteworthy findings; † = Not performed; † Mild, † Moderate, †† = Marked, * P ≤ 0.05, ** P ≤ 0.01

Table 2.6.7.13A Reproductive and Developmental Toxicity – Effects on Embryo-Fetal Development

Document Control Number: 930011508 (Continued)

Test Article: Dasatinib (BMS-354825)

Study No. DN04078

Daily Dose (mg/kg)		0 (Control)	2.5	5	10	20
Fetal Skeletal Anomalies: ^k (Continued):						
Forelimbs: Humeri, bent						
Fetal Incidence	N (%)	0	2 (1.7)	10 (25.0)**	b	b
Litter Incidence	N (%)	0	2 (9.5)	8 (42.1)**	b	b
Forelimbs: Radii, bent						
Fetal Incidence	N (%)	0	1 (0.8)	3 (7.5)**	b	b
Litter Incidence	N (%)	0	1 (4.8)	2 (15.8)	b	b
Hindlimbs: Femur, bent						
Fetal Incidence	N (%)	0	0	4 (10.0)**	b	b
Litter Incidence	N (%)	0	0	4 (21.0)**	b	b
Mean Ossification Sites per Fetus per Litter:						
No. Fetuses Examined	No. Litters Examined	154/22	118/21	40/19	b	b
Ribs (Total)		13.00	13.01	13.02	b	b
Sternebrae		5.63	5.62	5.13*	b	b
Forepaws: Carpals		0.00	0.00	0.00	b	b
Forepaws: Metacarpals		3.91	3.96	3.92	b	b
Forepaw: Phalanges		6.20	6.37	5.63	b	b
Hindpaws: Tarsals		0.00	0.00	0.00	b	b

Abbreviations: AUC = Area under the concentration-time curve; BMS = Bristol-Myers Squibb; C_{max} = Maximum observed concentration; GLP = Good Laboratory Practices; hr = Hour; kg = Kilogram; mg = Milligram; mL = Milliliter; mM = Millimolar; N or No. = Number; ng = Nanogram; -- = No noteworthy findings; † = Not performed; † Mild, † Moderate, †† = Marked, * P ≤ 0.05, ** P ≤ 0.01

Table 2.6.7.13A Reproductive and Developmental Toxicity – Effects on Embryo-Fetal Development

Document Control Number: 930011598 (Continued)

Test Article: Dasatinib (BMS-354825)

Study No. DN04078

Daily Dose (mg/kg)	0 (Control)	2.5	5	10	20
Mean Ossification Sites per Fetus per Litter: (Continued)					
Hindpaw: Metatarsals	4.01	4.10	4.00	b	b
Hindpaw: Phalanges	5.06	5.30	5.00	b	b

- a Mortality moribundity in 7 dams (2 found dead on Days 12 or 13 of gestation and 5 euthanized in moribund condition on Days 13 to 15 of gestation) preceded by agonal signs of soft loose feces, piloerection, brown-clear periorcular substance, and decreased motor activity.
- b There were no viable fetuses at 10 and 20 mg/kg day.
- c No inferential statistical analyses were conducted on clinical observation data; drug-related clinical observations included ungroomed coat, fecal- or urine-stained coat, brown/clear perinasal and/or perioral substance, and reduced/absent feces during Gestation Days 7 to 18.
- d Maternal necropsy observations of enlarged adrenals and gas-filled intestines.
- e For controls, group means are shown. For treated groups, percent differences from controls are shown, unless otherwise noted. Statistical significance is based on actual data (not on the percent differences).
- f N=16; excludes dams 5F0098 and 5F0099 which were found dead on Day 12 and 13 of gestation, respectively, and dams 5F0091, 5F0096, 5F0102, and 5F0104 which were euthanized in moribund condition on Days 13 to 14 of gestation.
- g N=15; excludes dams 5F0098 and 5F0099 which were found dead on Day 12 and 13 of gestation, respectively, and dams 5F0091, 5F0095, 5F0096, 5F0102, and 5F0104 which were euthanized in moribund condition on Days 13 to 15 of gestation.
- h N=21; excludes dam 5F0098 which was found dead on Day 12 of gestation.
- i Preimplantation loss calculated as: [(Corpora lutea - implantations) / corpora lutea] x 100.
- j Postimplantation loss calculated as: [(Dead + resorbed conceptuses) / implantations] x 100.
- k Skeletal anomalies noted exclusively in control fetuses are omitted from the listings below, but are included in the total incidence of affected fetuses and litters as well as the percent affected fetuses/litter. These anomalies included hypoplastic supraoccipital and/or interparietal; bifid thoracic arches and centra; and misshapen ilia.

Abbreviations: AUC = Area under the concentration-time curve; BMS = Bristol-Myers Squibb; Cmax = Maximum observed concentration; GLP = Good Laboratory Practices; hr = Hour; kg = Kilogram; mg = Milligram; mL = Milliliter; mM = Millimolar; N or No. = Number, ng = Nanogram; -- = No noteworthy findings; • = Not performed; • = Mild; • = Moderate; • = Marked; * P ≤ 0.05; ** P ≤ 0.01

Table 2.6.7.13B Reproductive and Developmental Toxicity – Effects on Embryo-Fetal Development

Report Title: Oral Study of Embryo-Fetal Development in Rabbits

Test Article: Dasatinib (BMS-354825)

Design similar to ICH 4.1.3? Yes

Duration of Dosing: Gestation Days 7 to 19

Study Number: DN04080

Day of Mating: Gestation Day 0

Document Control Number: 930010604

Species/Strain: New Zealand White Rabbit
Hra(NZW) SPF

Day of C-Section: Gestation Days 29

Location in Dossier: dn04080.pdf

Initial Age: 5.5 to 6 months at mating

Method of Administration: Oral, Gavage

Date of First Dose: 20-Sep-2004

Vehicle/Formulation: 80 mM sodium citrate buffer

GLP Compliance: Yes

Special Features: None

No Observed Adverse-Effect Level: F₀ Females: ≥ 6 mg/kg

F₁ Litters: ≥ 0.5 mg/kg

Daily Dose (mg/kg)	0 (Control)	0.5	2	6
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DOSS:

Toxicokinetics

AUC (ng·hr/mL)	•	44	248	834
Cmax (ng/mL)	•	14	63	227
No. Pregnant No. Assigned to Study - N/N (%)	20/22 (90.9)	22/22 (100)	20/22 (90.9)	21/22 (95.5)
No. Died or Sacrificed Moribund	0	0	0	0
No. Aborted or with Total Resorption of Litter	0	0	0	0
Clinical Observations	--	--	--	--

Abbreviations: AUC = Area under the concentration-time curve; BMS = Bristol-Myers Squibb; Cmax = Maximum observed concentration; GLP = Good Laboratory Practices; hr = Hour; kg = Kilogram; mg = Milligram; mL = Milliliter; mM = Millimolar; N or No. = Number; ng = Nanogram; SPF = Specific pathogen free; • = Not performed; -- = No noteworthy findings; * P ≤ 0.05; ** P ≤ 0.01.

Table 2.6.7.13B Reproductive and Developmental Toxicity – Effects on Embryo-Fetal Development

Report Title: 9300010604 (Continued)	Test Article: Dasatinib (BMS-354825)			
	Study No. 80			
Daily Dose (mg/kg)	0 (Control)	0.5	2	6
Doses: (Continued)				
Necropsy Observations	--	--	--	--
Body Weight (% ³) Gestation Day 20	3.85 kg	2%	2%	-1%
Body Weight (% ³) Gestation Day 29	3.98 kg	1%	2%	-1%
Body Weight Change (% ³) Gestation Days 7-20	0.17 kg	29%	18%	-12%
Body Weight Change (% ³) Gestation Days 7-29	0.29 kg	17%	24%	3%
Food Consumption (% ³) Gestation Days 7-20	157.8 grams/day	10% *	3%	-1%
Food Consumption (% ³) Gestation Days 7-29	145.3 grams/day	7%	4%	0%
Mean No. Corpora Lutea	9.7	9.3	10.2	9.9
Doses: (Continued)				
Mean No. Implantations	9.3	8.7	9.9	9.3
Litters:				
No. Evaluated	19 ^b	22	20	21
Mean No. Resorptions	0.4	0.1	0.4	0.5
No. of Litters with Dead Fetuses	0	0	0	0

Abbreviations: AUC = Area under the concentration-time curve; BMS = Bristol-Myers Squibb; Cmax = Maximum observed concentration; GLP = Good Laboratory Practices; hr = Hour; kg = Kilogram; mg = Milligram; mL = Milliliter; mM = Millimolar; N or No. = Number; ng = Nanogram; SPF = Specific pathogen free; ♦ = Not performed; --- = No noteworthy findings; * P < 0.05, ** P < 0.01.

Table 2.6.7.13B Reproductive and Developmental Toxicity – Effects on Embryo-Fetal Development

Report Title: 9300010604 (Continued)	Test Article: Dasatinib (BMS-354825)			
	Study No. 80			
Daily Dose (mg/kg)	0 (Control)	0.5	2	6
Litters: (Continued)				
Mean % Preimplantation Loss ^c	4.7	6.3	2.8	6.5
Mean % Postimplantation Loss ^d	4.0	3.2	3.5	6.0
Mean Fetal Body Weight/Litter (grams)	42.26	41.89	41.73	42.20
Fetal Sex Ratios (% male fetuses)	58.7	53.6	50.1	56.2
Summary of Gross External, Visceral, & Skeletal Anomalies:				
Total Affected Fetuses/Total Fetuses Evaluated - N/N (%)	32/170 (18.8)	58/190 (30.5) *	62/191 (32.5) **	67/185 (36.2) **
Total Affected Litters/Total Litters Evaluated - N/N (%)	16/20 (80.0)	16/22 (72.7)	18/20 (90.0)	17/21 (81.0)
Percent Affected Fetuses/Litter (Mean %)	22.5	27.9	32.7	32.7
Fetal Gross External Anomalies:				
No. Fetuses Examined/No. Litters Examined	170/20	190/22	191/20	185/21

All fetuses appeared normal at gross external examination

Abbreviations: AUC = Area under the concentration-time curve; BMS = Bristol-Myers Squibb; Cmax = Maximum observed concentration; GLP = Good Laboratory Practices; hr = Hour; kg = Kilogram; mg = Milligram; mL = Milliliter; mM = Millimolar; N or No. = Number; ng = Nanogram; SPF = Specific pathogen free; ♦ = Not performed; --- = No noteworthy findings; * P < 0.05, ** P < 0.01.

Table 2.6.7.13B Reproductive and Developmental Toxicity – Effects on Embryo-Fetal Development

Report Title: 9300010604 (Continued)		Test Article: Dasatinib (BMS-354825)			
		Study No. 80			
Daily Dose (mg/kg)		0 (Control)	0.5	2	6
Fetal Visceral Anomalies^c:					
No. Fetuses Examined/ No. Litters Examined		170/20	190/22	191/20	185/21
Ureters: Retrocaudal					
Fetal Incidence	N (%)	0	3 (1.6)	0	3 (1.6)
Litter Incidence	N (%)	0	2 (9.1)	0	2 (9.5)
Kidneys: Severe dilation					
Fetal Incidence	N (%)	0	0	0	1 (0.5)
Litter Incidence	N (%)	0	0	0	1 (4.8)
Gallbladder: Small					
Fetal Incidence	N (%)	0	0	0	1 (0.5)
Litter Incidence	N (%)	0	0	0	1 (4.8)

Abbreviations: AUC = Area under the concentration-time curve; BMS = Bristol-Myers Squibb; C_{max} = Maximum observed concentration; GLP = Good Laboratory Practices; hr = Hour; kg = Kilogram; mg = Milligram; mL = Milliliter; mM = Millimolar; N or No = Number; ng = Nanogram; SPI = Specific pathogen free; * = Not performed; -- = No noteworthy findings; * P ≤ 0.05, ** P ≤ 0.01.

Table 2.6.7.13B Reproductive and Developmental Toxicity – Effects on Embryo-Fetal Development

Report Title: 9300010604 (Continued)		Test Article: Dasatinib (BMS-354825)			
		Study No. 80			
Daily Dose (mg/kg)		0 (Control)	0.5	2	6
Fetal Skeletal Anomalies^f:					
No. Fetuses Examined/ No. Litters Examined		170/20	190/22	191/20	185/21
Skull: Nasals, supernumerary bone(s)					
Fetal Incidence	N (%)	1 (0.6)	0	4 (2.1)	1 (0.5)
Litter Incidence	N (%)	1 (5.0)	0	4 (20.0)	1 (4.8)
Skull: Nasals, hypoplastic					
Fetal Incidence	N (%)	0	0	3 (1.6)	0
Litter Incidence	N (%)	0	0	2 (10.0)	0
Skull: Frontals, supernumerary bone(s)					
Fetal Incidence	N (%)	4 (2.4)	4 (2.1)	5 (2.6)	1 (0.5)
Litter Incidence	N (%)	4 (20.0)	2 (9.1)	4 (20.0)	1 (4.8)
Skull: Anterior fontanel, supernumerary bone(s)					
Fetal Incidence	N (%)	0	0	2 (1.0)	0
Litter Incidence	N (%)	0	0	2 (10.0)	0
Skull: Interparietal, incomplete ossification					
Fetal Incidence	N (%)	0	0	0	1 (0.5)
Litter Incidence	N (%)	0	0	0	1 (4.8)

Abbreviations: AUC = Area under the concentration-time curve; BMS = Bristol-Myers Squibb; C_{max} = Maximum observed concentration; GLP = Good Laboratory Practices; hr = Hour; kg = Kilogram; mg = Milligram; mL = Milliliter; mM = Millimolar; N or No = Number; ng = Nanogram; SPI = Specific pathogen free; * = Not performed; -- = No noteworthy findings; * P ≤ 0.05, ** P ≤ 0.01.

Table 2.6.7.13B Reproductive and Developmental Toxicity – Effects on Embryo-Fetal Development
 Report Title: 9300010604 (Continued) Test Article: Dasatinib (BMS-354825)

Daily Dose (mg/kg)	Study No. 80			
	0 (Control)	0.5	2	6
Fetal Skeletal Anomalies^f: (Continued)				
Hyoid: Body, incomplete or not ossified				
Fetal Incidence N (%)	5 (2.9)	9 (4.7)	18 (9.4) *	10 (5.4)
Litter Incidence N (%)	3 (15.0)	2 (9.1)	6 (30.0)	3 (14.3)
Hyoid: Alvea, not ossified				
Fetal Incidence N (%)	0	1 (0.5)	0	0
Litter Incidence N (%)	0	1 (4.5)	0	0
Hyoid: Alvea, angulated				
Fetal Incidence N (%)	5 (2.8)	4 (2.1)	4 (2.1)	9 (4.8)
Litter Incidence N (%)	3 (15.0)	3 (13.6)	3 (15.0)	5 (23.8)

Abbreviations: AUC = Area under the concentration-time curve; BMS = Bristol-Myers Squibb; C_{max} = Maximum observed concentration; GLP = Good Laboratory Practices; hr = Hour; kg = Kilogram; mg = Milligram; mL = Milliliter; mM = Millimolar; N or No. = Number; ng = Nanogram; SPF = Specific pathogen free; * = Not performed; - = No noteworthy findings; * P ≤ 0.05, ** P ≤ 0.01.

Table 2.6.7.13B Reproductive and Developmental Toxicity – Effects on Embryo-Fetal Development
 Report Title: 9300010604 (Continued) Test Article: Dasatinib (BMS-354825)

Daily Dose (mg/kg)	Study No. 80			
	0 (Control)	0.5	2	6
Fetal Skeletal Anomalies^f: (Continued)				
Sternumbrae: Hyperplastic				
Fetal Incidence N (%)	1 (0.6)	0	5 (2.6)	0
Litter Incidence N (%)	1 (5.0)	0	4 (20.0)	0
Sternumbrae: Asymmetric				
Fetal Incidence N (%)	0	0	2 (1.0)	1 (0.5)
Litter Incidence N (%)	0	0	2 (10.0)	1 (4.8)
Sternumbrae: Dumbbell or irregularly-shaped				
Fetal Incidence N (%)	7 (4.1)	10 (5.5)	11 (5.8)	9 (4.8)
Litter Incidence N (%)	6 (30.0)	6 (27.3)	10 (50.0)	4 (19.0)
Sternumbrae: Bifid				
Fetal Incidence N (%)	2 (1.2)	4 (2.1)	6 (3.1)	2 (1.1)
Litter Incidence N (%)	2 (10.0)	4 (18.2)	5 (25.0)	2 (9.5)
Ribs: 7th cervical				
Fetal Incidence N (%)	1 (0.6)	1 (0.5)	1 (0.5)	20 (10.8) **
Litter Incidence N (%)	1 (5.0)	1 (4.5)	1 (5.0)	9 (42.9) **

Abbreviations: AUC = Area under the concentration-time curve; BMS = Bristol-Myers Squibb; C_{max} = Maximum observed concentration; GLP = Good Laboratory Practices; hr = Hour; kg = Kilogram; mg = Milligram; mL = Milliliter; mM = Millimolar; N or No. = Number; ng = Nanogram; SPF = Specific pathogen free; * = Not performed; - = No noteworthy findings; * P ≤ 0.05, ** P ≤ 0.01.

Table 2.6.7.13B Reproductive and Developmental Toxicity – Effects on Embryo-Fetal Development

Report Title: 93C0010604 (Continued)

Test Article: Dasatinib (BMS-354825)

Study No. 80

Daily Dose (mg/kg)		0 (Control)	0.5	2	6
Fetal Skeletal Anomalies¹: (Continued)					
Ribs: Fused					
Fetal Incidence	N (%)	2 (1.2)	1 (0.5)	0	0
Litter Incidence	N (%)	1 (5.0)	1 (4.5)	0	0
Ribs: Discontinuous					
Fetal Incidence	N (%)	0	0	0	1 (0.5)
Litter Incidence	N (%)	0	0	0	1 (4.8)
Vertebrae: Thoracic, arches, fused; centra, fused; centra, misaligned					
Fetal Incidence	N (%)	0	1 (0.5)	0	0
Litter Incidence	N (%)	0	1 (4.5)	0	0
Vertebrae: Thoracic, hemivertebrae					
Fetal Incidence	N (%)	2 (1.2)	2 (1.1)	0	0
Litter Incidence	N (%)	1 (5.0)	1 (4.5)	0	0
Vertebrae: Thoracic, centra, dumbbell or irregularly-shaped					
Fetal Incidence	N (%)	3 (1.8)	2 (1.1)	1 (0.5)	0
Litter Incidence	N (%)	1 (5.0)	1 (4.5)	1 (5.0)	0

Abbreviations: AUC = Area under the concentration-time curve; BMS = Bristol-Myers Squibb; C_{max} = Maximum observed concentration; GLP = Good Laboratory Practices; hr = Hour; kg = Kilogram; mg = Milligram; mL = Milliliter; mM = Millimolar; N or No. = Number; ng = Nanogram; SPF = Specific pathogen free; • = Not performed; -- = No noteworthy findings; * P ≤ 0.05; ** P ≤ 0.01.

Table 2.6.7.13B Reproductive and Developmental Toxicity – Effects on Embryo-Fetal Development

Report Title: 93C0010604 (Continued)

Test Article: Dasatinib (BMS-354825)

Study No. 80

Daily Dose (mg/kg)		0 (Control)	0.5	2	6
Fetal Skeletal Anomalies¹: (Continued)					
Vertebrae: Thoracic, centra, bifid					
Fetal Incidence	N (%)	1 (0.6)	1 (0.5)	0	0
Litter Incidence	N (%)	1 (5.0)	1 (4.5)	0	0
Vertebrae: Lumbar, arches, bifid					
Fetal Incidence	N (%)	1 (0.6)	26 (13.7)**	12 (6.3)**	29 (15.7)**
Litter Incidence	N (%)	1 (5.0)	9 (40.9)**	7 (35.0)*	10 (47.6)**
Pelvis: Pubes, incomplete or not ossified					
Fetal Incidence	N (%)	0	3 (4.2)**	4 (2.1)	5 (2.7)
Litter Incidence	N (%)	0	3 (13.6)	2 (10.0)	4 (19.0)
Mean Ossification Sites per Fetus per Litter:					
No. Fetuses Examined / No. Litters Examined		170/20	190/22	191/20	135/21
Ribs (Total)		12.56	12.57	12.55	12.61
Sternebrae		5.90	5.73	5.70	5.74
Forepaws: Carpals		0	0	0	0
Forepaws: Metacarpals		5.00	4.83	4.69	4.99
Forepaws: Phalanges		13.93	13.95 [§]	13.98	13.96

Abbreviations: AUC = Area under the concentration-time curve; BMS = Bristol-Myers Squibb; C_{max} = Maximum observed concentration; GLP = Good Laboratory Practices; hr = Hour; kg = Kilogram; mg = Milligram; mL = Milliliter; mM = Millimolar; N or No. = Number; ng = Nanogram; SPF = Specific pathogen free; • = Not performed; -- = No noteworthy findings; * P ≤ 0.05; ** P ≤ 0.01.

Table 2.6.7.13B Reproductive and Developmental Toxicity – Effects on Embryo-Fetal Development

Report Title: 9300013604 (Continued)

Test Article: Dasatinib (BMS-354825)

Daily Dose (mg/kg)	Study No. 80			
	0 (Control)	0.5	2	6
Mean Ossification Sites per Fetus per Litter: (Continued)				
Hindpaws: Tarsals	2.00	2.00	2.00	2.00
Hindpaws: Metatarsals	4.00	4.00	4.00	4.00
Hindpaws: Phalanges	12.00	12.00	12.00	12.00 ^f

a For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).

b Excludes litter 1F0023 which consisted of a single conceptus (1 male fetus that appeared normal).

c Preimplantation loss calculated as $[(\text{Corpora Lutea} - \text{implantations}) / \text{Corpora Lutea}] \times 100$.

d Postimplantation loss calculated as $[(\text{Dead} + \text{resorbed conceptuses}) / \text{Implantations}] \times 100$.

e Visceral anomalies noted exclusively in control fetuses (circumcorneal hemorrhagic of the eye; misshapen heart; enlarged aorta; stenosis of the pulmonary artery; absent interventricular septum) are omitted from the listings, but are included in the total incidence of affected fetuses and litters as well as the percent affected fetuses/litter.

f Skeletal anomalies noted exclusively in control fetuses (enlarged anterior fontanel; bifurcated ribs; misaligned cervical arches; cervical hemivertebrae; unilateral ossification of cervical centra; cervical centra misaligned; and thoracic arches misaligned) are omitted from the listings below, but are included in the total incidence of affected fetuses and litters as well as the percent affected fetuses/litter.

g Excludes fetuses 2F0041-R4 and 3F0031-L3 for which phalanges in the fore- or hindpaws could not be evaluated as the result of damage sustained during processing.

Abbreviations: AUC = Area under the concentration-time curve; BMS = Bristol-Myers Squibb; Cmax = Maximum observed concentration; GLP = Good Laboratory Practices; hr = Hour; kg = Kilogram; mg = Milligram; mL = Milliliter; nM = Nanomolar; N or No. = Number; ng = Nanogram; SPF = Specific pathogen free; • = Not performed; -- = No noteworthy findings; * P ≤ 0.05; ** P ≤ 0.01.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

The proposed indications for dasatinib are:

- Treatment of adults with chronic, accelerated, or blast phase chronic myeloid leukemia with resistance or intolerance to prior therapy including imatinib.
- Treatment of adults with Philadelphia chromosome-positive acute lymphoblastic leukemia and lymphoid blast chronic myeloid leukemia with resistance or intolerance to prior therapy.

Dasatinib (BMS-354825) is an inhibitor of multiple protein tyrosine kinases. It binds to the ATP binding site and, as shown in biochemical assays, it inhibits the following kinases at nM ranges: The SRC family of kinases (SRC, LCK, YES, FYN); BCR-ABL; c-KIT; EPHA2; and PDGF-Rβ. The IC50s ranged from 0.55 nM (c-SRC) to 28 nM (PDGF-Rβ).

Pharmacology studies conducted with dasatinib showed that it had anti-growth/ anti-tumor activity in CML and ALL cell lines as well as in CML tumor models. In addition, CML cell lines and tumor models resistant to imatinib were sensitive to dasatinib treatment. Over-expression of the SRC family of kinases, e.g. FYN, HCK, and LYN was detected in the imatinib-resistant cell lines produced in culture or in cell lysates obtained from imatinib-resistant CML patients (5-6 patients). This limited information

suggests that in a sub-population of imatinib-resistant CML patients, increased expression of SRC kinases may take place.

Based on the Secondary Pharmacology/Pharmacodynamics, dasatinib showed inhibitory effects on bone resorption in vitro and in vivo, under the conditions of the assays. This effect may be due to the inhibition of the SRC family of kinases; SRC kinase has been shown to be involved in osteoclast function.

Because LCK (a member of the SRC family) may be involved in the T cell signaling, the immunosuppressive potential of dasatinib to prevent graft rejection was tested in murine models of T cell proliferation and graft rejection. Dasatinib was shown to inhibit T-cell proliferation in a dose dependent manner. Moreover, oral administration of dasatinib resulted in reduced graft rejection, when dasatinib was administered continuously.

Safety Pharmacology studies revealed the potential for dasatinib to cause cardiovascular toxicities. Based on the hERG and rabbit Purkinje fiber assays, dasatinib has the potential to cause QT prolongation. Single dose safety pharmacology and toxicology studies in monkeys revealed the potential for dasatinib to increase systolic, diastolic, and arterial blood pressure. In addition, cardiovascular findings in the toxicology studies included: vascular and cardiac fibrosis, cardiac hypertrophy, myocardial necrosis, hemorrhage of the valves, ventricle, and atrium, and cardiac inflammation.

After oral administration, the C_{max} of dasatinib was reported 1-8 hrs (T_{max}) post-dose in rats and monkeys. Maximum plasma concentrations (C_{max}) of dasatinib were generally observed between 0.5 and 6 hrs (T_{max}) following oral administration in humans. The parent drug and the metabolites are distributed to several tissues, mainly by 4 hrs post-dose. The following tissues had tissue:plasma ratio of radioactivity greater than 1: GI tract, liver, adrenal glands, lungs, kidneys, spleen, thyroid, eyes, urinary bladder, bone marrow, heart, skeletal muscle.. Dasatinib is highly metabolized in rats, monkeys, and humans. Only involvement of CYP3A4 has been verified in vivo and appears to play a major role in human metabolism. Elimination of dasatinib takes place mainly in the first 48 hrs post-dose. Elimination is mostly hepatic/biliary.

Single doses of dasatinib in rats and monkeys resulted in overlapping toxicities in the GI tract, lymphocytic/ hematopoietic system, liver, kidneys. Cardiotoxicity was evident in rats and presented with ventricular necrosis, valvular/ ventricular/ atrial hemorrhage, and cardiac hypertrophy. There was a tendency for increased systolic and diastolic blood pressure in monkeys. Thrombocytopenia was reported in rats; however, hemorrhage and bruising was more evident in monkeys. Ecchymosis was observed in monkeys over numerous sites of the body.

Repeat-dose studies in rats and monkeys resulted in toxicities in multiple tissues, many of which were seen at sub-therapeutic exposures. Findings were seen in the GI tract, lymphocytic/ hematopoietic system, kidneys, heart, liver, adrenals, reproductive organs,

thyroid, pancreas, lung, and bile duct. Electrolyte imbalance was also noted; this may be due to the nephrotoxicity and/or GI toxicity in the animals.

Mechanistic studies to assess the potential effect of dasatinib on platelet function were conducted in order to determine the cause of the cutaneous hemorrhage observed in the toxicology studies. As excerpted from the summary of non-clinical studies, in vitro, dasatinib inhibited platelet aggregation in human, monkey, and rat platelet-rich plasma at concentrations of 0.5 and 5 $\mu\text{g/mL}$.

Dasatinib was clastogenic to CHO cells, in the absence or presence of S9 metabolic activation. Dasatinib was not mutagenic in the bacterial reverse mutation assays (Ames Test) using *S. typhimurium* strains TA98, TA100, TA1535, TA1537, and for *E. coli* strain WP2 *uvrA*. BMS-354825 did not cause chromosomal damage in the rat bone marrow micronucleus test, under the conditions of the study.

The effects of dasatinib on male and female fertility have not been studied. However, results of repeat-dose toxicity studies in multiple species indicate the potential for dasatinib to impair reproductive function and fertility. Effects evident in male animals included reduced size and secretion of seminal vesicles, and immature prostate, seminal vesicle, and testis. The administration of dasatinib resulted in uterine inflammation and mineralization in monkeys, and cystic ovaries and ovarian hypertrophy in rodents. Dasatinib was teratogenic in both rats and rabbits. Embryo-fetal effects, i.e. lethality (rats) and/or abnormalities (rats and rabbits), started to manifest at doses that did not cause maternal toxicities. In addition, in both studies embryo-fetal lethality and/or abnormalities started to manifest at sub-therapeutic exposures. In rats, the lowest dose of 2.5 mg/kg/day (15 mg/m²/day) resulted in embryo-fetal toxicities. This dose had maternal AUC of 105 ng/hr/mL, which is 0.3 fold the human AUC in females at the recommended dose of 70 mg BID. In rabbits the lowest dose of 0.5 mg/kg/day (6 mg/m²/day) caused embryo-fetal toxicities. This dose had a maternal AUC of 44 ng.hr/mL, 0.1 fold the human AUC in females at the recommended dose of 70 mg BID. Embryo-fetal toxicities included: skeletal malformations over several sites (scapula; humerus; femur; radius; ribs; clavicles), reduced ossification over several sites (sternbrae; thoracic, lumbar, and sacral vertebrae; forepaw phalanges; pelvis; and hyoid body), edema, microhepatia.

Unresolved toxicology issues: None

In human, metabolite M20 (4-OH-chloromethylphenyl dasatinib) was detected in significant amount (13%) in the plasma. Rats had no detectable amounts of M20 in the plasma. In monkeys, the plasma level of M20 was 2.8% of radioactivity. Therefore, toxicology studies conducted may not have adequately assessed toxicities of M20 in humans. Additional non-clinical studies with unique human metabolites are not required at this time due to the life threatening conditions of the patients. Additional studies may be required if dasatinib is developed for other indications.

Recommendations: based on the non-clinical studies reviewed, dasatinib is approvable for the proposed indication.

Reviewer Signature _____
Haleh Saber, Ph.D.
Pharmacologist

Supervisor Signature _____ Concurrency Yes X No _____
David E. Morse, Ph.D.
Supervisory Pharmacologist

APPENDIX/ATTACHMENTS

NONE

Reference List

- (1) Donato NJ, Wu JY, Stapley J, Gallick G, Lin H, Arlinghaus R, et al. BCR-ABL independence and LYN kinase overexpression in chronic myelogenous leukemia cells selected for resistance to STI571. *Blood* 2003 Jan 15;101(2):690-8.
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/s/

Haleh Mahloogi
6/27/2006 12:29:54 PM
PHARMACOLOGIST

David Morse
6/27/2006 12:36:40 PM
PHARMACOLOGIST