CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

212489Orig1s000

NON-CLINICAL REVIEW(S)

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration

Division of Neurology 1
Division of Pharmacology/Toxicology
Office of Neuroscience
Center for Drug Evaluation and Research

Date: April 23, 2020

From: Lois M. Freed, Ph.D.
Supervisory Pharmacologist

Subject: NDA 212-489 (Ongentys, opicapone, BIA 9-1067)

NDA 212-489 was submitted by Neurocrine Biosciences, Inc. on April 26, 2019, to support marketing approval of opicapone, a reversible catechol o-methyl transferase (COMT) inhibitor, as adjunctive treatment to levodopa/carbidopa in patients with Parkinson's disease experiencing "off" episodes. Clinical development of opicapone was initiated by Bial-Portela & Ca, S.A. under IND 104380; sponsorship of the IND was transferred to Neurocrine on March 20, 2017.

A full battery of nonclinical studies was submitted to support the NDA. These studies have been reviewed by Dr. McKinney (Review and Evaluation of Pharmacology/Toxicology Studies, NDA 212-489, LuAnn McKinney, D.V.M, April 22, 2020), who references reviews conducted under the IND (Pharmacology/Toxicology Review, IND 104380, Terry S. Peters, D.V.M., March 24, 2009). Dr. McKinney has concluded the nonclinical data support approval of the NDA.

The pharmacology studies conducted by the sponsor characterize opicapone as a COMT inhibitor, with K_i values of 0.15 and 0.016 nM in rat and human, respectively. Opicapone is extensively metabolized in animals and humans; however, in vivo metabolism is qualitatively different among species. In humans, the major circulating metabolite is BIA 9-1103 (sulfate conjugate; inactive), whereas in animals, the major circulating metabolite is BIA 9-1079 (active; rat: IC_{50} for COMT = 429 nM vs 110-224 nM for opicapone).

The pivotal toxicity studies were conducted in CD-1 mouse, Wistar rat, New Zealand White rabbit, and cynomolgus monkey. Standard study designs were employed.

In the subchronic and chronic toxicity studies in rat (up to 6 months) and monkey (up to 52 weeks) and in the carcinogenicity studies in mouse and rat, opicapone was tested at doses up to 1000 mg/kg/day, which was identified as the NOAEL in all three species. No drug-related tumors were observed in mouse or rat. Toxicokinetic data are not available for mouse. In rat,

plasma exposures (AUC) at 1000 mg/kg/day were 67.94-65.22 µg*hr/mL in the 26-week study and 58.40-86.90 µg*hr/mL in the 2-year carcinogenicity study. For comparison, plasma AUC in humans at the recommended human dose (50 mg/day) is 2.43 µg*hr/mL.

Opicapone was negative in a standard battery of genetic toxicology (Ames, in vitro chromosomal aberration in peripheral human lymphocytes, in vivo CD-1 mouse micronucleus) assays.

In the reproductive and developmental toxicology studies in rat, opicapone was tested at oral doses up to 1000 mg/kg/day, using standard study designs. No adverse effects on fertility or on embryofetal (EFD) or pre- and postnatal development (PPND) were observed. In the PPND study, effects on neurobehavioral development were not assessed using a complex learning and memory task, such as the Biel or Cincinnati water maze, and were, therefore, marginally acceptable. Deaths in two offspring of high-dose dams in the PPND study, although unusual, were not likely drug-related. They occurred only during the post-weaning period, and there was no commonality in clinical signs or necropsy findings between the only two affected offspring. Plasma exposure data were not collected in these studies; however, in an EFD dose-ranging study, plasma opicapone AUC on gestation day 17 was 87.6 µg*hr/mL. In a separate study in which a single oral dose of [^{14}C]-opicapone was administered to lactating Wistar rat on postnatal day 11, levels of radioactivity in milk were similar to those in maternal plasma at 4 and 12 hrs postdose (milk: plasma ratios of 0.933 and 1.08).

In the EFD study in rabbit (0, 100, 175, and 225 mg/kg/day), increases in structural (blood vessel and skeletal) abnormalities were observed. The sponsor did not consider these drug-related; however, the incidences of multiple fetal findings were significantly increased at the mid and high doses or at all doses. While fewer findings were observed at the low dose, plasma exposures at the low and mid doses were similar. Therefore, a clear NOAEL could not be identified. Plasma exposures were 0.37, 0.26, and 0.81 µg/mL for C_{max} and 1.02, 0.79, and 4.52 µg*hr/mL for AUC at low, mid, and high doses, respectively.

Adverse development effects of opicapone given in combination with levodopa/carbidopa (LD/CD) were not assessed; however, LD/CD is known to cause visceral and skeletal malformations in rabbits. This information should be included in labeling because opicapone is to be indicated as an adjunct to LD/CD.

Conclusions and Recommendations

The nonclinical studies of opicapone provided by the sponsor are adequate to support approval of the NDA for the proposed indication, with appropriate labeling.

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/s/

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	212489
Supporting document/s:	00001
Applicant's letter date:	04/26/19
CDER stamp date:	04/26/19
Product:	Opicapone
Indication:	Parkinson's disease
Applicant:	Neurocrine Biosciences, Inc.
Review Division:	Neurology Products 1
Reviewer:	LuAnn McKinney, DVM, DACVP
Supervisor:	Lois M. Freed, PhD
(Acting) Division Director:	Eric Bastings, MD
Project Manager:	Stacy M. Metz

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1 Executive Summary

1.1 Recommendations

1.1.1 Approvability

From a nonclinical perspective, this application is approvable.

1.1.2 Additional Nonclinical Recommendations

NA

1.1.3 Labeling

The proposed labeling placed the fertility section under section 8.1, with margins measured in mg/m^2. This should be moved to Section 13 (Carcinogenesis, Mutagenesis and Impairment of Fertility). Safety margins should be expressed in comparisons to the AUC at the MRHD.

Of note: The sponsor proposed using the clinical $AUC_{(0\text{-}t\ last)}$ which was 2.02 ug*hr/mL (N=16), rather than $AUC_{(0\text{-}24)}$ which was 2.43 ug*hr/mL (N=9). The Clinical Pharmacology reviewer (personal communication) found that, in the absence of accumulation, either parameter would be appropriate. Although the two values offer slightly different margins, given the difference in the number of subjects, the $AUC_{(0\text{-}t\ last)}$ is acceptable.

For studies of short duration, such as fertility and embryo development, the calculated exposure margins to the NOAEL in rat (1000 mg/kg/day) should be based on AUC at Study Day 28 (100.6 ug*hr/mL).

1.2 Brief Discussion of Nonclinical Findings

BIA 9-1067 (Opicapone) is a selective, reversible inhibitor of catechol-O-methyltransferase (COMT). In vivo, monkeys administered 100 mg/kg/day PO for 14 days, BIA 9-1067 reduced erythrocyte COMT activity by 70% and resulted in a 2-fold increase in levodopa systemic exposure and a 5-fold reduction in the AUC and C_{max} of the levodopa metabolite 3-O-methyldopa (3-OMD). BIA 9-1067 inhibited metabolism of levodopa to 3-O-methyl-dopa in rats orally administered 1000 mg/kg/day of BIA 9-1067 and levodopa+carbidopa (20+5, 50+12.5, or 120+30 mg/kg/day) for 13 weeks.

At concentrations up to 10 µM BIA 9-1067 had no relevant effects on hERG-mediated currents in HEK cells or on action potential of isolated Purkinje fibers. Respiratory function was studied in M Wistar rats; at 30 mg/kg PO, BIA 9-1067 produced no test

article-related effects over 6 hours of post-dose observation. There were no cardiovascular effects in telemetered Beagle dogs administered ascending doses or repeatedly for 4 weeks, at 60, 200, and 600 mg/kg/day PO. No effects on ECG parameters were reported in cynomolgus monkeys administered BIA 9-1067 at daily oral doses of 100, 300, or 1000 mg/kg for 52 weeks. In a modified Irwin study in male Wistar rats administered 30, 300 or 1000 mg/kg PO, slight excitation was seen at the LD and sedation was seen for 1 hour in MD and up to 2 hours in HD animals.

Oral absorption and systemic distribution were rapid in rats administered [14C]-BIA 9-1067 (10 mg/kg), with an average T_{max} of 1-4 hours. Tissue distribution (based on radioactivity) was maximal at 4 hours post-oral administration. Minimal-to-no drug was detected in the CNS. Metabolism was largely through reduction in nonclinical species and through sulfation in human. The main metabolite found in human (BIA 9-1103) was found in rat, mouse, and monkey; plasma exposure to BIA 9-1103 exceeded clinical concentrations in rats dosed with BIA 9-1067 at the NOAEL (1000 mg/kg/day). BIA 9-1079, the major metabolite in animal species, was found at very low concentrations in humans. CYP2C8 and CYP2C9 were inhibited by BIA 9-1067 and by metabolites BIA 9-1079 and BIA 9-1103.

Excretion was mainly fecal: in all species tested, orange- or yellow-stained feces were reported, which was attributed to the presence of the yellow-colored test article.

In toxicity studies in mice, rats, dogs, monkeys, and minipigs, BIA 9-1067 was administered orally in 0.2% hydroxypropyl methylcellulose (HPMC). The maximum feasible dose was 1000 mg/kg and, in most species, was the NOAEL. Non-adverse findings were largely limited to transient inactivity, sporadic emesis, diarrhea or pasty stools, and transient decreases in body weight gains that resolved after Study Week 1 or 2. In the 6-month study in rat, the MD (500 mg/kg/day) was the NOAEL due to increased bilirubin levels (in the absence of histologic changes) at the HD of 1000 mg/kg/day. Marked inappetence with marked body weight loss was seen in rabbits at doses ≥ 175 mg/kg/day; the NOAEL was 100 mg/kg/day. When BIA 9-1067 was administered in DMSO intravenously to rats, doses ≥ 8 mg/kg were acutely fatal. In a study of BIA 9-1067 (1000 mg/kg/day) in combination with a high dose of levodopa/carbidopa (120/30 mg/kg/day), adverse pancreatic acinar apoptosis in F and mammary hypertrophy in M were seen.

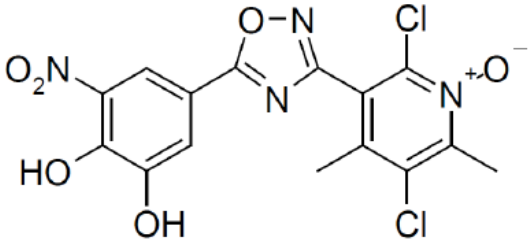
No adverse effects on reproductive function were seen in rats dosed up to 1000 mg/kg/day or in fetuses of rabbits dosed at 225 mg/kg/day. Minor variations in rat and rabbit fetuses were seen in embryofetal development studies which were greater than concurrent controls, but at incidences within the testing laboratory's historical database. BIA 9-1067 (up to 1000 mg/kg/day PO) administration did not affect male fertility. When administered at 1000 mg/kg/day PO to female rats from conception through weaning, 2 F1 M were euthanized at 34 and 62 days of age. In the same study, at the NOAEL of 500 mg/kg/day there were no adverse effects on behavior, learning and memory, fertility or implantation or numbers of live embryos in the F1 generation.

BIA 9-1067 and metabolite BIA 9-1079 were found to be neither mutagenic nor clastogenic a battery of in vitro and in vivo assays. BIA 9-1067 was negative in 2-year

bioassay/carcinogenicity studies in Wistar rat (HD 1000 mg/kg/day) and CD-1 mouse (HD 750 mg/kg/day).

2 Drug Information

2.1 Drug

CAS Registry Number	92387
Generic Name	Opicapone
Code Name	BIA910767
Chemical Name	2,5-dichloro-3-(5-(3,4-dihydroxy-5-nitrophenyl)-1,2,4-oxadiazol-3-yl)-4,6-dimethylpyridine-1-oxide
Molecular Formula/Molecular Weight	??
Structure or Biochemical Description	Sponsor's figure: 
Pharmacologic Class	catechol-O-methyltransferase (COMT) inhibitor

2.2 Relevant INDs, NDAs, and DMFs

IND 104380
DMF (b) (4) (LOA provided from DMF holder)

2.3 Clinical Formulation

2.3.1 Drug Formulation

Sponsor's table: Composition of 25 and 50 mg capsules

Table 1: Composition of Opicapone, 25 mg Capsules

Component	Quality Standard	Function	Weight (mg/unit)	% (w/w)
Opicapone	In-house	Drug Substance	25.0	(b) (4)
Lactose monohydrate	USP/NF		(b) (4)	
Starch, pregelatinized	NF			
(b) (4)				
Sodium starch glycolate, (b) (4)	NF			
Magnesium stearate[c]	NF			
				100
Hard gelatin capsules, Size 1; light blue opaque cap/light pink opaque body; axially printed with 'OPC' over '25' in blue ink, on both the cap and body	Pharmaceutical[d]			1 capsule
(b) (4)				

[d] Specification provided in 3.2.P.4.1.

2.3.2 Comments on Novel Excipients

None

2.3.3 Comments on Impurities/Degradants of Concern

None

2.4 Proposed Clinical Population and Dosing Regimen

Adjunctive therapy (to levodopa/carbidopa) in Parkinson's disease patients suffering "off" episodes. The recommended dose is 50 mg PO once daily.

2.5 Regulatory Background

IND 104380 was submitted on MAR 10, 2011, by Bial-Portela & Ca, S.A. c/o PharmaNet. A May Proceed letter was signed on JUN 27, 2011. On March 21, 2019, the agency was notified of the transfer of ownership of IND 104380 to Neurocrine Biosciences, Inc.

3 Studies Submitted

3.1 Studies Reviewed

Primary and safety pharmacology, PK/ADME, single and repeat dose toxicology (up to chronic) and combination (with LD and CD) toxicology, developmental and reproduction, genetic toxicology, and carcinogenicity studies were reviewed.

3.3 Previous Reviews Referenced

Special Protocol Assessment for 2-year carcinogenicity studies in rat and mouse, to include reviews of 90-day toxicity studies in CD-1 mouse and Wistar rat (Nonclinical review by Terry Peters, DVM, March 24, 2009).

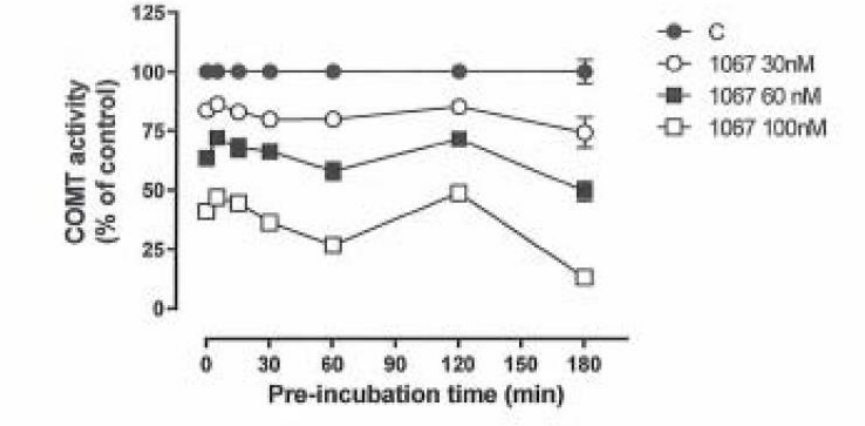
4 Pharmacology

4.1 Primary Pharmacology

BIA 9-1067 is an inhibitor of catechol-O-methyltransferase (COMT). Similar to deopa-decarboxylase enzymes, COMT is inactivates catechol compounds such as adrenaline, epinephrine or levodopa. When Parkinson's disease patients are administered the standard-of-care levodopa in combination with inhibitors of dopa-decarboxylase (DDIs), COMT becomes the major catechol-metabolizing enzyme and catalyzes levodopa to 3-O-methyldopa (3-OMD), resulting in sub-therapeutic levels of levodopa. In vitro, BIA 9-1067 inhibited recombinant human solubilized COMT (S-COMT) inactivation of the catechol adrenaline with high affinity and low dissociation constants. In vivo, BIA 9-1067 reduced COMT activity in erythrocytes, liver, and kidney in monkey, mouse and rat. Administered in combination with levodopa (LD) and the decarboxylase inhibitor such as carbidopa (CD) or benserazide, BIA 9-1067 increased levodopa levels and decreased 3-OMD levels in rat and monkey. When BIA 9-1067 was administered with CD/LD in animal models of Parkinson's disease, Parkinsonian motor symptoms were reduced, compared to vehicle or CD/LD alone.

BIA 9-1067 inhibition in vitro is selective and reversible, with a Ki of 0.016 nM in human and 0.154 nM in rat. BIA 9-1067 binds human COMT at a Kd of 0.19 pM. In a pre-incubation study, BIA 9-1067 inhibited human S-COMT activity in a dose and time-related manner.

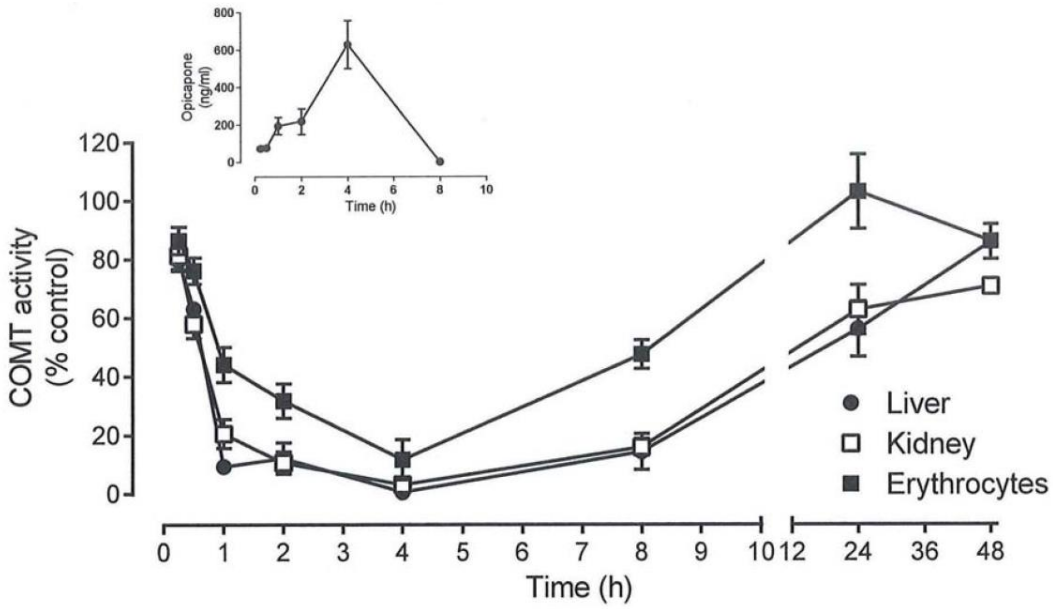
Sponsor's figure:



1067 = opicapone; c = control; COMT = catechol-O-methyltransferase; S-COMT = soluble catechol-O-methyltransferase.

When 3 mg/kg BIA 9-1067 was administered PO to mice and rats, COMT activity was inhibited in liver of mice and kidney, liver, and erythrocytes of rats in a dose-related manner for up to 96 hours.

Sponsor's figure:



COMT = catechol-O-methyltransferase; SEM = standard error of the mean
Time dependence of COMT inhibition following administration of 3 mg/kg opicapone (polymorph A, N=4-10/timepoint). Mean ± SEM. Inset is opicapone exposure in plasma. Study SRMJB110913.

In monkey, BIA 9-1067 (100 mg/kg/day PO for 14 days) reduced erythrocyte COMT activity by 77% compared to control. In a second study, BIA 9-1067 was administered to Cynomolgus monkeys with cannulas implanted into the substantia nigra, dorsal striatum, and prefrontal cortex (100 mg/kg for 14 days, with a cross-over with vehicle for an additional 14 days). The administration resulted in a 2-fold increase of levodopa systemic exposure and a 5-fold reduction in the AUC and C_{max} of 3-OMD.

Sponsor's table:

Plasma Levels AUC (µg•min/mL)		
Analyte	Opicapone (mg/kg/day)	
	0	100
Levodopa	324 ± 58.0	638 ± 45.1*
3-OMD	548 ± 130	112 ± 38.4*

*P < 0.05

Although not statistically significant, decreased 3-OMD levels were found, notably in the substantia nigra and the dorsal striatum, and at lesser levels in the prefrontal cortex.

Sponsor's table:

Analyte	Opicapone (mg/kg/day)	
	0	100
Dorsal Striatum AUC Levels (µg•min/mL)		
Levodopa	2.03 ± 0.37	3.35 ± 0.81
3-OMD	8.21 ± 3.29	1.68 ± 1.00
DOPAC	18.7 ± 12.0	16.8 ± 6.49
HVA	381 ± 54.4	305 ± 33.6
***Substantia Nigra* AUC Levels (µg•min/mL)**		
Levodopa	1.24 ± 0.51	1.72 ± 0.41
3-OMD	4.17 ± 1.91	0.59 ± 0.38
DOPAC	0.97 ± 0.59	4.24 ± 1.00
HVA	103 ± 23.8	145 ± 15.6
MOPEG	0.69 ± 0.48	0.74 ± 0.43
Prefrontal Cortex AUC Levels (µg•min/mL)		
Levodopa	1.34 ± 0.29	3.07 ± 1.17
3-OMD	4.72 ± 1.64	1.96 ± 1.31
HVA	27.4 ± 1.29	25.4 ± 4.03

In a 13-week toxicity study in rat, when BIA 9-1067 was administered PO in combination with levodopa/carbidopa, 3-OMD was not measurable.

In MPTP-lesioned cynomolgus monkeys with stable Parkinsonian-like symptoms, BIA 9-1067 was found to augment the effects of L-DOPA/benserazide (a DDI) on motor activity. BIA 9-1067 PO alone had no effect on Parkinsonian-like behavior.

4.2 Secondary Pharmacology

In a series of comprehensive radioligand binding and enzyme assays, BIA 9-1067 (concentration 10 µM) interaction was limited to phosphatase PP2A, kinase ZAP70, and TSPO by 76.7%, 49.0%, and 82.2%, respectively; there was no binding to a panel of 5-HT receptors.

In vitro effects of the major human metabolite BIA 9-1103 were inhibition of radioligand binding in the TSPO assay by 88.2% and reduced phosphodiesterase 5A1 activity by 54.9%, with an IC_{50} of 31.0 µg/mL,

4.3 Safety Pharmacology

Safety Pharmacology studies were performed in vitro (hERG currents and Purkinje fibers) and in vivo (in rat, dog, and monkey). BIA 9-1067 had no effect on hERG mediated currents at concentrations up to 10 µM. No significant effects of BIA 9-1067 on action potential parameters in dog Purkinje fibers were noted up to 10 µg/mL.

Sponsor's tables: In vitro safety pharmacology of BIA 9-1067

hERG channel inhibition	HEK 293 cells	In vitro	Opicapone: 0 (DMSO), 3, 10, 30, 100 µM E-4031: 100 nM	3 cells treated/ condition	3 µM (1.24 µg/mL): None. 10 µM (4.13 µg/mL): None. 30 µM (12.4 µg/mL): Reduced hERG tail current of 13% relative to control (87.27 ± 1.40%, p<0.01 vs vehicle) observed. 100 µM (41.3 µg/mL): Reduced hERG tail current of 26% relative to control (74.2 ± 2.14%, p<0.01 vs vehicle) observed. The IC_{50} value for opicapone was 389 µM (161 µg/mL). E-4031: Reduced hERG tail current of 93% relative to control (7.48 ± 1.42%) observed.	Yes

Sponsor's table: in vitro studies of the major human metabolite, BAI 9-1103

Organ Systems Evaluated	Species / Strain	Method of Admin.	Test Article Concentration	Gender and No. per Group	Noteworthy Findings	GLP	Study No.
Action potential	Purkinje fibers isolated from Beagle dogs	In vitro	BIA 9-1103: 0 (vehicle), 1, 3, 10 µg/mL[a] (expressed as base)	M 4	1 µg/mL: None. 3 µg/mL: None. 10 µg/mL: At 30 mins lengthened APD_{60} (+6%, p<0.01) and APD_{90} (+5%, p<0.01) observed.	Yes	11-0533
hERG channel inhibition	HEK 293 cells	In vitro	BIA 9-1103: 0 (vehicle), 1, 3, 10, 30 µg/mL[a] E-4031: 100 nM	3 cells/ treated condition	1 µg/mL: None. 3 µg/mL: None. 10 µg/mL: None. 30 µg/mL: 14% inhibition of hERG tail current (NS). E-4031: Marked inhibition of hERG tail current of 94% (5.85 ± 0.89% vs control, N=6 cells, p<0.01).	Yes	A0539

APD_{60} = action potential duration at 60% repolarization; APD_{90} = action potential duration at 90% repolarization; E-4031 = reference selective I_{kr} blocker; GLP = Good Laboratory Practice; HEK 293 = human embryonic kidney cells; hERG = human ether-à-go-go-related gene; I_{kr} = rapid delayed rectifier current; M = male; NS = not statistically significant.
[a] BIA 9-1103 batch no. PC110527 was used in both studies; this batch contained opicapone as a contaminant at 9%.

In a GLP single ascending dose cardiovascular study and a 28-day toxicity study in Beagle dogs, both at oral doses of 60, 200 and 600 mg/kg/day PO, BIA 9-1067 had no effect on arterial blood pressure or heart rate. In electrocardiogram (ECG) assessments, no effects on PR interval, QT interval, or corrected QT interval were reported. Likewise, no effects on ECG parameters were reported in cynomolgus monkeys administered BIA 9-1067 at daily oral doses of 100, 300, or 1000 mg/kg for 52 weeks.

Respiratory function was studied in Wistar rats. At 30 mg/kg PO, BIA 9-1067 produced no test article-related effects over 6 hours of post-dose observation. At 300 and 1000 mg/kg, there was slight and transient depressant effects on respiratory parameters immediately post-dose and a decrease in the respiration rate in 1/4 and 3/4 animals throughout the 6-hour observation period.

In a modified Irwin screen in male Wistar Han rats, at 30 mg/kg, incidental and nonspecific effects included slight excitatory effects (excitation and increased reactivity to touch in 4 of 4 and 1 of 4 animals respectively) observed 60 minutes post-dose. A transient increase in sedation was seen in 1 of 4 animals 60 minutes after a 300 mg/kg dose and in 4/4 animals 120 min after a 1000 mg/kg dose.

There were no test article-related effects on GI transit time or renal function in rats after single oral doses of up to 1000 mg/kg.

Metabolites

BIA 9-1103 (1-30 µg/mL), the major human metabolite, did not show any significant effects on hERG channel inhibition. No effects on action potential parameters in dog Purkinje fibers were seen at 1 and 3 µg/mL.

BIA 9-1079, the major metabolite in nonclinical species, had no effect on hERG mediated currents at concentrations up to 10 µM.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Pharmacokinetics

The half-life of BIA 9-1067 was generally less than 4 hours in mice and rats. In monkeys, the half-life ranged from 0.66 to 2.5 hours at 100 mg/kg/day and at dose levels of 1000 mg/kg/day increased to 2.5 to 5 hours. After repeat-dose administration of doses of 500 and 1000 mg/kg/day, there was no notable accumulation of BIA 9-1067.

Sponsor's table: PK in species used in toxicology studies

Species and Route	Dose (mg/kg/day)	Duration of Dosing	Pharmacokinetic Parameter				Study No.
			C_{max} (µg/mL)		AUC_{0-t} (µg•h/mL)		
			Male	Female	Male	Female	
Human (oral)	50 mg/day	14 days	0.506[a]		10.9[a] [b]		NBI-OPC-1706
Mouse (oral gavage)	1800	Day 2	0.18[c]	1.14[c]	NC	NC	792471
Mouse (oral gavage)[d]	1000	Day 1	0.26	NA	2.81	NA	SRAL110222
Rat (oral gavage)	500	Week 13	8.18	1.95	72.10	16.70	D38007
	1000	**Week 13**	**11.50**	**3.11**	**182.00**	**35.30**	
Monkey (oral gavage)	1000	Day 7	1.08[a]		5.80[a]		SRAL070509

AUC_{0-24}=area under the concentration-time curve from time zero to 24 hours; AUC_{0-t}=area under the concentration-time curve from time zero to the time of the last measurable concentration; C_{max}=maximal plasma concentration; NA=not applicable; NC=not calculated.

[a] Mean value of males and females combined.

[b] AUC_{0-24}

[c] Plasma concentrations 1 h after the last dose as determined in TK study to support micronucleus test.

[d] Single oral dose PK study in male mice only.

Notes: Data presented are mean values for male and female animals on the last day when TK sampling occurred. Bolded text indicates NOAEL doses.

ADME:

Distribution:

In a quantitative whole-body autoradiography study, after oral administration of [14C]-BIA 9-1067 (10 mg/kg) to Wistar male rats, distribution was relatively rapid, and measurable levels of radioactivity were present in the majority of tissues at 1 h post-dose. Maximum levels were in the majority of tissues at 4 h post-dose; the highest tissue levels were found in the liver, kidney cortex, and renal medulla (7163, 4243, and 3806 ng equiv/g, respectively). Concentrations in brain were found to be below the limit of quantification at all time points. When administered PO to pregnant rats at 10 mg/kg on gestation day 17, [$_{14}$C]-opicapone-related radioactivity distributed to fetal tissues within one hour and persisted in fetal tissues through 48 hours post-dose.

Metabolism:
Metabolites of BIA 9-1067 (opicapone) detected in vitro were found to be formed in vivo through 5 major metabolic pathways after oral administration:
- Sulfation to form BIA 9-1103 (opicapone 3-O-sulfate), N-oxide reduction to form BIA 9-1079 (pyridine N-oxide reduced opicapone), glucuronidation to form BIA 9-1106 (opicapone 3-O-glucuronide) and BIA 9-1107 (glucuronide of BIA 9-1079), methylation to form BIA 9-1100 (3-O-methyl opicapone), and BIA 9-1104 (4-O-methyl opicapone).

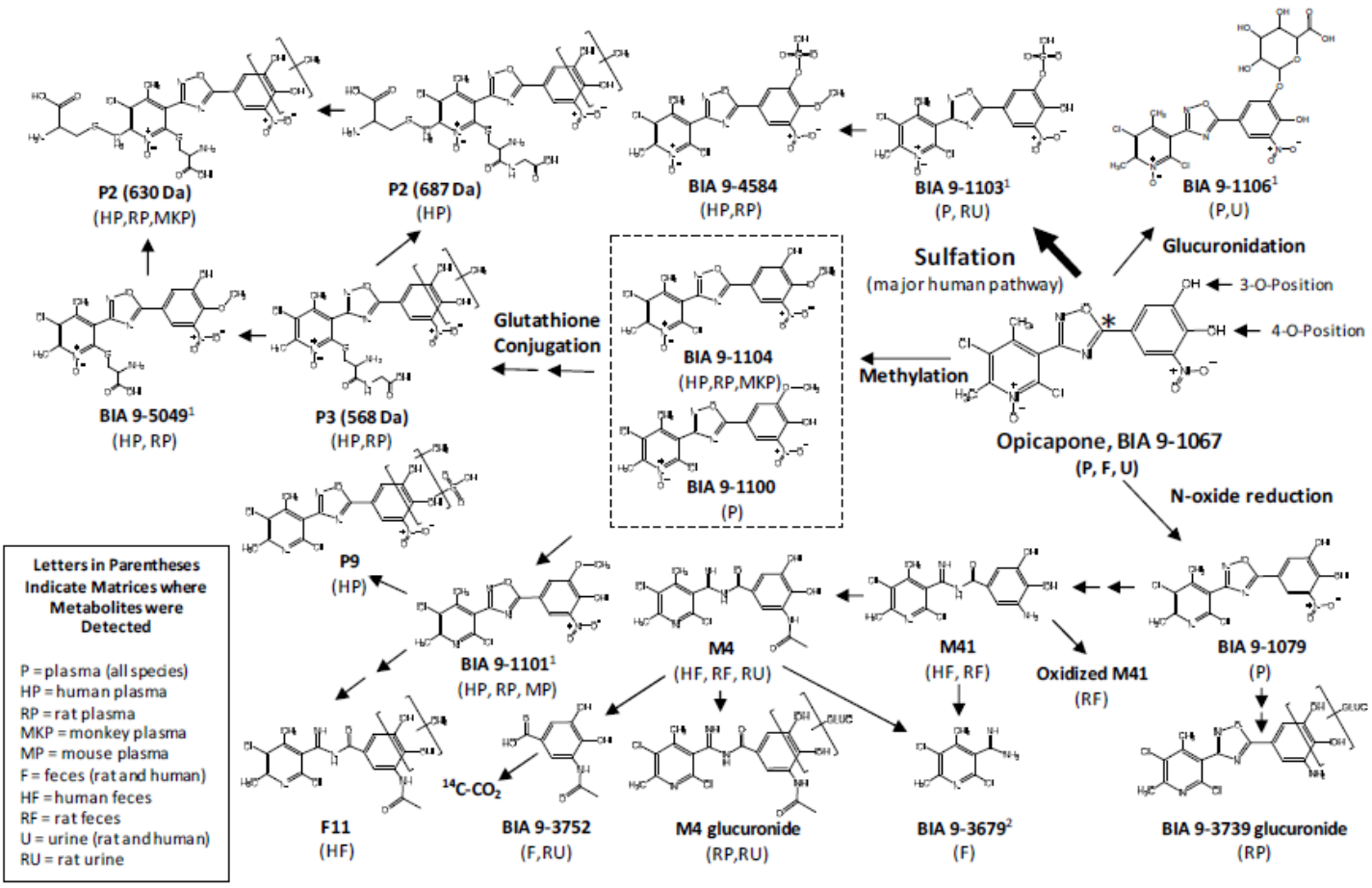
Sponsor's table: Comparative metabolism among species,

	Metabolic transformation	Human	Monkey	Dog	Rat (Wistar)	Guinea Pig	Mouse (NMRI)	Hamster (syrian)
BIA 9-1079	Reduction	NE	+	+	+	+	+	+
BIA 9-1100	Methylation	-	-	+	+	-	+	-
BIA 9-1104	Methylation	-	-	-	-	NE	-	NE
BIA 9-1101	Methylation	-	+	+	+	+	+	-
BIA 9-1102	Methylation	-	-	NE	-	NE	-	NE
BIA 9-1103	Sulfation	+	+	-	+	-	+	-
BIA 9-1105	Sulfation	+	-	-	+	+	+	+
BIA 9-1106	Glucuronidation	+	+	-	+	-	+	+
BIA 9-1107	Glucuronidation	+	+	-	+	-	+	-

NE = not evaluated

Sponsor's figure: Metabolic pathway of BIA 9-1067 in rat:

Proposed Metabolic Pathways for Opicapone



In *in vitro* studies in human hepatic microsomes, BIA 9-1067 inhibited CYP2C8 (K_i =0.9 ug/mL) and CYP2C9 (K_i =18 ug/mL). The major nonclinical metabolite (BIA 9-1079) caused marked inhibition of CYP2C8 (K_i = 0.19 ug/mL and IC_{50} = 0.78 ug/mL) and moderate inhibition of CYP2C9. The major human metabolite (BIA 9-1103) inhibited CYP2C8 (IC_{50} =6.7 ug/mL) and CYP2C9 (IC_{50} = 20.7 µg/mL).

BIA 9-1067 inhibited OAT1, OAT3, OATP1B1 and OATP1B3-mediated substrate accumulation (IC_{50} = 9.18, 4.36, 0.45, and 1.40 µg/mL, respectively) but did not inhibit OCT1 and OCT2-mediated substrate accumulation or BCRP.

Metabolites:

BIA 9-1103 is found in mice, rats, and monkey. In rat, after daily administration of 1000 mg/kg/day of BIA 9-1067 for 13 weeks, BIA 9-1103 exposures [C_{max} 11500 ng/mL (M) and 3110 ng/mL (F)] exceeded the clinical exposures (AUC 506 ng/mL) at the MRHD of 50 mg/day. BIA 9-1079 was the most abundant circulating metabolite in vivo in monkey, mice, and rats. Low levels of BIA 9-1079, (1% of total drug-related radioactivity) were found in human. BIA 9-1106, the most abundant metabolite in mice and also found in rat and monkey, was found at low levels in humans (<2.7% of total drug-related radioactivity). Metabolites BIA 9-1104 and BIA 9-1100 were found at 6.59% and 2.96% of

total circulating drug-related radioactivity, respectively, in human and were found in vivo in rat and monkey (see table below).

Sponsor's table:

Table 48: Mean (Standard Deviation) Opicapone and Metabolite Pharmacokinetic Parameters Following Once-Daily Administration of 50 mg Opicapone to Healthy Caucasian Subjects for 10 Days (Study BIA-91067-126)

Parameter	Opicapone	BIA 9-1079	BIA 9-1100	BIA 9-1101	BIA 9-1104	BIA 9-1106	BIA 9-1103
C_{max} (µg/mL)	1.55 ± 0.65	BLQ	BLQ	BLQ	BLQ	0.07 ± 0.04	0.607 ± 0.26
t_{max} (h)[a]	1.5 (0.5 – 3.0)	NE	NE	NE	NE	1.5 (0.75-4.0)	5.0 (4.0-10.0)
AUC_{0-t} (µg·h/mL)	4.00 ± 1.77	NE	NE	NE	NE	0.17 ± 0.07	NR
$t_{½}$ (h)	1.4 ± 0.7	NE	NE	NE	NE	1.56 ± 0.9	105 ± 10.8

AUC_{0-t}=area under the concentration-time curve from time zero to the time of the last measurable concentration; BLQ=below the limit of quantification; C_{max}=maximal plasma concentration; NE=not estimable; NR=not reported. $t_{½}$=terminal half-life.

[a] Median (range).

Sponsor's table:

Comparison of Representative Plasma Opicapone and Metabolite Exposures across Studies

Species	Dose Level (mg/kg)	Regimen[a]	Formulation	Plasma AUC_{0-t} (µg×h/mL)					
				OPC	BIA 9-1079	BIA 9-1100	BIA 9-1101	BIA 9-1103	BIA 9-1106
Mouse[b]	1000	Single dose	0.0082% HPMC	60.0	5.75	BLQ	BLQ	2.81	36.2
Rat	3	Single dose[c]	0.5% CMC	2.42	1.97	5.15	5.25	1.45	0.488
Rat	10	Single dose	0.2% HPMC	9.12	0.758	4.68	0.307	1.11	2.57
Rat[d]	1000	Single dose	0.0082% HPMC	45.0	4.09	7.92	Trace	32.6	27.6
Rat (male)	1000	Multiple dose	0.2% HPMC	121	11.8	ND	ND	182	45.6
Rat (female)	1000	Multiple dose	0.2% HPMC	122	12.9	ND	ND	35.3	63.4
Monkey[e]	1000	Multiple dose	0.2% HPMC	8.33	15.1	BLQ	0.772	5.80	4.54

AUC_{0-t}=area under the concentration-time curve from time zero to the time of the last measurable concentration; BLQ=below the limit of assay quantification; CMC=carboxymethylcellulose; HPMC=hydroxypropyl methylcellulose; ND=not determined; OPC=opicapone.

[a] Sampling for multiple-dose regimens was after the last dose.

[b] BIA 9-1105 was evaluated but not detected.

[c] (b) (4)

[d] Trace levels of BIA 9-1105 were observed.

[e] Trace levels of BIA 9-1105 were observed; BIA 9-1102 and BIA 9-1104 were evaluated but not detected.

Excretion:

In rats, the highest drug-related radioactivity after a single oral dose of [^{14}C]-BIA 9-1067 were in the eliminating organs (kidney and liver) and in the contents of the GI tract.

Fecal excretion was the primary route of elimination of total radioactivity following both oral and IV administration of [$_{14}$C]-OPC; urinary excretion was minor. The most abundant metabolite in human urine was a glucuronide conjugate BIA 9-1106 (1.72% of the administered radioactivity).

Sponsor's table: Excretion in rat

Table 42: Mean Cumulative Excretion of Total Radioactivity Following Single Oral and Intravenous Administration of [^{14}C]-Opicapone in Rats at Target Doses of 10 and 1 mg/kg, Respectively [Study No. ZNA15658.001]

Route	Urine (%)		Feces (%)		Total (%)	
	0-24 h	0-120 h[a]	0-24 h	0-120 h	0-24 h	0-120 h[a]
Oral	3.5	5.5	86.3	92.3	91.3	100.7
IV	4.5	8.4	50.1	72.8	56.4	89.0

IV=intravenous.

[a] Includes cage wash.

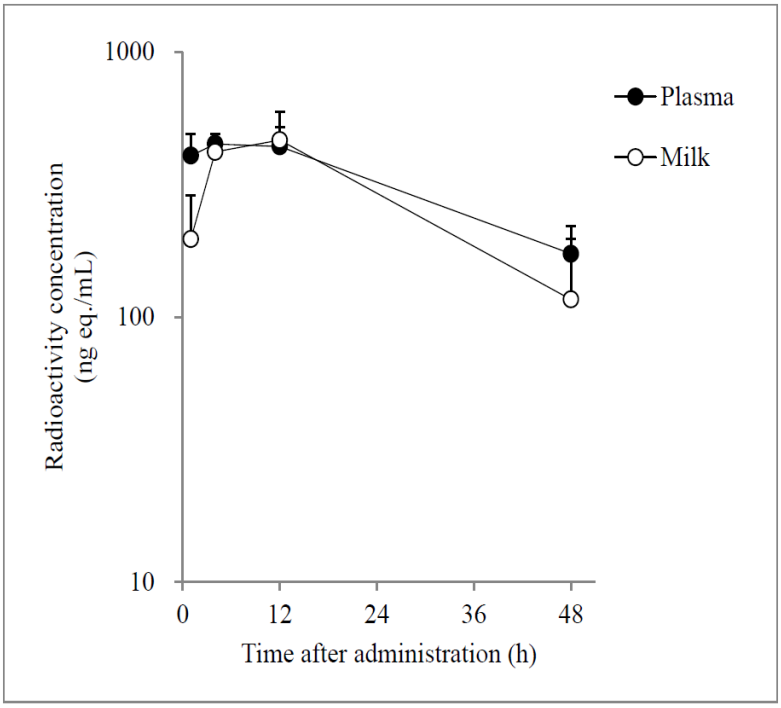
Excretion in milk:

Three Crlj:WI lactating rats, each with 5 pups, were administered a single oral gavage dose of [$_{14}$C]-BIA 9-1067 (10 mg/5MBq/kg) at 11 days postpartum. 0.5 U/mL oxytocin in physiological saline was intraperitoneally administered to dams at about 30 min before milk collection at 1, 4, 12 and 48 h post dose.

The maximum radioactivity concentration in milk (465 ng eq./mL) was at 12 h post-dose and was 1.08-fold that in plasma. The rate of elimination of radioactivity in milk was similar to that in plasma.

Sponsor's figure: Radioactivity in milk and plasma

Concentration-time profiles of radioactivity in milk and plasma after single oral administration of 10 mg/kg ^{14}C-opicapone to lactating rats



5.2 Toxicokinetics

Toxicokinetics are discussed in the toxicity studies. The following table summarizes exposures (AUC) among nonclinical species.

Sponsor's table: Summary BIA 9-1067 exposures at NOAEL among nonclinical species

Species	Study Duration	NOAEL Dose (mg/kg/day)	Effect at LOAEL	AUC_{0-t} (µg×h/mL)
General Toxicity Studies				
Mouse (male)	90 days	1000	NA	28.53[b]
Mouse (female)	90 days	1000	NA	56.86[b]
Rat (male)	28 days	1000	NA	104.39
Rat (female)	28 days	1000	NA	100.62
Rat (male)	13 weeks	1000	NA	121
Rat (female)	13 weeks	1000	NA	122
Rat (male)	26 weeks	500	1000 – stomach erosions; increased bilirubin	59.18
Rat (female)	26 weeks	500	1000 – increased bilirubin	41.61
Monkey (male)	28 days	1000	NA	22.49
Monkey (female)	28 days	1000	NA	18.01
Monkey (male)	52 weeks	1000	NA	20.90
Monkey (female)	52 weeks	1000	NA	16.20
Carcinogenicity Studies				
Mouse (male)[b]	104 weeks	1000/750	NA	9.13[c]
Mouse (female)[b]	104 weeks	1000/750	NA	20.40[c]
Rat (male)	104 weeks	1000	NA	58.4[c]
Rat (female)	104 weeks	1000	NA	86.9[c]

AUC=area under the plasma concentration-time curve; AUC_{0-6h}=area under the plasma concentration-time curve from time 0 to 6 hours postdose; AUC_{0-t}=area under the plasma concentration-time curve from time zero to the time of the last measurable concentration; LOAEL=low observed adverse effect level NA=not applicable; NOAEL=no observed adverse effect level. b Mean AUC_{0-6h} c AUC at 26 weeks.

6 General Toxicology

6.1 Single-Dose Toxicity

Acute toxicity studies were performed to determine MTDs in mice, rats, dogs, monkeys, and minipigs. Animal numbers were limited, and no control groups were included in the studies.

When BIA 9-1067 was administered PO to CD-1 mice (250 mg/kg to 3 F, 1000 mg/kg to 3 F and 2000 mg/kg to 3/sex) the MTD was greater than 2000 mg/kg. Clinical signs were limited to occasional hunched posture and piloerection.

When BIA 9-1067 was administered PO to Crl:CD(SD) rats (250 mg/kg to 3 F, 1000 mg/kg to 3 F and 2000 mg/kg to 3/sex), the no effect level was at the HD.

BIA 9-1067 was administered IV to Crl:CD(SD) rats at 1, 5, or 10 mg/kg in DMSO. DMSO caused red stained urine at all doses. The formulation was adverse at the MD and caused acute lethality at the HD.

Sponsor's table: IV doses in DMSO

Dose (mg/kg)	Noteworthy Findings	
	Mortality	Clinical Signs of Toxicity
1	0/2 F	Subdued behavior for up to 4 h after dosing, hunched appearance and red staining in urine and around vaginal area in both animals 15 min after dosing.
5	0/3 M; 0/3 F	Labored breathing, staggering, subdued behavior and increased breathing rate in all males immediately after dosing with red staining on bedding observed 1.25 h after dosing. Red staining in urine and around vaginal area in females 10 min after dosing.
10	1/1 F	The animal died immediately after dosing and clinical signs observed prior to death included convulsions, prostration and labored breathing.

F=female; M=male

In a second single-dose IV study (2 or 8 mg/kg), 8 mg/kg was acutely fatal, but no effects were attributed to BIA 9-1067 at 2 mg/kg throughout the 14-day observation time.

Sponsor's table:

Males

Dosage (mg/kg)	Sign	Time after Dosing on Day 1						Days 2-15
		At dosing	20 min	¾ h	2 h	3 h	3¾ h	
2	NAD	10-12			10-12	10-12	10-12	10-12
	Red staining in urine		10-12	10-12				

Females

Dosage (mg/kg)	Sign	Time after Dosing on Day 1						Days 2-15
		At dosing	5 min	10 min	½ h	¾ h	2-3¾ h	
2	NAD	1-3					1-3	1-3
	Red staining in urine				1-3			
8	Found dead		4					
	NAD						5,6	5,6
	Subdued behaviour	4-6	6	5,6	5,6			
	Laboured breathing		5,6	5,6				
	Red discharge (nose)				6			
	Red staining in cage				5,6	5,6		

NAD = No abnormalities detected

When BIA 9-1067 was administered PO to 1 male and 4 female Beagle dogs at ascending doses of 100, 200, 400, 800 and 1000 mg/kg, 24 hours apart, the NOEL was at 600 mg/kg; the MTD was determined to exceed 1000 mg/kg.

When BIA 9-1067 was administered by oral gavage to monkeys (1/sex) at ascending doses of 100, 200, 500, 600, and 1000 mg/kg, the MTD exceeded 1000 mg/kg.

BIA 9-1067, administered by oral gavage at ascending doses of100, 200, 400, 800, and 1000 mg/kg PO to 1 M and 4 F Gottingen minipigs, was well tolerated at all dose levels.

6.2 Repeat-Dose Toxicity

6.2.1

Study title: 28-Day oral toxicity (Gavage) study in the CD-1 mouse

Study no.:	B18404
Study report location:	eDR 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	FEB 21, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BIA 9-1067/31879-1-2/98.0%

Key Study Findings:

Neither macroscopic nor microscopic findings were found to differ among groups.

NOAEL: HD (1000 mg/kg/day)
Cmax: M:12313; F: 26867 ng/mL
$AUC_{(0-t)}$: M: 35066; F: 62764 ng*h/mL
T_{max}: M and F - 0.5hr

Methods

Doses:	0(vehicle), 100, 500, or 1000 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	20 mL/kg
Formulation/Vehicle:	0.2% hydroxypropyl methylcellulose
Species/Strain:	Mouse, Crl: CD-1 (ICR) BR
Number/Sex/Group:	10/sex
Age:	6 weeks
Weight:	M: 23.8-31.9 g; F: 21.3-26.8 g
Satellite groups:	TK: 3/sex C; 9/sex dosed
Unique study design:	None
Deviation from study protocol:	None were reported

Observations and Results

Mortality:

Study Days 1, 15, 30: 3 HDM; Study day 1: 1 MDF and 1 HDF - largely attributed to gavage errors.
Study Day 21: 1 TK HDM and 1 TK HDF - attributed to bleeding procedures.

Clinical Signs:
At the MD and HD, hunched posture and shivering with ruffled fur were observed during first week of dosing. This was possibly related to the high volume of test article.

Body Weights:
Mean body weight gain was reduced in HDM and MDM during Study Week 2. No differences in absolute body weights among groups were reported.

Food Consumption:
No differences among groups were reported.

Hematology, coagulation, clinical chemistry, urinalysis:
No differences among groups were reported.

Gross Pathology:
No test article-related effects were reported.

Organ Weights:
No differences among groups were reported.

Histopathology:
Adequate Battery: yes
Pathology report: Pathologist's GLP compliance statement signed and dated
Peer Review: No
Histological Findings:

No test article-related findings were reported.
Esophageal inflammation and hemorrhage confirmed gavage error-associated morbidity in HDM; pleural inflammation in LD and HDM was also possibly related to gavage error.

Toxicokinetics:

Exposure to BIA 9-1067 was higher than to the metabolite BIA 9-1079, and AUC_{0-t} and C_{max} increased dose-proportionally in M but less than dose-proportionally in F; the half-life was similar in M and F.

Metabolite BIA 9-1079 increased with dose in M and F in a less than dose-proportional manner.

Sponsor's table: TK of BIA 9-1067 on Study days 1 and 28

Day	Group	Dose [mg/kg]	Ratio*	AUC_{0-t} [ng•h/mL]	Ratio*	AUC_{0-t} / Dose	Ratio*	C_{max} [ng/mL]	Ratio*	C_{max}/ Dose	Ratio*	$t_{½, z}$ [h]	Ratio*
males													
Day 1	2	100	-	2117	-	21.2	-	1032	-	10.3	-	[2.4]	-
	3	500	5.0	7644	3.6	15.3	0.7	5220	5.1	10.4	1.0	0.63	-
	4	1000	2.0	21500	2.8	21.5	1.4	9743	1.9	9.74	0.9	1.7	2.7
Group4/Group 2			10		10		1.0		9.4		0.9		-
Day 28	2	100	-	3989	-	39.9	-	2170	-	21.7	-	5.6	-
	3	500	5.0	10863	2.7	21.7	0.5	4757	2.2	9.51	0.4	[4.2]	-
	4	1000	2.0	35066	3.2	35.1	1.6	12313	2.6	12.3	1.3	3.0	-
Group4/Group 2			10		8.8		0.9		5.7		0.6		0.5
females													
Day 1	2	100	-	6741	-	67.4	-	3113	-	31.1	-	2.4	-
	3	500	5.0	16289	2.4	32.6	0.5	12767	4.1	25.5	0.8	0.85	0.4
	4	1000	2.0	42723	2.6	42.7	1.3	13367	1.0	13.4	0.5	1.7	2.0
Group4/Group 2			10		6.3		0.6		4.3		0.4		0.7
Day 28	2	100	-	8205	-	82.1	-	6197	-	62.0	-	[3.3]	-
	3	500	5.0	30301	3.7	60.6	0.7	10850	1.8	21.7	0.4	[3.3]	-
	4	1000	2.0	62764	2.1	62.8	1.0	26867	2.5	26.9	1.2	2.2	-
Group4/Group 2			10		7.6		0.8		4.3		0.4		-

* Ratio group 3/2 and group 4/3
[…] unreliable, poor loglinear correlation

Sponsor's table: TK of metabolite BIA 9-1079 on Study Days 1 and 28

Day	Group	Dose [mg/kg]	Ratio*	AUC_{0-t} [ng•h/mL]	Ratio*	AUC_{0-t} / Dose	Ratio*	C_{max} [ng/mL]	Ratio*	C_{max}/ Dose	Ratio*	$t_{½, z}$ [h]	Ratio*
males													
Day 1	2	100	-	2341	-	23.4	-	502	-	5.02	-	2.3	-
	3	500	5.0	2672	1.1	5.34	0.2	943	1.9	1.89	0.4	[3.7]	-
	4	1000	2.0	6371	2.4	6.37	1.2	1050	1.1	1.05	0.6	4.3	-
Group4/Group 2			10		2.7		0.3		2.1		0.2		1.9
Day 28	2	100	-	3867	-	38.7	-	503	-	5.03	-	[3.7]	-
	3	500	5.0	6432	1.7	12.9	0.3	878	1.7	1.76	0.3	8.5	-
	4	1000	2.0	13412	2.1	13.4	1.0	1400	1.6	1.40	0.8	[12]	-
Group4/Group 2			10		3.5		0.3		2.8		0.3		-
females													
Day 1	2	100	-	3098	-	31.0	-	469	-	4.69	-	2.6	-
	3	500	5.0	3043	1.0	6.09	0.2	1045	2.2	2.09	0.4	[4.5]	-
	4	1000	2.0	6481	2.1	6.48	1.1	946	0.9	0.946	0.5	2.9	-
Group4/Group 2			10		2.1		0.2		2.0		0.2		1.1
Day 28	2	100	-	3528	-	35.3	-	478	-	4.78	-	[6.3]	-
	3	500	5.0	7329	2.1	14.7	0.4	922	1.9	1.84	0.4	[16]	-
	4	1000	2.0	8956	1.2	8.96	0.6	1690	1.8	1.69	0.9	5.9	-
Group4/Group 2			10		2.5		0.3		3.5		0.4		-

* Ratio group 3/2 and group 4/3
[…] unreliable, poor loglinear correlation

6.2.2

Study number B18393: BIA 9-1067: 90-day oral toxicity (gavage) study in the CD-1 mouse

Summarized from the nonclinical review of Terry Peters, DVM, March 24, 2009.

BIA 9-1067 was administered (0, 100, 500, 0r 1000 mg/kg/day) by oral gavage to 10 6-week old CD-1 mice per sex in a vehicle of 0.2% hydroxypropyl methylcellulose for 90 days. The NOAEL was the HD of 1000mg/kg/day. Mean exposures at that dose were $AUC_{(0-t)}$: M - 28530 and F - 56860 ng*h/mL; C_{max}: M - 8147 and F - 22600 ng/mL; and the T_{max} was 0.5 h in M and F. Exposures to parent (BIA-1067) exceeded those of metabolite BIA 1079.

6.2.3

Study title: 28-day oral toxicity (gavage) study in the Wistar Rat

Study no.:	A73980
Study report location:	eDR 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	DEC 7, 2006
GLP compliance:	Yes (OECD)
QA statement:	Yes
Drug, lot #, and % purity:	BIA 9-1067/MA-0343.06/98.4% and DL060418/ 97.27%

Key Study Findings:
In all dosed groups there was a slight increase in the T_{helper} ($CD3_+/CD4_+$) lymphocyte populations, with no alteration in function or response to sheep RBC's.

NOAEL: HD (1000 mg/kg/day)
$AUC_{(0-t)}$: M: 24561; F: 16831 ng*h/mL
C_{max}: M: 14967; F: 11983 ng/mL
T_{max} M and F: 0.5 h

Methods

Doses:	0(vehicle), 100, 500, or 1000 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	20 mL/kg
Formulation/Vehicle:	0.2% hydroxypropyl methylcellulose
Species/Strain:	Rat, HanRcc:WIST (SPF)
Number/Sex/Group:	10
Age:	6 weeks
Weight:	M: 132 – 164 g; F: 111 - 131
Satellite groups:	TK: C-3/sex/Dosed: 9/sex Immuno-toxciology: 6/sex
Unique study design:	Immuno-toxicologic phenotyping
Deviation from study protocol:	Minor, with no impact on study results

Observations and Results

Mortality:
None

Clinical Signs:
White and opaque right eyes (OD) seen in some TK animals was attributed to blood sampling technique.
Orange feces in all MD and HD was attributed to the color of the test article

Body Weights and Food Consumption:
No test article-related effects were reported.

Ophthalmoscopy:
No test-article-related effects were reported.

Hematology, Coagulation, Clinical Chemistry, Urinalysis:
No differences among groups were reported.

Gross Pathology:
No test article-related effects were reported.

Organ Weights:
Liver weights (absolute and relative to body weight) were slightly decreased in HDM and relative liver weights in MDM. This corresponds to liver glycogen in C and LD and reduced glycogen in MD and HD.

Histopathology:
Adequate Battery: Yes
Signed and dated pathology report: Yes
Peer Review: No
Histological Findings:

Increased liver glycogen (slight to marked) was noted in C and LDM and decreased severity of glycogen (minimal to moderate) was reported in MDM and HDM. F animals were not affected.

Toxicokinetics:
The t_{max} of BIA 9-1067 was 0.5-1.0 hr and of metabolite BIA 9-1079 was 1.6-4.4 hours. Exposure to parent exceeded metabolite on Study Day 1 and after 28 days of dosing. There was no accumulation of BIA 9-1067. Gender did not affect the TK of parent; however, the half-life of BIA 9-1079 tended to be shorter in F.

Sponsor's table: TK male and female

Day	Group	Dose [mg/kg]	Ratio*	AUC_{0-t} [ng•h/mL]	Ratio*	AUC_{0-t} / Dose	Ratio*	C_{max} [ng/mL]	Ratio*	C_{max}/ Dose	Ratio*	$t_{½, z}$ [h]	Ratio*
males													
Day 1	2	100	-	19669	-	197	-	4710	-	47	-	2.7	-
	3	500	5.0	59979	3.0	120	0.6	15203	3.2	30	0.6	[1.9]	-
	4	1000	2.0	96332	1.6	96	0.8	18467	1.2	18	0.6	2.6	-
Group4/Group 2			10		4.9		0.5		3.9		0.4		1.0
Day 29	2	100	-	24561	-	246	-	14967	-	150	-	1.7	-
	3	500	5.0	68362	2.8	137	0.6	22333	1.5	45	0.3	2.5	1.5
	4	1000	2.0	104385	1.5	104	0.8	29333	1.3	29	0.7	4.4	1.8
Group4/Group 2			10		4.3		0.4		2.0		0.2		2.6
females													
Day 1	2	100	-	11571	-	116	-	3613	-	36	-	[2.9]	-
	3	500	5.0	45497	3.9	91	0.8	13493	3.7	27	0.7	1.6	-
	4	1000	2.0	67031	1.5	67	0.7	19600	1.5	20	0.7	2.0	1.3
Group4/Group 2			10		5.8		0.6		5.4		0.5		-
Day 29	2	100	-	16831	-	168	-	11983	-	120	-	1.8	-
	3	500	5.0	51261	3.0	103	0.6	23867	2.0	48	0.4	1.6	0.9
	4	1000	2.0	100622	2.0	101	1.0	28933	1.2	29	0.6	[3.3]	-
Group4/Group 2			10		6.0		0.6		2.4		0.2		-

* Ratio group 3/2 and group 4/3
[…] unreliable, poor loglinear correlation

6.2.4

Study number B18382: BIA 9-1067: 90-day oral toxicity (gavage) study in the Wistar rat

Summarized from the nonclinical review of Terry Peters, DVM, March 24, 2009.

BIA 9-1067 was administered (0, 100, 500, 0r 1000 mg/kg/day) by oral gavage to 10 per sex 6-week old HanRcc: WIST (SPF) rats in a vehicle of 0.2% hydroxypropyl methylcellulose for 90 days. An additional 9/sex animals were sampled for TK and 5/sex C and HD were held for a 4-week recovery period.

The NOEL and MFD was the HD of 1000 mg/kg/day. Mean exposures were slightly higher in males than females at all doses. At the NOEL: $AUC_{(0-t)}$: M - 29007 and F - 27127 ng*h/mL; C_{max}: M - 6377 and F - 6173 ng/mL; and the T_{max} was 4 h in M and 2 h in F. Exposures to parent (BIA-1067) exceeded those of metabolite BIA 1079.

Exposures to test article increased in a dose proportional manner at Day 1. The increase was less than dose proportional at the end of dosing, No accumulation of parent or metabolites was reported.

Sponsor's table: TK of BIA 9-1067

BIA 9-1067		day 1		week 4		week 8		week 13	
Dose (mg/kg/day)		500	1000	500	1000	500	1000	500	1000
Male									
C_{max}	(ng/mL)	23100	34200	20100	20200	29100	24300	18100	28600
$C_{max\ norm}$	[1]	46.2	34.2	40.2	20.2	58.2	24.3	36.2	28.6
t_{max}	(h)	1.0	1.0	0.50	0.50	0.50	1.0	0.50	0.50
$AUC_{0\text{-}t}$	(ng.h/mL)	70600	126000	47700	83600	73500	108000	65400	121000
$AUC_{0\text{-}t\ norm}$	[2]	141	126	95.4	83.6	147	108	131	121
$AUC_{0\text{-}inf}$	(ng.h/mL)	71700	131000	47900	83700	75400*	137000*	65600	149000
$t_{½,z}$	(h)	2.1	2.7	1.6	2.3	3.6*	13	2.6	10
Female									
C_{max}	(ng/mL)	25900	43800	22600	31300	21100	41200	23200	42000
$C_{max\ norm}$	[1]	51.8	43.8	45.2	31.3	42.2	41.2	46.4	42.0
t_{max}	(h)	0.50	0.50	0.50	0.50	1.0	0.50	0.50	0.50
$AUC_{0\text{-}t}$	(ng.h/mL)	53200	106000	47600	94000	50100	110000	58300	122000
$AUC_{0\text{-}t\ norm}$	[2]	106	106	95.2	94.0	100	110	117	122
$AUC_{0\text{-}inf}$	(ng.h/mL)	54900*	109000	47700	95000	59600*	119000	59400*	125000
$t_{½,z}$	(h)	1.9*	2.9	2.6	3.8	7.4*	7.9	3.6*	4.4

[1] (ng/mL)/(mg/kg/day)
[2] (ng.h/mL)/(mg/kg/day)
* unreliable, $r^2 < 0.900$ and/or AUC%extr > 20%

Sponsor's table: TK of major metabolite BIA 9-1079

BIA 9-1079		day 1		week 4		week 8		week 13	
Dose (mg/kg/day)		500	1000	500	1000	500	1000	500	1000
Male									
C_{max}	(ng/mL)	1330	1100	689	574	611	661	512	593
$C_{max\ norm}$	[1]	2.66	1.10	1.38	0.574	1.22	0.661	1.02	0.593
t_{max}	(h)	8.0	8.0	4.0	4.0	8.0	12	12	4.0
$AUC_{0\text{-}t}$	(ng.h/mL)	16500	15400	8530	9110	11600	12400	9150	11800
$AUC_{0\text{-}t\ norm}$	[2]	33.0	15.4	17.1	9.11	23.2	12.4	18.3	11.8
$AUC_{0\text{-}inf}$	(ng.h/mL)	19300	17800	9920	10700	84100*	n.c.	n.c.	35600*
$t_{½,z}$	(h)	7.1	7.0	8.0	7.8	100*	n.c.	n.c.	40
Female									
C_{max}	(ng/mL)	971	644	508	511	510	581	628	847
$C_{max\ norm}$	[1]	1.94	0.644	1.02	0.511	1.02	0.581	1.26	0.847
t_{max}	(h)	2.0	8.0	4.0	8.0	4.0	4.0	8.0	12
$AUC_{0\text{-}t}$	(ng.h/mL)	11900	11400	7310	8690	7660	10300	11600	12900
$AUC_{0\text{-}t\ norm}$	[2]	23.8	11.4	14.6	8.69	15.3	10.3	23.2	12.9
$AUC_{0\text{-}inf}$	(ng.h/mL)	12500*	15500*	8400	12700*	9910*	13600*	17100*	n.c.
$t_{½,z}$	(h)	6.0*	11	7.2	13	11	11	13	n.c.

[1] (ng/mL)/(mg/kg/day)
[2] (ng.h/mL)/(mg/kg/day)
* unreliable, $r^2 < 0.900$ and/or AUC%extr > 20%
n.c. not calculated

Sponsor's table: TK of major human metabolite BIA9-1103

BIA 9-1103		day 1		week 4		week 8		week 13	
Dose (mg/kg/day)		500	1000	500	1000	500	1000	500	1000
Male									
C_{max}	(ng/mL)	5730	10600	6740	8620	14900	13200	8180	11500
$C_{max\ norm}$	[1]	11.5	10.6	13.5	8.62	29.8	13.2	16.4	11.5
t_{max}	(h)	2.0	2.0	2.0	2.0	2.0	1.0	2.0	1.0
AUC_{0-t}	(ng.h/mL)	43200	90900	43500	74700	74000	163000	72100	182000
$AUC_{0-t\ norm}$	[2]	86.4	90.9	87.0	74.7	148	163	144	182
AUC_{0-inf}	(ng.h/mL)	44900	98400	47200	80600	81400	199000*	81200	278000*
$t_{½,z}$	(h)	4.9	6.3	7.5	6.1	7.3	10*	7.6	16
Female									
C_{max}	(ng/mL)	1330	3260	1240	3010	1710	3430	1950	3110
$C_{max\ norm}$	[1]	2.66	3.26	2.48	3.01	3.42	3.43	3.90	3.11
t_{max}	(h)	2.0	2.0	1.0	2.0	1.0	2.0	2.0	1.0
AUC_{0-t}	(ng.h/mL)	9410	18400	8940	19400	12500	30700	16700	35300
$AUC_{0-t\ norm}$	[2]	18.8	18.4	17.9	19.4	25.0	30.7	33.4	35.3
AUC_{0-inf}	(ng.h/mL)	9570	18700	9070	20000	13000	38000	17600	40000
$t_{½,z}$	(h)	4.0	4.0	3.8	4.3	5.2	9.8	5.4	8.3

[1] (ng/mL)/(mg/kg/day)
[2] (ng.h/mL)/(mg/kg/day)
* unreliable, $r^2 < 0.900$

Sponsor's table: TK of metabolite BIA 9-1106

BIA 9-1106		day 1		week 4		week 8		week 13	
Dose (mg/kg/day)		500	1000	500	1000	500	1000	500	1000
Male									
C_{max}	(ng/mL)	8130	14800	7560	7820	8950	8130	5870	9840
$C_{max\ norm}$	[1]	16.3	14.8	15.1	7.82	17.9	8.13	11.7	9.84
t_{max}	(h)	1.0	1.0	0.50	0.50	0.50	1.0	1.0	0.50
AUC_{0-t}	(ng.h/mL)	24200	48600	14700	24000	21900	35800	19600	45600
$AUC_{0-t\ norm}$	[2]	48.4	48.6	29.4	24.0	43.8	35.8	39.2	45.6
AUC_{0-inf}	(ng.h/mL)	24500	49400	14700	24700	22200*	43200	21000*	53500
$t_{½,z}$	(h)	1.8	2.0	1.5	2.4	3.1*	11	3.1*	8.6
Female									
C_{max}	(ng/mL)	8940	12500	5630	12400	8710	23200	8300	23100
$C_{max\ norm}$	[1]	17.9	12.5	11.3	12.4	17.4	23.2	16.6	23.1
t_{max}	(h)	0.50	0.50	0.50	0.50	1.0	0.50	0.50	0.50
AUC_{0-t}	(ng.h/mL)	17800	36600	13100	31700	18300	51900	22000	63400
$AUC_{0-t\ norm}$	[2]	35.6	36.6	26.2	31.7	36.6	51.9	44.0	63.4
AUC_{0-inf}	(ng.h/mL)	17900	37200	13400*	31800	19000*	53600	22200*	63900
$t_{½,z}$	(h)	4.2	2.0	2.3*	2.8	2.4*	5.7	3.2*	3.3

[1] (ng/mL)/(mg/kg/day)
[2] (ng.h/mL)/(mg/kg/day)
* unreliable, $r^2 < 0.900$

6.2.5

Study title: 26-week oral (gavage) toxicity study in the Wistar rat

Study no.:	A73980
Study report location:	eDR 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	JUN 23, 2008
GLP compliance:	Yes (OECD GLP)
QA statement:	Yes
Drug, lot #, and % purity:	BIA 9-1067/39129-1-10/97.9%

Key Study Findings:

During dosing a shift to immature reticulocytes was seen in HDM and HDF. The relative and absolute numbers of reticulocytes were slightly decreased in HDM and HDF after the recovery period.

Bilirubin levels were increased during dosing; after recovery, bilirubin levels were still increased in males and females similar to Weeks 13 and 26. During dosing, non-adverse Increases in electrolyte concentrations (sodium, potassium, chloride, calcium and phosphorus) and decreased albumin and increased globulin levels as well as increased lactate dehydrogenase levels were seen.

(NOAEL) MD (500 mg/kg/day) Based on bilirubin levels in HD.

Methods

Doses:	0(vehicle), 100, 500, or 1000 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	20mL/kg
Formulation/Vehicle:	0.2% hydroxypropyl methylcellulose
Species/Strain:	Rat, HanRcc: WIST(SPF)
Number/Sex/Group:	20/sex; 1/sex C and HD recovery (4 weeks)
Age:	6 weeks
Weight:	114.2-160.7 g
Satellite groups:	TK 3/sex C; 9/sex dosed
Unique study design:	NA
Deviation from study protocol:	Minor with no impact on study results

Observations and Results

Mortality:
None

Clinical Signs:

Yellow discoloration of feces was seen in all dosed groups and was attributed to color the test article.

Body Weights and Food Consumption:
No differences among groups were reported.

Ophthalmoscopy:
No test article-related effects were reported.

Hematology: (Study weeks 13 and 26 and after 4-week recovery)

Compared to C: Slight decreases in hemoglobin parameters were noted in MDM, HDM, and HDF and a shift to less mature reticulocytes noted at the HD in both M and F. A 32% decrease in neutrophils in HDM and an 80% increase in eosinophils in HDF were also reported.

After recovery: Compared to C, a slight decrease in hemoglobin distribution in HDM and decreased reticulocytes in HDM and HDF were reported.

Coagulation:
Compared to C, a slight increase in prothrombin time (3.5-5%) in HDM and HDF were reported. After recovery, PT and PTT were slightly decreased.

Clinical Chemistry:
Changes in bilirubin, compared to C, were considered to be adverse.

- Study Week 13: Increased bilirubin in HDM (+15.8%) and HDF (+38.8%)
- Study Week 26: Increased bilirubin in HDF (+14.4%)
- After recovery: bilirubin remained increased in HDM (+27.5%) and HDF (37.7%).
- Alkaline phosphatase was increased (+19.6%) in HDF.

Phosphorus was increased in HDM (+16.9%) and in LDF (+18.5%), in MDF (+16.7%), and in HDF (+26.9%).

In Study Week 26: Increased aspartate aminotransferase levels were reported in all test item-treated males (+22.6% LDM; +20.8% MDM, +19.3% HDM), and +29.7% in LDF. After recovery, AST and ALT levels were less than C.

Urinalysis:
No test-article related effects were reported.

Gross Pathology:
No test-article related effects were reported.

Organ Weights:
Absolute and relative liver weights were reduced in all dosed M and F groups. Absolute and relative spleen weights were reduced in all F groups and in HDM and absolute and relative adrenal weights were reduced in MD and HDF.

Sponsor's table:

Table: Percent change of selected organ weights (compared to controls)

	100 mg/kg/day		500 mg/kg/day		1000 mg/kg/day	
	Males	**Females**	**Males**	**Females**	**Males**	**Females**
Spleen abs.		-18.5%**		-20.0%**	-11.1%*	-15.4%*
to bw		-16.7%**		-12.5%#	-12.5%**	-12.5%#
to brain		-19.6%**		-17.5%**	-11.7%*	-14.6%*
Liver abs.	-15.4%**	-15.3%**	-14.0%**	-14.7%**	-9.3%**	-10.8%**
to bw	-13.1%**	-12.4%**	-12.2%**	-6.9%*	-10.1%**	-5.5%#
to brain	-16.2%**	-16.0%**	-14.2%**	-12.9%**	-9.9%**	-9.1%*
Adrenals abs.				-15.7%**		-18.1%**
to bw				-9.7%#		-12.9%*
to brain weight				-13.1%**		-16.4%**

*: $p<0.05$; **: $p<0.01$; #: not significant; Dunnett-test

Histopathology:
Adequate Battery Yes
Pathology report: Yes
Peer Review: Yes
Histological Findings:

Limited to acanthosis, parakeratosis, and erosions of the forestomach of HDM

Toxicokinetics:
No accumulation was seen. Exposure increased with dose but less than dose-proportionally. There were no significant differences between M and F.

Sponsor's table: TK of BIA 9-1067

BIA 9-1067 Exposure: Effects of Dose									
Occasion	Group	Dose [mg/kg]	Ratio	AUC_{0-t} [ng•h/mL]	Ratio	C_{max} [ng/mL]	Ratio	$t_{½, z}$ [h]	Ratio
Males									
Day 1	2	100	-	17467	-	4137	-	n.c.	-
	3	500	5.0	33634	1.9	8280	2.0	8.8	n.c .
	4	1000	2.0	50622	1.5	16467	2.0	4.0*	n.c.
Group4/Group 2			10		2.9		4.0		n.c.
Week 13	2	100	-	15006	-	8817	-	1.7	-
	3	500	5.0	38600	2.6	16733	1.9	1.5	0.9
	4	1000	2.0	54037	1.4	18800	1.1	2.5	1.7
Group4/Group 2			10.0		3.6		2.1		1.5
Week 26	2	100	-	14953	-	7187	-	1.6	-
	3	500	5.0	59182	4.0	19467	2.7	7.8*	n.c.
	4	1000	2.0	67942	1.1	19733	1.0	2.4	n.c.
Group4/Group 2			10.0		4.5		2.7		1.5
Females									
Day 1	2	100	-	14019	-	4290	-	1.8	-
	3	500	5.0	31450	2.2	10500	2.4	1.8	1.0
	4	1000	2.0	49310	1.6	23367	2.2	2.2*	n.c.
Group4/Group 2			10		3.5		5.4		n.c.
Week 13	2	100	-	12413	-	10797	-	0.95	-
	3	500	5.0	53416	4.3	26833	2.5	2.6	2.7
	4	1000	2.0	62538	1.2	31400	1.2	1.9	0.7
Group4/Group 2			10.0		5.0		2.9		2.0
Week 26	2	100	-	11266	-	7663	-	1.3	-
	3	500	5.0	41607	3.7	27133	3.5	1.4	1.1
	4	1000	2.0	65223	1.6	23333	0.9	2.7*	n.c.
Group4/Group 2			10.0		5.8		3.0		n.c.

n.c. Not calculated

* Unreliable, poor loglinear correlation for λz ($r^2 < 0.900$)

Metabolite 9-1079 levels peaked between 1 and 6 hours, increasing in a less than dose-proportional manner, but there was no accumulation.

Sponsor's table: TK of metabolite BIA 9-1079

BIA 9-1079 Exposure: Effects of Dose									
Occasion	Group	Dose [mg/kg]	Ratio	AUC_{0-t} [ng•h/mL]	Ratio	C_{max} [ng/mL]	Ratio	$t_{½, z}$ [h]	Ratio
Males									
Day 1	2	100	-	19727	-	1863	-	n.c.	-
	3	500	5.0	25583	1.3	2120	1.1	n.c.	n.c.
	4	1000	2.0	40082	1.6	3067	1.4	n.c.	n.c.
Group4/Group 2			10		2.0		1.6		n.c.
Week 13	2	100	-	16348	-	1204	-	9.0	-
	3	500	5.0	28619	1.8	3047	2.5	5.9	0.7
	4	1000	2.0	47516	1.7	4473	1.5	6.7	1.1
Group4/Group 2			10.0		2.9		3.7		0.7
Week 26	2	100	-	15116	-	1141	-	n.c.	-
	3	500	5.0	34112	2.3	2640	2.3	13	n.c.
	4	1000	2.0	46504	1.4	4050	1.5	10	0.8
Group4/Group 2			10.0		3.1		3.5		n.c.
Females									
Day 1	2	100	-	11949	-	1453	-	n.c.	-
	3	500	5.0	19804	1.7	2297	1.6	3.3	n.c.
	4	1000	2.0	31934	1.6	2543	1.1	7.2	2.2
Group4/Group 2			10		2.7		1.8		n.c.
Week 13	2	100	-	7531	-	1045	-	3.8	-
	3	500	5.0	28202	3.7	3120	3.0	6.2	1.6
	4	1000	2.0	37855	1.3	4150	1.3	6.4	1.0
Group4/Group 2			10.0		5.0		4.0		1.7
Week 26	2	100	-	7579	-	973	-	4.2	-
	3	500	5.0	19980	2.6	2450	2.5	4.7	1.1
	4	1000	2.0	35034	1.8	3660	1.5	6.7	1.4
Group4/Group 2			10.0		4.6		3.8		1.6

n.c. Not calculated

Stability:

After 7 days at room temperature, the formulation contained ±20% of the nominal content.

Homogeneity:

The formulations were found to be homogenous.

6.2.6

Study title: 4-week oral (gavage) toxicity in the Cynomolgus monkey with a 2-week recovery period.

Study no.:	SO3352
Study report location:	eDR 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	JAN 22, 2008
GLP compliance:	Yes: OECD (b) (4)
QA statement:	Yes
Drug, lot #, and % purity:	BIA 9-1067/39129-1-12/97.5%

Key Study Findings:

Sporadic pasty feces, diarrhea, and occasional emesis were noted in all dosed groups.

The NOAEL was at the HD of 1000 mg/kg/day (the MFD).

- T_{max}: 1-4 hr (median 4 hr.)
- Half-life: M - 3.5; F-1.7 hr.
- $AUC_{(0-t)}$: M - 27965; F- 22490 ng*h/mL
- C_{max}: M - 8258; F - 6848 ng/mL

Methods

Doses:	0(vehicle), 100, 300, or 1000 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	10mL/kg
Formulation/Vehicle:	0.2% hydroxypropyl methylcellulose
Species/Strain:	Cynomolgus monkey (M. fascicularis) Captive-bred from Viet Nam
Number/Sex/Group:	5/sex C and HD; 3/sex LD and MD
Age:	2.3-3.0
Weight:	2.3-2.8 Kg
Satellite groups:	NA
Unique study design:	Recovery: 2/sex C and HD
Deviation from study protocol:	Minor with no impact on study results

Dose: In an MTD study, 1000 mg/kg/day was the NOAEL and the MFD, and the dose could not be increased without exceeding a 10-mL/kg administration volume.

Observations and Results

Mortality:
None

Clinical Signs:
Sporadic occurrences of pasty feces, diarrhea, and discolored (yellow) feces were reported in all dosed groups. Emesis immediately post-dose was also noted in dosed animals.

Body Weights:
No test article-related effects were reported.

Food Consumption:
Not reported.

Ophthalmoscopy, Electrocardiography:
No test article-related effects were reported.

Hematology, Coagulation, and Urinalysis:
No test article-related effects were reported.

Clinical Chemistry:
Test article-related effects were limited to reduced triglyceride levels in the HDM and MDM, compared to CM.

Gross Pathology:
No test article-related effects were reported.

Organ Weights:
No test article-related effects were reported.

Histopathology:
Adequate Battery: Yes
Pathology report: Yes
Peer Review: No
Histological Findings:
No test article-related findings were reported.

Toxicokinetics:
NOAEL: HD (1000 mg/kg/day)

- T_{max}: 1-4 hr (median 4hr.)
- Half-life: M - 3.5; F-1.7 hr.
- $AUC_{(0-t)}$: M - 27965; F- 22490 ng*h/mL
- C_{max}: M - 8258; F - 6848 ng/mL

Sponsor's table: TK of BA 9-1067

	Group	2	3	4
Dose	**[mg/kg]**	**100 mg/kg**	**300 mg/kg**	**1000 mg/kg**
Males			**Day 1**	
C_{max}	[ng/mL]	964 ± 191	2575 ± 1698	8258 ± 8812
t_{max} (range)	[h]	(1)	(1)	(1-2)
AUC_{0-t}	[ng•h/mL]	2319 ± 300	3922 ± 933	27965 ± 26957
$AUC_{0-inf.}$	[ng•h/mL]	2593 ± 379	4110 ± 967	28389 ± 26835
$t_{½, z}$	[h]	1.4 ± 0.2	1.6 ± 0.8	3.5 ± 1.5
			Week 4	
C_{max}	[ng/mL]	828 ± 331	1364 ± 623	6848 ± 5865
t_{max} (range)	[h]	(1)	(1-2)	(2)
AUC_{0-t}	[ng•h/mL]	1314 ± 128	2321 ± 624	22490 ± 13887
$AUC_{0-inf.}$	[ng•h/mL]	1418 ± 67	[2030]	25941 ± 14853
$t_{½, z}$	[h]	0.89 ± 0.33	[0.62]	1.7 ± 0.3

[…] unreliable, only one valid estimate per group

Sponsor's table: TK of metabolite BA 9-1079

	Group	2	3	4
Dose	**[mg/kg]**	**100 mg/kg**	**300 mg/kg**	**1000 mg/kg**
Females			**Day 1**	
C_{max}	[ng/mL]	1170 ± 82	1062 ± 198	2533 ± 801
t_{max} (range)	[h]	(1)	(1)	(2)
AUC_{0-t}	[ng•h/mL]	1762 ± 66	2475 ± 1095	11291 ± 4382
$AUC_{0-inf.}$	[ng•h/mL]	1828 ± 77	[2974]	12643 ± 4729
$t_{½, z}$	[h]	1.1 ± 0.7	[1.5]	4.5 ± 0.7
			Week 4	
C_{max}	[ng/mL]	836 ± 268	1236 ± 558	4294 ± 3434
t_{max} (range)	[h]	(1)	(1)	(1-4)
AUC_{0-t}	[ng•h/mL]	1039 ± 326	2298 ± 1468	18005 ± 13120
$AUC_{0-inf.}$	[ng•h/mL]	1293	1517	16475 ± 5286
$t_{½, z}$	[h]	0.78	1.0	3.4 ± 0.3

[…] unreliable, only one valid estimate per group

Stability:

Stability and homogeneity of the administered formulation was not assessed.

6.2.7

Study title: 13-week oral (gavage) toxicity study in the Cynomolgus monkey with a 4-week recovery period.

Study no.:	S128924
Study report location:	eDR 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	APR 16, 2008
GLP compliance:	Yes: OECD
QA statement:	Yes
Drug, lot #, and % purity:	BIA 9-1067/39129-1-12/97.5%

Key Study Findings:

No effect level (NOEL): HD (1000 mg/kg/day)

Methods

Doses:	0 (vehicle), 100, 300 or 1000 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0 2% hydroxypropyl methylcellulose
Species/Strain:	Cynomolgus monkey (*Macaca fascicularis*), captive bred from China.
Number/Sex/Group:	Main study 4/sex; Recovery 2/sex C and HD
Age:	3.4 - 4.0 years
Weight:	2.2-3.87 kg
Satellite groups:	NA
Unique study design:	NA
Deviation from study protocol:	None reported

Observations and Results

Mortality:

None

Clinical Signs:

Limited to sporadic emesis, soft or pasty feces, and yellow-discolored feces (attributed to presence of the test item)

Body Weights:

No test article-related effects were reported.

Food Consumption:

Not reported

Ophthalmoscopy:
No test article-related effects were reported.

Electrocardiography:
No test article-related effects were reported.

Hematology and Coagulation:
No test article-related effects were reported.

Clinical Chemistry:
No test article-related effects were reported.

Urinalysis:
No test article-related effects were reported.

Gross Pathology:
No test-article related changes were reported.

Organ Weights:
Absolute and relative (to body weight) lung weights were greater in HDF than CF.
No other test-article related effects were reported.

Histopathology:
Adequate Battery: Yes
Pathology report: Yes
Peer Review: Yes, (b) (4) senior pathologist
Histological Findings:
No test article-related changes were reported.

Toxicokinetics:
There was high interanimal variability; however, exposures increased with dose and were largely dose-proportional after the first dose and less than dose-proportional after 13 weeks of dosing. The t_{max} was 1-4 hours.

Sponsor's table: TK in M

	Group	2	3	4
Dose		**100 mg/kg/day**	**300 mg/kg/day**	**1000 mg/kg/day**
Males			**Day 1**	
C_{max}	[ng/mL]	1166 ± 650	3223 ± 947	5017 ± 2723
t_{max} (range)	[h]	1	1-2	1-2
AUC_{0-t}	[ng•h/mL]	1306 ± 782	7260 ± 2297	16729 ± 6486
AUC_{0-inf}	[ng•h/mL]	1414 ± 1323	[5644]	19519 ± 8464
$t_{½, z}$	[h]	0.84 ± 0.01	[1.2]	6.5 ± 2.6
			Week 13	
C_{max}	[ng/mL]	1080 ± 724	3515 ± 1937	5405 ± 1531
t_{max} (range)	[h]	1	1-2	1-4
AUC_{0-t}	[ng•h/mL]	1843 ± 1182	7203 ± 3980	23162 ± 5588
AUC_{0-inf}	[ng•h/mL]	2368 ± 1561	6449 ± 1118	25939 ± 3861
$t_{½, z}$	[h]	1.1 ± 0.2	1.6 ± 0.8	3.0 ± 1.4

[...] unreliable, only one valid estimate per group

Sponsor's table: TK in F

	Group	2	3	4
Dose		**100 mg/kg/day**	**300 mg/kg/day**	**1000 mg/kg/day**
Females			**Day 1**	
C_{max}	[ng/mL]	1040 ± 590	4338 ± 455	5540 ± 3092
t_{max} (range)	[h]	1	1-2	1-2
AUC_{0-t}	[ng•h/mL]	1264 ± 848	7577 ± 1619	24795 ± 12353
AUC_{0-inf}	[ng•h/mL]	1339 ± 1048	8380 ± 1074	25668 ± 12554
$t_{½, z}$	[h]	0.93 ± 0.44	1.4 ± 0.8	5.2 ± 0.89
			Week 13	
C_{max}	[ng/mL]	2501 ± 1094	4010 ± 2592	6168 ± 2905
t_{max} (range)	[h]	1	1	1-4
AUC_{0-t}	[ng•h/mL]	3547 ± 1751	9079 ± 4504	33636 ± 13743
AUC_{0-inf}	[ng•h/mL]	3684 ± 2123	7292 ± 3871	38462 ± 15099
$t_{½, z}$	[h]	1.1 ± 0.3	1.6 ± 0.2	3.9 ± 0.7

Metabolite BIA 9-1079 had higher exposures (AUC and C_{max}) than parent. The differences were greater at lower doses.

Sponsor's table: TK of metabolite BIA 1079 in M and F:

	Group	2	3	4
Dose	**[mg/kg/day]**	**100 mg/kg/day**	**300 mg/kg/day**	**1000 mg/kg/day**
Males			**Day 1**	
C_{max}	[ng/mL]	717 ± 185	1950 ± 562	1760 ± 221
t_{max} (range)	[h]	1-4	2-4	1-4
AUC_{0-t}	[ng•h/mL]	3122 ± 1555	20004 ± 7178	23188 ± 3757
$AUC_{0-inf.}$	[ng•h/mL]	3270 ± 1867	25144 ± 4710	n.c.
$t_{½, z}$	[h]	3.0 ± 1.2	6.2 ± 1.4	14 ± 4
			Week 13	
C_{max}	[ng/mL]	1438 ± 470	2228 ± 500	2150 ± 314
t_{max} (range)	[h]	2-4	2-4	2-4
AUC_{0-t}	[ng•h/mL]	10203 ± 5968	21056 ± 3268	28068 ± 5242
$AUC_{0-inf.}$	[ng•h/mL]	10477 ± 6138	22021 ± 2974	28874 ± 5439
$t_{½, z}$	[h]	4.2 ± 0.6	5.1 ± 1.0	9.9 ± 2.9
Females			**Day 1**	
C_{max}	[ng/mL]	919 ± 196	1903 ± 587	2642 ± 555
t_{max} (range)	[h]	1-2	1-2	2
AUC_{0-t}	[ng•h/mL]	4191 ± 2588	14821 ± 5574	36544 ± 9348
$AUC_{0-inf.}$	[ng•h/mL]	4362 ± 2602	14122 ± 6402	[21899]
$t_{½, z}$	[h]	2.4 ± 1.3	5.0 ± 1.2	16 ± 7
			Week 13	
C_{max}	[ng/mL]	1590 ± 525	2428 ± 822	2347 ± 301
t_{max} (range)	[h]	1-2	1-4	1-4
AUC_{0-t}	[ng•h/mL]	9737 ± 6011	23946 ± 8022	37775 ± 10496
$AUC_{0-inf.}$	[ng•h/mL]	11931 ± 6759	25919 ± 9172	[28505]
$t_{½, z}$	[h]	3.8 ± 1.8	5.9 ± 1.4	21 ± 13

[…] unreliable, only one valid estimate per group

n.c. Not calculated

Stability: The test item content was within the range of ± 20%.

Homogeneity:
The formulations were found to be homogenous.

6.28

Study title: 52-week oral (gavage) toxicity study in the Cynomolgus monkey with an 8-week recovery period.

Study no.:	S13825
Study report location:	eDR 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	NOV 11, 2008
GLP compliance:	Yes (OECD)
QA statement:	Yes
Drug, lot #, and % purity:	BIA 9-1067/39129-1-11/97.5-97.7% BIA 9-1067/39129-1-3/97.5-97.7%

Key Study Findings:

Occasional emesis was noted in HDF up to 4.5 hours post-dose. Occasional emesis (within 15 min of administration) and increased salivation were observed in all groups.

Discoloration of feces, attributed to presence of test item, was noted in LD and MD groups during Study Week 1 and throughout dosing in HD groups. Feces were normal in color during recovery.

Body weight gain and Absolute body weight in HDF was greater than CF.

NOEL in M: HD (1000 mg/kg/day)
NOAEL in F: HD (1000 mg/kg/day)

Methods

Doses:	0 (vehicle), 100, 300, or 1000 mg/kg/day
Frequency of dosing:	Daily
Route of administration:	Gastric gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.2% hydroxypropyl methylcellulose
Species/Strain:	Cynomolgus monkey (*Macaca fascicularis*) Captive bred form Viet Nam
Number/Sex/Group:	Main: 4/sex; recovery: 2/sex C and HD
Age:	2.4-2.9 years
Weight:	1.91-2.4 kg
Satellite groups:	NA
Unique study design:	NA
Deviation from study protocol:	None reported

Dose Level	Vehicle	BIA 9-1067		
	Group 1 0 mg/kg/day	Group 2 100 mg/kg/day	Group 3 300 mg/kg/day	Group 4 1000 mg/kg/day
Male animals	1-4	7-10	11-14	15-18
Male recovery animals	5-6			19-20
Female animals	21-24	27-30	31-34	35-38
Female recovery animals	25-26			39-40

Observations and Results

Mortality:
One MDM died 3 hours post-dose after inhaling vomitous during dosing.

Clinical Signs:
Diarrhea, pasty fees, yellow discoloration of feces, were seen in all dosed groups. No discoloration was seen after the 4th day of recovery.

Sporadic salivation was noted in HD and MD groups; the incidence increased from Study Week 30 through the end of dosing. Occasional emesis up to 15 minutes post-dose was noted in all groups. Emesis was not reported during recovery.

Body Weights:
Mean body weight gain tended to be greater in dosed F, compared to CF. The percentage gain was greater than C in LDF (49%) and HDF (57%), Absolute body weight in HDF was greater than CF from Study Week 36 through the end of dosing.

Food Consumption:
Not reported

Ophthalmoscopy:
No test article-related effects were reported.

ECG:
No test article-related effects were reported.

Hematology, Coagulation, Clinical Chemistry, Urinalysis:
No test article-related effects were reported.

Gross Pathology:
No test article-related changes were reported.

Organ Weights:
No test article-related changes were reported.

Histopathology:

Adequate Battery: Yes
Pathology report: Yes (signed and dated)
Peer Review: Yes (b) (4) senior pathologist)
Histological Findings:
No test article-related effects were reported.

Toxicokinetics:
Exposures to parent increased in a greater than dose-proportional manner; exposure to the metabolite increased in a generally dose-proportional manner. No differences were noted between M and F.

The half-life of parent BIA 9-1067 increased with dose and duration of dosing. T_{max} generally ranged from 1.0-2.0 hrs. and increased with dose; no accumulation was seen.

The half-life of metabolite BIA 9-1079 ranged from 3-28 hours and tended to increase with dose and duration of dosing; however, in Study Week 52 the half-life in HDF was markedly reduced to 1.4 hr and the exposure ($AUC_{(0-t)}$) was half that seen at Study Week 26. The T_{max} ranged from 1 to 8 hours; no accumulation was found.

Sponsor's table: TK of BIA 9-1067

		Males			Females		
Dose [mg/kg]		100	300	1000	100	300	1000
		Day 1			Day 1		
C_{max}	[ng/mL]	1080	2260	9420	1210	1610	8800
t_{max} (range)	[h]	(1)	(1)	(1-2)	(1)	(1)	(1-2)
AUC_{0-t}	[ng•h/mL]	1390	2970	32100	1330	3620	34400
AUC_{0-inf}	[ng•h/mL]	1620	3430	38000	[1020]	3500	35200
$t_{½,z}$	[h]	0.76	1.6	5.0	0.67	2.5	4.4
		Week 13			Week 13		
C_{max}	[ng/mL]	501	1460	6990	748	1070	5220
t_{max} (range)	[h]	(1)	(1-2)	(2)	(1)	(1-2)	(1-2)
AUC_{0-t}	[ng•h/mL]	646	2450	30000	827	1890	15300
AUC_{0-inf}	[ng•h/mL]	625	3550	30300	1160	2080	18200
$t_{½,z}$	[h]	0.88	0.99	2.5	0.72	1.4	2.4
		Week 26			Week 26		
C_{max}	[ng/mL]	528	1130	3720	673	1030	3420
t_{max} (range)	[h]	(1)	(1-2)	(2-4)	(1)	(1-2)	(2-4)
AUC_{0-t}	[ng•h/mL]	799	2690	21600	1180	2710	17800
AUC_{0-inf}	[ng•h/mL]	1140	3100	27100	1180	2730	13300
$t_{½,z}$	[h]	0.66	1.7	3.3	0.80	1.5	2.6
		Week 52			Week 52		
C_{max}	[ng/mL]	1040	1210	4440	1260	1110	2920
t_{max} (range)	[h]	(1)	(1)	(2)	(1)	(1-2)	(1-2)
AUC_{0-t}	[ng•h/mL]	1650	2620	20900	2270	3400	16200
AUC_{0-inf}	[ng•h/mL]	2020	2790	21600	2680	3890	16900
$t_{½,z}$	[h]	1.2	1.5	4.7	1.4	2.5	4.7

[ ] based on 1 value only

Sponsor's table: TK of metabolite BIA 9-1079

		Males			Females		
Dose [mg/kg]		100	300	1000	100	300	1000
		Day 1			Day 1		
C_{max}	[ng/mL]	850	874	2220	724	1140	2570
t_{max} (range)	[h]	(1-2)	(1-2)	(1-2)	(1-2)	(1-2)	(2)
AUC_{0-t}	[ng•h/mL]	4040	5790	27100	3320	9310	30000
AUC_{0-inf}	[ng•h/mL]	4850	7150	32400	4030	9710	[35900]
$t_{½,z}$	[h]	3.7	5.5	10.0	3.1	5.1	[8.6]
		Week 13			Week 13		
C_{max}	[ng/mL]	910	1180	2390	766	1320	2860
t_{max} (range)	[h]	(1-2)	(1-2)	(2-4)	(1-2)	(1-2)	(2-8)
AUC_{0-t}	[ng•h/mL]	4170	8440	35800	2840	10600	36400
AUC_{0-inf}	[ng•h/mL]	4220	9000	33700	3280	11000	40100
$t_{½,z}$	[h]	3.3	4.5	15	2.8	4.6	7.0
		Week 26			Week 26		
C_{max}	[ng/mL]	914	1310	2850	824	1410	3460
t_{max} (range)	[h]	(1-2)	(1-2)	(2-8)	(1-2)	(2-4)	(2-8)
AUC_{0-t}	[ng•h/mL]	5300	13200	41300	3540	11500	57100
AUC_{0-inf}	[ng•h/mL]	5870	14300	[44800]	4020	11800	[66200]
$t_{½,z}$	[h]	3.6	5.6	12	2.3	4.0	15
		Week 52			Week 52		
C_{max}	[ng/mL]	1360	1440	2310	1210	1420	2920
t_{max} (range)	[h]	(1-2)	(1-4)	(2-4)	(2)	(2)	(2-4)
AUC_{0-t}	[ng•h/mL]	6420	11400	36800	6470	13900	36100
AUC_{0-inf}	[ng•h/mL]	6890	11800	68000	7930	14800	34500
$t_{½,z}$	[h]	3.6	4.9	28	3.6	5.9	1.4

[ ] based on 1 value only

Stability:
The concentration of test article was within 20% of nominal content in the formulations after 7 days at room temperature.

Homogeneity:
The formulations were found to be homogenous.

7 Genetic Toxicology

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

BIA 9-1067 was tested for mutagenic activity in an Ames assay using *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98 and TA 100 and *Escherichia col*i WP2*uvr*A, with and without Aroclor-1254® induced rat S9. Concentrations ranged from 17 to 5000 µg per plate. Cytotoxicity and precipitation were seen at the highest concentration. No increase in numbers of revertant colonies were seen with or without S9.

In cultures treated with positive controls, with and without S9, the numbers of revertant colonies were up to 100-fold those of the vehicle (DMSO) control plates and were within

the range of the historical control data for the laboratory. The study was determined to be valid, and BIA 9-1067 was found to be negative for mutagenicity.

The metabolite BIA 9-1079 was tested for mutagenic activity in an Ames assay using *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98 and TA 100 and *Escherichia coli* WP2*uvr*A, with and without Aroclor-1254® induced rat S9. Concentrations ranged from 17 to 5000 µg per plate. Cytotoxicity (thinning of the lawn) was seen at ≥ 500 µg/plate. No increase in numbers of revertant colonies were seen both with and without S9.

In cultures treated with positive controls, with and without S9, the numbers of revertant colonies were up to 100-fold those of the vehicle (DMSO) control plates and were within the range of the historical control data for the laboratory. The study was determined to be valid, and BIA 9-1079 was found to be negative for mutagenicity.

The metabolite BIA 9-1103 was tested for mutagenic activity in an Ames assay using *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98 and TA 100 and *Escherichia coli* WP2*uvr*A, with and without Aroclor-1254® induced rat S9. Doses ranged from 17 to 5000 µg per plate. Precipitation was not seen at any concentration and cytotoxicity (thinning of the lawn) was seen in the absence of S9 at the highest dose. No increase in numbers of revertant colonies were seen both with and without S9.

V In cultures treated with positive controls, with and without S9, the numbers of revertant colonies were up to 100-fold those of the vehicle (DMSO) control plates and were within the range of the historical control data for the laboratory. The study was determined to be valid, and BIA 9-1079 was found to be negative for mutagenicity.

7.2 *In Vitro* Chromosomal Aberration Assays in Mammalian Cells

BIA 9-1067 was tested in vitro for clastogenicity in human peripheral blood lymphocytes in DMSO as vehicle, with and without Aroclor-1254 induced rat S9 and a NADPH-generating system. Positive controls were cyclophosphamide and mitomycin C. Test article concentrations ranged from 20 to 5000 µg/mL. BIA 9-1067 was cytotoxic at 313 µg/mL in the presence of S9 mix and at ≥78 µg/mL in the absence of S9. The number of structural aberrations due to treatment with BIA 9-1067 were within the 95% confidence limits for a negative response and considered to be negative. However, cultures treated with the cytotoxic concentration of 78 ug/mL, in the absence of S9 mix and harvested at 53h, had a numerical increase in endo-reduplicated chromosomes and polyploidy.

Sponsor's table: Polypoid data – 25 h of treatment, without S-9, harvested at 53 hours.

Treatment Group	Conc. (µg/mL)	Decoded No.	No. Of Diploid Cells	No. of Polyploid Cells		Frequency of Polyploid Cells	Judge
				Normal	Endoploid		
Dimethyl-sulphoxide	1%	91	300	0	1	0.33	-
		92	300	1	0	0.33	-
BIA 9-1067	20	93	300	1	0	0.33	-
		94	300	0	0	0.00	-
	39	95	300	1	1	0.66	-
		96	300	1	0	0.33	-
	78	97	300	7	4	3.54	+
		98	300	3	4	2.28	+

The positive controls resulted in increased structural aberrations up to 20-fold that of vehicle control-treated colonies and were judged to be positive findings. The studies were determined to be valid, and BIA 9-1067 to be not clastogenic.

Metabolite BIA 9-1079 was tested in vitro for clastogenicity in human peripheral blood lymphocytes, with and without Aroclor-1254 induced rat S9 and a NADPH-generating system. Positive controls were cyclophosphamide and mitomycin C. Test article concentrations ranged from 8 to 4000 µg/mL. BIA 9-1079 was cytotoxic at ≥125 ug/mL. The number of structural aberrations due to treatment with BIA 9-1079 were within the 95% confidence limits for a negative response and considered to be negative. Treatment with the positive control articles resulted in increased structural and numerical aberrations up to 20-fold that of vehicle control-treated colonies and were judged to be positive findings. The studies were determined to be valid, and BIA 9-1079 to be not clastogenic.

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

BIA 9-1067

M and F CD-1 mice were dosed PO at 0 h and 24 h with 0, 0 (vehicle), 450, 900, or 1800 mg/kg/day BIA 9-1067 or 50 mg/kg/day of cyclophosphamide (positive control). Marrow was harvested at 48 hours post-dose. No micronucleus induction was detected in bone marrow erythrocytes of mice dosed with BIA 9-1067; increased micronuclei were seen in mice dosed with cyclophosphamide.

Plasma levels of BIA 9-1067 and BIA 1079 were measured 1 and 2 hours after the second dose in HD M and F. At 24 h post-dose, the concentrations of BIA 9-1067 and metabolite BIA 9-1079 in HDF (74333 ng/mL ng/mL and 679 ng/mL, respectively) exceeded HDM. At 24 h + 1h, the concentration of BIA 9-1067 in HDF was 57700 ng/mL and that of the metabolite was 938 ng/mL, also exceeding HDM. At 24+2h post-dose,

levels of BIA 9-1067 were similar (45033 and 50367 ng/mL, respectively) in M and F. Levels of BIA 9-1079 were 834 and 488 ng/mL in M and F, respectively.

BIA 9-1079

M and F CD-1 mice were dosed PO at 0 h and 24 h with 0 (vehicle), 500, 1000, or 2000 mg/kg/day BIA 9-1079 at 20 mL/kg in 1.0% methylcellulose or, 50 mg/kg/day cyclophosphamide (positive control). Marrow was harvested at 48 hours post initial dose. No micronucleus induction was detected in bone marrow erythrocytes of mice dosed with BIA 9-1079; increased micronuclei were seen in mice dosed with cyclophosphamide.

Plasma levels of BIA 1079 were measured 0.5, 1, and 2 hours after the second dose in HD M and F. The highest concentrations of BIA 9-1079 were at 0.5 hours post-dose in HDM (74800 ng/mL) and in HDF (62467 ng/mL).

8 Carcinogenicity

8.1

Study title: BIA 9-1067: 104-week oncogenicity (gavage) study in the CD1-Mouse

Study no.:	C30740
Study report location:	4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 29, 2009
GLP compliance:	Yes (OECD)
QA statement:	Yes
Drug, lot #, and % purity:	BIA 9-1067/Batch (study days)/% 39129-1-5 (used until day 135)/97.6% 54516-2-1 (days 136 to 182)/100% 54516-2-3 (days 183 to 343)/100% 62321-2-2 (days 344 to 483)/100% 62321-2-3 (days 484 to 651)/100% 62321-2-4 (days 652 to end)/100%
CAC concurrence:	Yes: Minutes March 25, 2009 Dose reduction (email) July 02, 2009 Early termination (email) November 02, 2010

Key Study Findings

No test-article related neoplastic findings were reported.

Early deaths (22 HDM, 22 HDF, 7 MDM, and 8 MDF through Study Week 10) were attributed to the large dosing volume (20 mL/kg) and the particle size in batch 39129-1-5. Multiple subsequent batches with smaller particle size were used from Study Day 136

through the end of the dosing period; subsequent to the change from the original batch, mortality in MD and HD groups was similar to C.

Although the mortality at the HD was similar to C after Study Day 136, losses in the first portion of the study resulted in fewer survivors at the HD, and dosing was stopped in Study Week 85 (HDM) and Study Week 93 (HDF) when the number survivors was reduced to 20/sex/group. Although marked losses of HDF and HDM occurred early in the study, adequate numbers did survive to 104 weeks to support the adequacy of the study.

Adequacy of Carcinogenicity Study
Dose: Based on the results of 90-day dose-ranging study performed at (b) (4) (Study Number B18393), 1000 mg/kg/day of BIA 9-1067 was established as the no-observed-adverse-effect-level (NOAEL). Because dose formulations at higher concentrations were not feasible, 1000 mg/kg was selected as the HD.

Appropriateness of Test Models
The mouse is toxicologically relevant and an appropriate model.

Evaluation of Tumor Findings
No test-article related neoplastic findings were reported.

Methods

Doses:	0 (vehicle), 0 (vehicle), 100, 500/375, or 1000/750 mg/kg/day (reductions in Study Week 10)
Frequency of dosing:	Daily (84 weeks HDM and 93 weeks HDF)
Dose volume:	20 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	HPMC (0.2% in distilled water)
Basis of dose selection:	The HD was the NOAEL in a 90-day study; due to particle size, 1000 mg/kg is the MFD.
Species/Strain:	Mouse - Crl: CD-1 (ICR) BR
Number/Sex/Group:	56 C1, 50 C2, 65 LD, MD, and HD
Age:	6 weeks
Animal housing:	Individual
Dual control employed:	Yes: both HPMC 0.2%
Satellite groups:	TK: 3/sex C and 9/sex dosed groups.
Deviation from study protocol:	Reduced dose in MD and HD (Study Day

Observations and Results

Mortality
Twenty-two HDM, 22 HDF, 7 MDM, and 8 MDF died or were euthanized moribund from Study Day 8; the deaths before Day 135 were largely attributed to the viscosity (particle size: d(90) ≤195.3 µm) and dose volume (20 mL/kg) needed to achieve the high dose.

The particle size of subsequent batches was lower (d(90) ≤25 µm to d(90) ≤69.92 µm) and resulted in mortality similar to C.

Sponsor's table: Mortality in main study and TK animals

Group / Dose Level	Sex	No. of Early Deaths / Group Size	Spontaneous Death [%]	Killed *in extremis* [%]	Mortality Rate [%]
1 / 0 mg/kg/day	Male	15 (17)* / 56	6	9	27 (30)*
	Female	33 / 56	12	21	59
2 / 0 mg/kg/day	Male	18 / 50	7	11	36
	Female	23 (24)** / 50	10	13	46 (48)**
3 / 100 mg/kg/day	Male	24 / 65	16	8	37
	Female	43 / 65	18	25	66
4 / 500/375 mg/kg/day	Male	40 / 65	22	18	62
	Female	40 / 65	20	20	62
5/ 1000/750 mg/kg/day	Male	48 / 65	35	13	74
	Female	50 / 65	28	22	77

* Two animals, nos. 23 and 45, died following trauma
** One animal, no. 384, died following trauma.

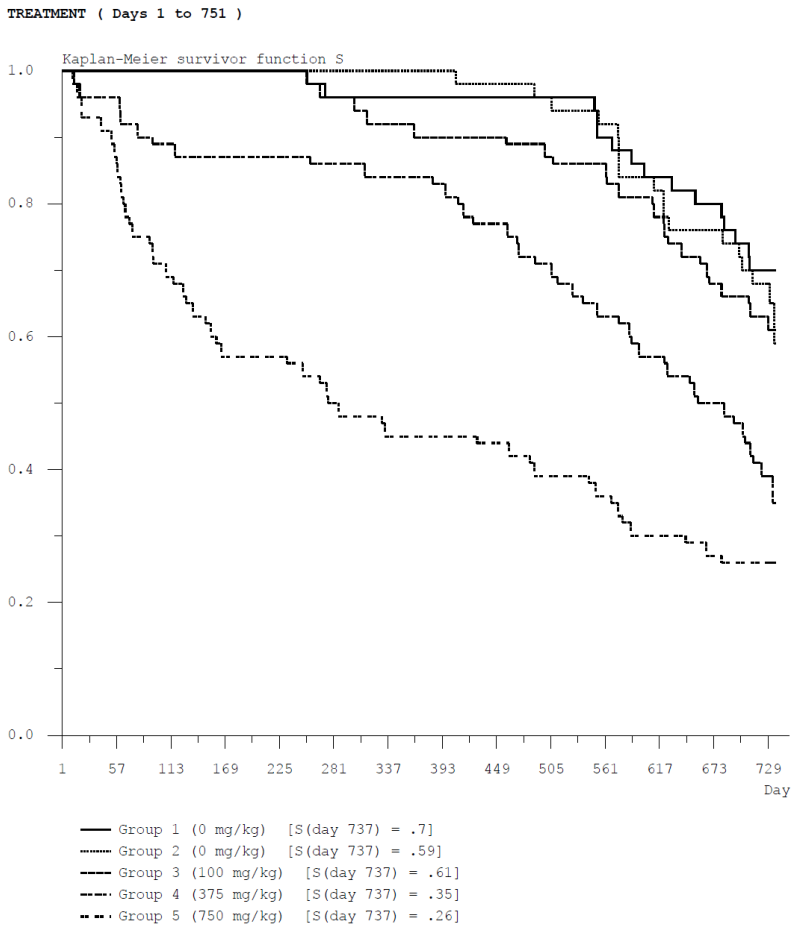
Eleven HDM, 18 HDF, 4 MDM, and 5 MDF were euthanized or found dead from Study Day 8 (HDF) with evidence of gastroenteric dilation and/or esophageal perforations.

At the recommendation of the Executive CAC, the doses were reduced in Study Week 10 in HD (from 1000 mg/kg to 750 mg/kg) and MD (from 500 mg/kg/d to 375 mg/kg/day) groups.

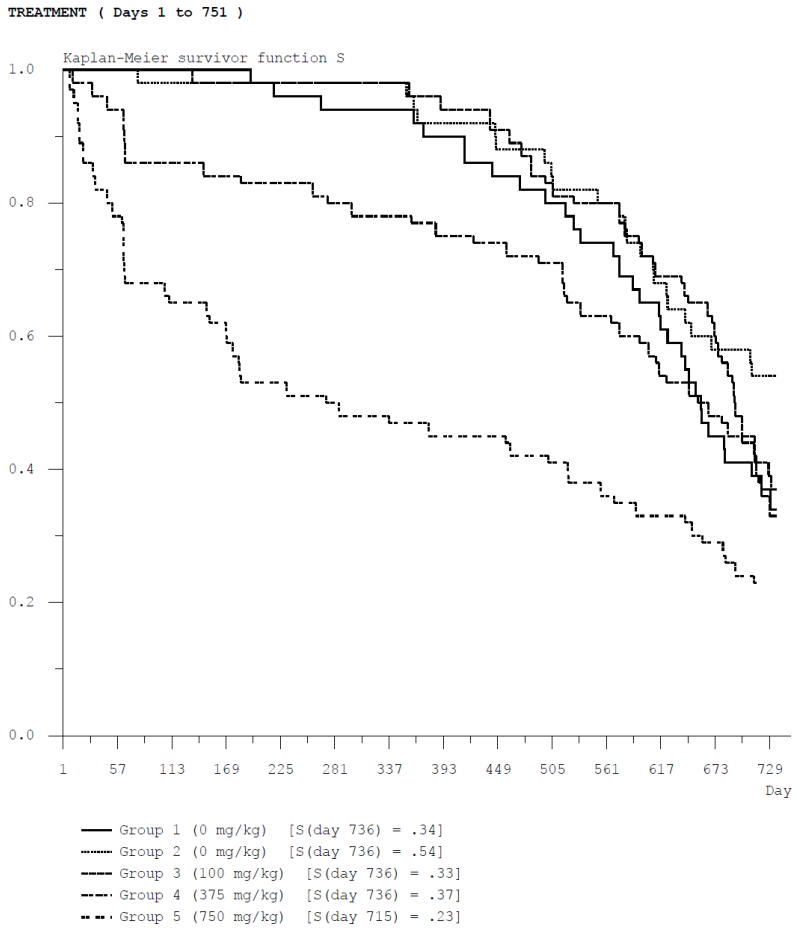
Because of continued losses, attributable to viscosity of the test article and difficulty in administration, the test item batch was changed to a series of batches with smaller particle sizes on Study Day 136.

Although the mortality at the HD was similar to C after Study Day 136, losses in the first portion of the study resulted in fewer survivors in the HD groups. Dosing was stopped in Study Week 85 (HDM) and Study Week 93 (HDF) when survivors/sex/group was reduced to 20 (as recommended by the Executive CAC); 17 HDM and 15 HDF survived to terminal necropsy.

Sponsor's figure: Mortality in M



Sponsor's figure: Mortality in F



Sponsor's table: deaths prior to (until Day 63) and after dose reduction (until Day 135) in MD and HD groups, and after switch to drug batches with smaller particle sizes (after Day 135)

Table 2 Mortality until Day 63*

Group / Dose Level	Sex	No. of Early Deaths / Group Size	Spontaneous Death [%]	Killed *in extremis* [%]	Mortality Rate [%]
4 / 500/375 mg/kg/day	Male	4 / 65	3	1	6
	Female	5 / 65	3	2	8
5/ 1000/750 mg/kg/day	Male	11 / 65	9	2	17
	Female	18 / 65	13	5	28

* One female (no. 384 of group 2) died following trauma. No further deaths occurred in groups 1 to 3.

Table 3 Mortality until Day 135

Group / Dose Level	Sex	No. of Early Deaths / Group Size	Spontaneous Death [%]	Killed *in extremis* [%]	Mortality Rate [%]
2 / 0 mg/kg/day	Male	0 / 50	0	0	0
	Female	1 (2)* / 50	0	1	2 (4)*
3 / 100 mg/kg/day	Male	0 / 65	0	0	0
	Female	1 / 65	1	0	2
4 / 500/375 mg/kg/day	Male	7 / 65	6	1	11
	Female	8 / 65	5	3	12
5/ 1000/750 mg/kg/day	Male	22 / 65	19	3	34
	Female	22 / 65	16	6	34

* One female (no. 384 of group 2) died following trauma. No deaths occurred in group 1.

Table 4 Mortality in Allocation A and B animals after day 135

Group / Dose Level	Sex	No. of Early Deaths / Group Size	Spontaneous Death [%]	Killed *in extremis* [%]	Mortality Rate [%]
1 / 0 mg/kg/day	Male	15 (17)* / 56	6	9	27 (30)*
	Female	33 / 56	12	21	59
2 / 0 mg/kg/day	Male	18 / 50	7	11	36
	Female	22 / 50	10	12	44
3 / 100 mg/kg/day	Male	24 / 65	16	8	37
	Female	42 / 65	17	25	65
4 / 500/375 mg/kg/day	Male	33 / 65	17	16	51
	Female	32 / 65	15	17	49
5/ 1000/750 mg/kg/day	Male	26 / 65	16	10	40
	Female	28 / 65	12	16	43

* Two animals, nos. 23 and 45, died following trauma

Clinical Signs

A consistent dose-related finding of yellow feces was attributed to the presence of drug. From Study Day 8 onward, esophageal reflux during administration was noted in many MD and HD animals and some LD animals. Signs noted before death/euthanasia were weakness, hunched posture, ruffled fur, breathing noises, labored breathing, abdomen swelling, pallor, visible weight loss and reduced body temperature. Similar, but transient, signs were noted sporadically in surviving MD and HD animals prior to eliminating test item batch #39129-1-5.

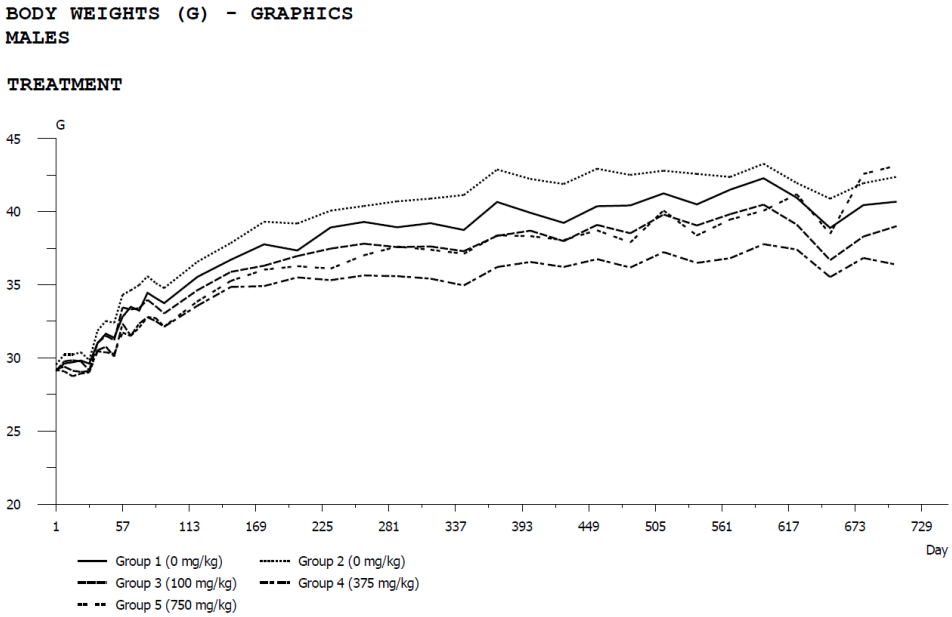
Body Weights

Dose-related decreases in body weight, compared to controls, was reported in males (~9-10% at MD, 5-7% at HD [through Study Week 38])

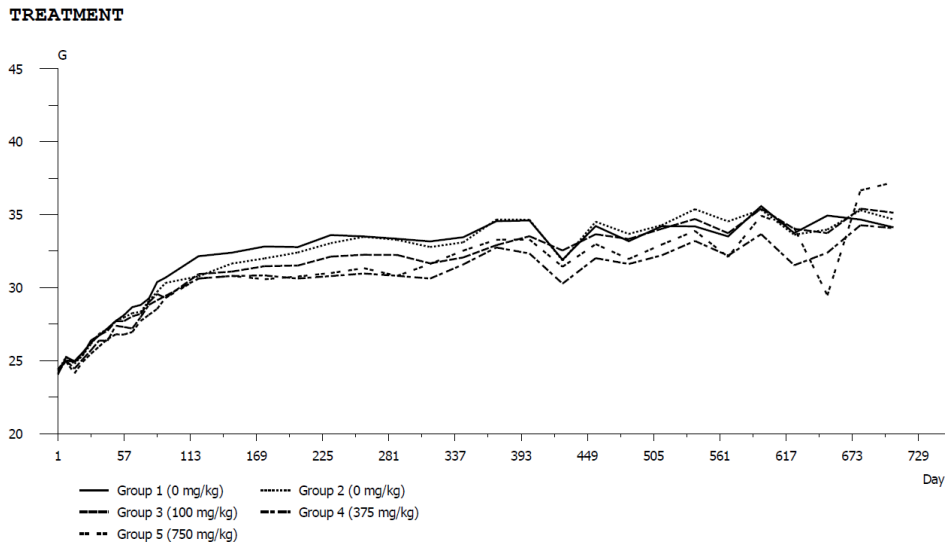
- Males: MD (~9-10%) and HD (5-7% less than CM) in Study Weeks 7,10,12-18, 34, and 38.
- Females: MD (~7-11%) and HD (7-10% [through Study Week 94]).

When dosing was suspended in HDM (Week 85) and HDF (Study Week 93), body weights and body weight gains increased.

Sponsor's figures: Body weights in M



Sponsor's figure: Body weights in F



Food Consumption
No differences among groups were reported.

Hematology
Increased reticulocytes and hemoglobin concentration distribution width was seen in HDM and HDF. Absolute increase in eosinophils was seen in HDM and HDF and in the relative number of eosinophils in MDM and LDM.

Clinical Chemistry
Dose-related increases in glucose (94-97%) and potassium (16-18%) were observed in HDM and HDF.

Urinalysis
A slight increase in the number of erythrocytes in the urine was noted in all dosed F groups but was of no toxicological significance.

Toxicokinetics
BIA 9-1067 and metabolite BIA 9-1079:

BIA9-1067 exposures were higher in F compared to M, at all dose levels and decreased with multiple doses over time in both M and F. (Sponsor's tables, below.)

Table 5 Toxicokinetic Parameters of BIA 9-1067 in Mice

BIA 9-1067		week 13			week 26		
Dose [mg/kg/day]		100	375	750	100	375	750
Male							
C_{max}	[ng/mL]	1750	4720	3630	1150	2460	2370
t_{max}	[h]	1.0	1.0	1.0	1.0	2.0	1.0
AUC_{0-6h}	[ng.h/mL]	3650	11700	14700	2340	8220	9130
$AUC_{0-inf.}$	[ng.h/mL]	4100	14000	30400*	2600	13000	16800*
$t_{½, z}$	[h]	1.7	2.1	5.7	1.6	4.5	4.8
Female							
C_{max}	[ng/mL]	4720	6010	15300	2770	2750	6830
t_{max}	[h]	1.0	1.0	1.0	1.0	2.0	1.0
AUC_{0-6h}	[ng.h/mL]	8120	14400	46200	6280	9680	20400
$AUC_{0-inf.}$	[ng.h/mL]	9590*	16000	71000	9630*	16000	35000
$t_{½, z}$	[h]	1.9*	1.8	2.6	3.3*	5.2	4.4

* Unreliable value (see details in the toxicokinetic appendix)

Table 6 Toxicokinetic Parameters of BIA 9-1079 in Mice

BIA 9-1079		week 13			week 26		
Dose [mg/kg/day]		100	375	750	100	375	750
Male							
C_{max}	[ng/mL]	634	1090	1740	179	226	353
t_{max}	[h]	2.0	2.0	2.0	1.0	6.0	2.0
AUC_{0-6h}	[ng.h/mL]	2910	5400	7510	634	1140	1580
AUC_{0-inf}	[ng.h/mL]	5000	9900	12000	3680*	n.c.	n.c.
$t_{½, z}$	[h]	4.0	4.1	3.3	18*	n.c.	n.c.
Female							
C_{max}	[ng/mL]	609	845	1440	147	160	435
t_{max}	[h]	2.0	2.0	1.0	6.0	6.0	6.0
AUC_{0-6h}	[ng.h/mL]	2920	4300	6630	577	682	1600
AUC_{0-inf}	[ng.h/mL]	n.c.	n.c.	12000	n.c.	n.c.	n.c.
$t_{½, z}$	[h]	n.c.	n.c.	3.6	n.c.	n.c.	n.c.

* Unreliable value (see details in the toxicokinetic appendix)

Table 33 Ratio of Metabolite/Parent Exposure in Male Mice

Ratio of metabolite/parent exposure (Male)				
Parameter	Dose [mg/kg/day]	Parent [µM.h]	Metabolite [µM.h]	Ratio M/P
AUC_{0-6h}	Week 13			
	100	8.83	7.32	0.83
	375	28.4	13.6	0.48
	750	35.7	18.9	0.53
	Week 26			
	100	5.67	1.60	0.28
	375	19.9	2.88	0.14
	750	22.1	3.99	0.18
C_{max}	Week 13			
	100	4.23	1.60	0.38
	375	11.4	2.73	0.24
	750	8.79	4.4	0.50
	Week 26			
	100	2.78	0.45	0.16
	375	5.95	0.57	0.10
	750	5.72	0.89	0.16

Table 34 Ratio of Metabolite/Parent Exposure in Female Mice

Ratio of metabolite/parent exposure (Female)				
Parameter	Dose [mg/kg/day]	Parent [µM.h]	Metabolite [µM.h]	Ratio M/P
AUC_{0-6h}	Week 13			
	100	19.7	7.35	0.37
	375	34.8	10.8	0.31
	750	112	16.7	0.15
	Week 26			
	100	15.2	1.45	0.10
	375	23.4	1.72	0.07
	750	49.3	4.04	0.08
C_{max}	Week 13			
	100	11.4	1.53	0.13
	375	14.5	2.13	0.15
	750	37.0	3.63	0.10
	Week 26			
	100	6.70	0.37	0.06
	375	6.66	0.40	0.06
	750	16.5	1.09	0.07

Organ Weights:
No differences among groups were reported.

Gross Pathology
No test item related changes were reported; however, in animals died or euthanized in extremis:

- 11 HDM, 18 HDF, 4 MDM, and 5 MDF were euthanized or found dead from Study Day 8 (HDF) with evidence of gastroenteric dilation and/or esophageal perforations.
- 25 of 48 HDM, 25 of 50 HDF, 6 of 40 MDM, and 6 of 40 MDF had distension of parts or all of the gastrointestinal system with gas.

Histopathology

Neoplastic
There were no test-article related neoplastic changes. The most common neoplasms were noted in the lungs, liver (males), hemo-lymphoreticular system, Harderian gland, and uterus and were considered to be within the range of the biological variation in mice of this age.

Non Neoplastic
There were no toxicities related to the parent drug or metabolites. Non-neoplastic findings were related to either administration or formulation of the test article. Non-neoplastic changes were largely gross and histologic lesions of the upper respiratory gastrointestinal tracts. The large particle size in the initial batch resulted in a viscous formulation that was administered at a relatively high volume and resulted in post-dosing reflux and substantial morbidity. These findings and interpretation were confirmed by an independent peer review pathologist.

MD and HD: Drug-related degenerative (erosion of the lining epithelium and submucosa) and inflammatory changes with associated exudate, were seen in the nasal cavity, pharynx, larynx, trachea and lungs.

Erosion, inflammation, and exudates were also seen in the airways of some control animals, indicating that 20 mL/kg of the vehicle (0.2% hydroxypropyl methylcellulose) could cause reflux in some animals.

The dosing volume (20 mL/kg/day) was high; gavage administration was reported to be "difficult" and post-dosing reflux was frequently observed at administration. Changes were compatible with gastrointestinal reflux of the dose formulation into the airways leading to degenerative and inflammatory lesions of the affected organs.

Sponsor's table:

Table 8 Lesions in the Airways, Esophagus and Intestinal Tract

	Males						Females				
Group	**1**	**2**	**3**	**4**	**5**		**1**	**2**	**3**	**4**	**5**
Animals Exam.	**56**	**50**	**65**	**65**	**65**		**56**	**50**	**65**	**65**	**65**
Pharynx											
Erosion	1	1		1	10**		1	1	1	1	10**
Plug in lumen	2	2	6	7	6		5	2	4	5	11
Inflammation					4		1	1	1	1	7*
Larynx											
Erosion	5	3	6	15**	27**		2	3	5	10*	17**
Inflammation	4	1	2	1	2				3		7*
Trachea											
Erosion		1	5*	11**	12**		2	4	2	9*	9*
Inflammation	1			3			2		1		3
Lungs											
Alv. Edema		1	2	3	1		3	1		3	3
Alv. Hemorrhage	1	2	6	8*	11**		6	3	5	9	13
Alv. Emphysema		1	2	5	6*		1	1	3	5	2
Bronchopneumonia					1		1		5		2
Bronchi Amorph.		1	2	9**	13**		5	2	6	10	15*
Mat.				3	5*					2	9**
Inflammation											
Nasal Cavity											
Erosion	4		3	10	33**			4	1	9**	28**
Inflammation	6	6	11	33**	39**		2	1	4	27**	33**
Plug	17	23	30	35**	50**		9	15	8	24*	41**
Hyperplasia, Mucosal			5*	15**	7**				2	5*	5*

Esophagus											
Perforation				3	1					2	4
Inflammation	3		1	4	4		2		2	4	5
Traumatic Lesion		1	4	2	6*		1	1		2	5
Amorphous Material		1	1	1	7*		1	4	2	7*	11**
Duodenum											
Dilation					6*					2	10**
Jejunum											
Dilation		1	1		6*					3	12**
Ileum											
Dilation		1			8**					3	11**
Caecum											
Dilation		1								1	7**
Colon											
Dilation		1			6*					2	8**
Rectum											
Dilation											1

Fisher's Exact Test (One-Sided): $p<0.05$*; $p<0.01$**

Peer Review:
An adequate pathology peer review was performed by a qualified toxicologic pathologist, and the final diagnoses were agreed between the peer review pathologist and the study pathologist.

Summary:
There were no drug-related neoplasias. Neoplastic findings were largely of tumors common to aged mice, with no relationship to administration of the test article. The FDA biostatistical review (HeiPei Chen, PhD) and the sponsor's statistical analysis found no tumor incidences different from controls.

Twenty-two HDM, 22 HDF, 7 MDM, and 8 MDF died or were euthanized moribund from Study Day 8 through Study Day 136, with evidence of gastroenteric dilation and/or esophageal perforations and regurgitation/aspiration of the test item/vehicle. At the recommendation of the Executive CAC, the doses were reduced in Study Week 10 in HD (from 1000 mg/kg to 750 mg/kg) and MD (from 500 mg/kg/d to 375 mg/kg/day) groups; the dosing volume remained 20 mL/kg.

Because of continued losses, the test item batch was changed on Study Day 136 to a series of batches with smaller particle size than the original batch. Dosing was stopped in Study Week 85 (HDM) and Study Week 93 (HDF) when the number of survivors was reduced to 20/sex/group. Although the mortality at the HD and MD was similar to C after Study Day 136, losses in the first portion of the study resulted in fewer survivors in the HD groups. 17 HDM and 15 HDF survived to terminal necropsy.

Non-neoplastic changes were largely post mortem findings of gross and histologic lesions of the upper respiratory gastrointestinal tracts. The large particle size in the initial batch resulted in a viscous formulation that caused untoward. These findings and interpretation were confirmed by an independent peer review pathologist.

8.2

Study title: BIA 9-1067: 104-Week oncogenicity (gavage) study in the Wistar rat

Study no.:	C30751
Study report location:	4.2.3.4.1 eDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	APR 14, 2009
GLP compliance:	(b) (4) OECD compliance statement (pg. 1976/4029)
QA statement:	Yes
Drug, lot #, and % purity:	BIA 9-1067; Batches number 54516-2-1 (until Day 193), 54516-2-3 (Days 194 to 364), 62321-2-2 (Days 365 to 500), 62321-2-3 (Days 501 to 665), 62321-2-4 (Day 666 to end) 100% purity (by HPLC) in all batches
CAC concurrence:	Yes (Minutes MAR 25, 2009)

Key Study Findings
No neoplasias or hyperplasias were related to administration of the test-article.

Mortality in HDM, MDM, and MDF were largely attributed to regurgitation/aspiration of test item/vehicle.

Consistent clinical signs were limited to yellow-stained feces, attributed to the presence of test article.

Adequacy of Carcinogenicity Study
Sufficient numbers of animals were dosed for not less than 104 weeks and survived to terminal necropsy.

- 23 HDM (45 %) and 34 HDF (67%)
- 24 MDM (47%) and 31 MDF (61%)
- 34 LDM (67%) and 36 LDF (71%)
- 38 C-2M (74%) and 37 C-2 F (73%)
- 35 C-1 M (67%) and 34 C-1 F (69%)

Appropriateness of Test Models
The Wistar Han rat is toxicologically relevant and an appropriate model.

Evaluation of Tumor Findings
There were no neoplasms attributed to administration of the test article.

Of the 185 animals which died prematurely or were sacrificed *in extremis*, 78 had adenoma of the pars distalis (pituitary). Tumors of the pituitary gland were found at terminal necropsy in 87 animals. The highest incidence (73%) was in LDF.

According to the sponsor's statistical analysis, the "Conventional Peto Test" of all neoplastic lesions with an incidence equal or >5% in at least one sex/dose group revealed negative trends or one-tailed p-values >0.005/>0.025 for common/rare neoplasms, respectively.

The FDA reviewing biostatistician (HePei Chen, PhD) found: "No statistically significant dose response relationship or pairwise comparison in tumor data was noted in the reviewer's analysis for both male and female rats."

Methods

Doses:	0 (vehicle), 0 (vehicle), 100, 500, 1000 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	20 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Hydroxypropyl methylcellulose (0.2% in bi-distilled water)
Basis of dose selection:	The HD was the NOAEL in a 90-day study; due to particle size, 1000 mg/kg is the MFD.
Species/Strain:	Wistar, [HanRcc: WIST(SPF)]
Number/Sex/Group:	51
Age:	6 weeks
Animal housing:	Individual
Dual control employed:	Both control groups received vehicle only
Satellite groups:	TK: C 3/sex; Dosed 9/sex
Deviation from study protocol:	Minor, no impact on study results

Observations and Results

Mortality

A total of 184 main study animals died spontaneously or were killed *in extremis* during treatment and there was one accidental death in a CF. Regurgitation/aspiration of the test item/vehicle were found to be the cause of death or moribund condition in 1 CM-1,1 CF-1, 8/51 MDM, 3/51 MDF, 13/51 HDM, and 5/51 HDF.

Survived to terminal necropsy:
23 HDM (45%), 34 HDF (67%); 24 MDM (47%), 31 MDF (61%); 34 LDM (67%), 36 LDF (71%); 38 C-2M (74%), 37 C-2F (73%); 35 C-1M (67%);34 C-1F (69%).

Sponsor's table:

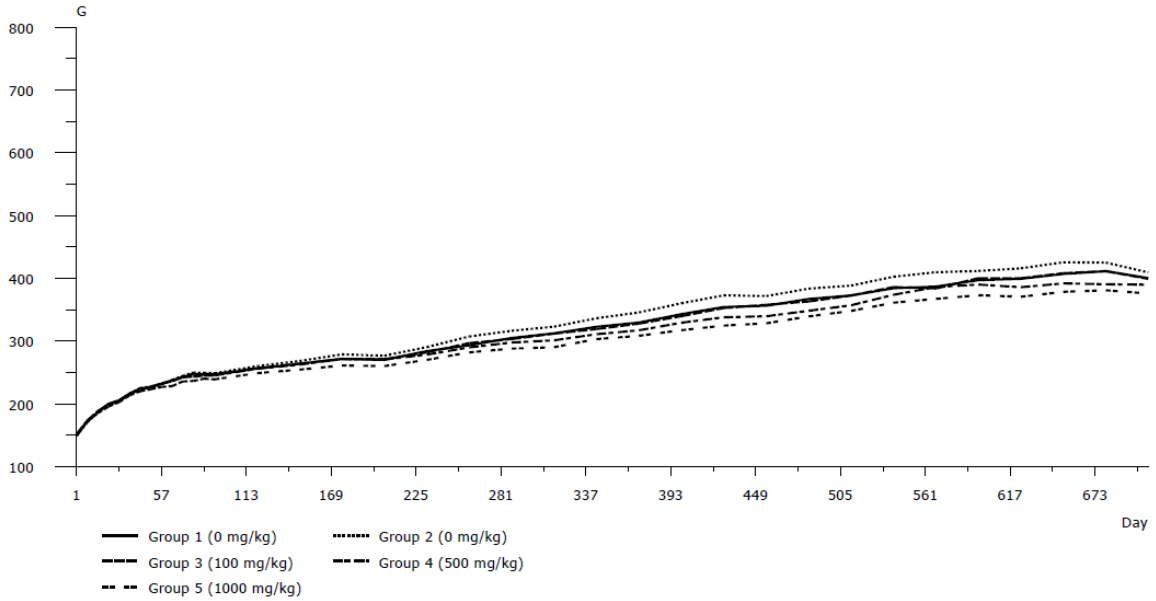
Group / Dose Level	Sex	No. of Early Deaths / Group Size	Spontaneous Death [%]	Killed *in Extremis* [%]	Mortality Rate [%]
1 / 0 mg/kg/day	Male	16 / 51	6	10	31
	Female	17* / 51	5	12	33
2 / 0 mg/kg/day	Male	13 / 51	7	6	25
	Female	14 / 51	7	7	27
3 / 100 mg/kg/day	Male	17 / 51	9	8	33
	Female	15 / 51	5	10	29
4 / 500 mg/kg/day	Male	27 / 51	14	13	53
	Female	20** / 51	8	12	39
5 / 1000 mg/kg/day	Male	28 / 51	23	5	55
	Female	17 / 51	9	8	33

* One further female (no. 314) died due to an accident.
** One further female (no. 505) from allocation B died spontaneously.

Clinical Signs
Yellowish discoloration of feces was noted in all MD and HD male and females during the dosing period, with some variability in the incidence. The sponsor attributed this finding presence of the (yellow) test item.

Body Weights
The mean body weight of HDF at the end of treatment was 6.1% lower than that of the CF. This effect, although slight, was considered to be test item-related.

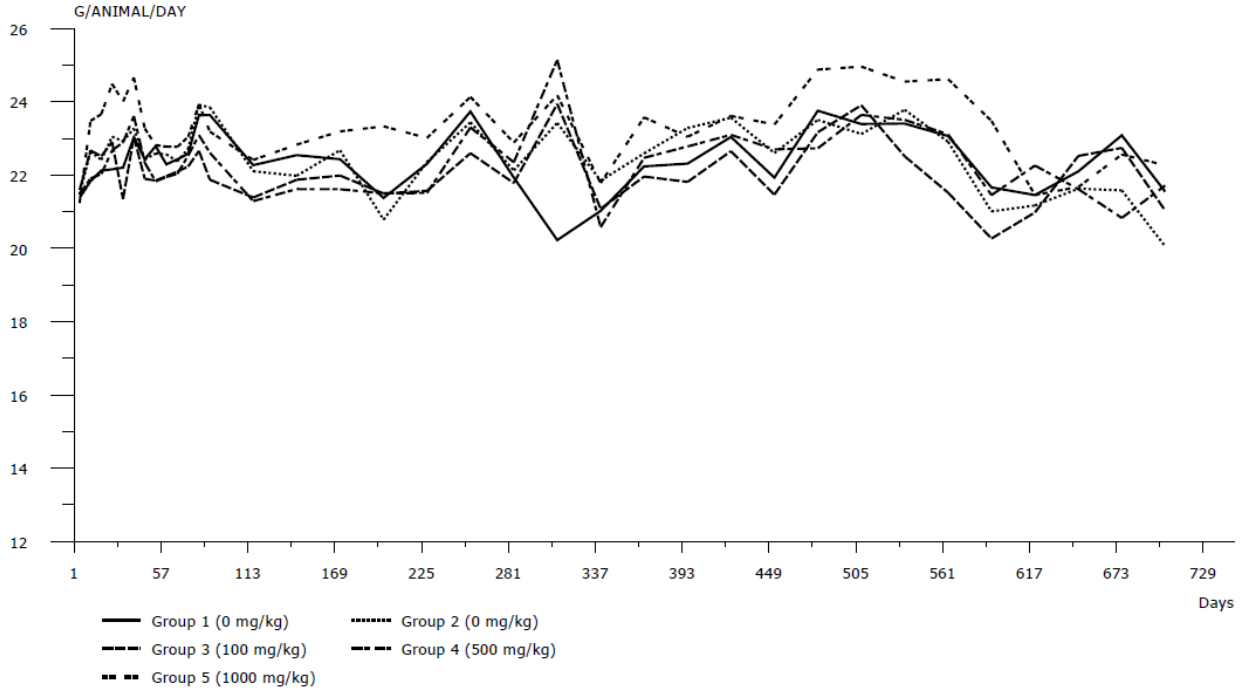
Sponsor's figure: Body weights in F



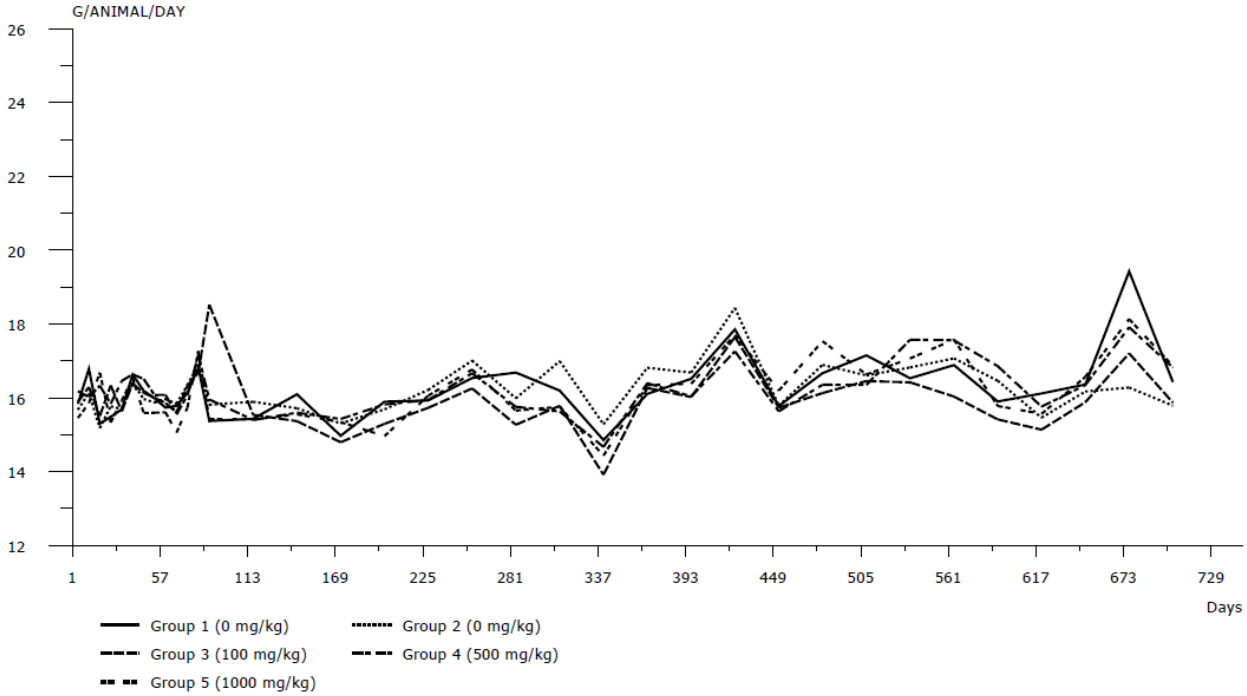
Food Consumption

The overall food consumption until Study Week 101 in HDM was 4.0% higher than in CM. Food consumption in HDF was 3.8% higher than in controls.

Sponsor's figure: Food consumption in M



Sponsor's figure: Food consumption in F



Ophthalmoscopy

No test article related findings were reported.

Clinical chemistry
Non-adverse (slight) test-item related changes, compared to C, were increased prothrombin time in HD and MDF, increased AST in HDM, and increased LDH, phosphorus, and albumin in HDF

There was a slight decrease in urine specific gravity at all doses in males.

Organ weights
Compared to C there were decreased absolute spleen weights in HD, MD and LDF and decreased weights in M and F in all dosed groups.

Gross Pathology
The most frequent findings were of brain compression resulting from diffusely enlarged pituitary glands and /or a nodule in the pituitary gland.

Histopathology
All findings were either common in rats of this strain and age or were associated with the gavage administration of the test item formulation.

Neoplastic:
There were no drug-related neoplasias.
Of the 185 animals which died prematurely or were sacrificed *in extremis*, 78 had adenoma of the pars distalis (pituitary). Tumors of the pituitary gland were found at terminal necropsy in 87 additional animals. The highest incidence (73%) was in LDF

Non Neoplastic
There were no toxicities related to the parent drug or metabolites. The cause of death or moribund condition in 1 CM, 1 CF, 8 MDM, 3 MDF, 13 HDM, and 5 HDF was related to gavage administration and viscosity of the formulation. Changes seen at postmortem were of foreign material (characteristic of gavage or regurgitation of test compound) in nose, pharynx, trachea, larynx and/or lungs and broncho-pneumonia/aspiration pneumonia, and/or inflammation of parts of the upper respiratory tract.

Peer Review
An adequate pathology peer review was performed by a qualified toxicologic pathologist, and the final diagnoses were agreed between the peer review pathologist and the study pathologist.

Toxicokinetics:
Data were collected in satellite animals (3/sex C and 9/sex dosed groups) on Study Day 1, after Study Week 13, and after Study Week 26.

BIA 9-1067 (parent): The overall 10-fold dose-range from 100 to 1000 mg/kg/day resulted in less than dose proportional $AUC_{0\text{-}t}$ and C_{max} ratios in both sexes at all three time points.

Exposure (AUC) was lower in F than M, except after Week 26 when exposures in HDF slightly exceeded those in HDM. There was some accumulation (AUC) in M and F, at all dose levels from Study Week 1 through Study Week 26.

Sponsor's table:

BIA 9-1067		Week 1			After Week 13			After Week 26		
Dose [mg/kg/day]		100	500	1000	100	500	1000	100	500	1000
Male										
C_{max}	[ng/mL]	2340	8110	12300	5060	12200	11400	4410	11600	14700
t_{max}	[h]	2.0	1.0	1.0	0.50	0.50	1.0	0.50	0.50	0.50
AUC_{0-t}	[ng.h/mL]	8830	23000	61900	15800	55000	92200	13900	43300	58400
$AUC_{0-inf.}$	[ng.h/mL]	n.c.	32700*	62300	15900	56000	115000	14300	45900	63800
$t_{½, z}$	[h]	n.c.	3.0	3.1	3.0	4.2	10	4.0	6.0	6.7
Female										
C_{max}	[ng/mL]	1550	8640	13800	4270	12700	9570	3270	9600	28700
t_{max}	[h]	1.0	1.0	1.0	1.0	0.50	1.0	1.0	0.50	0.50
AUC_{0-t}	[ng.h/mL]	4500	20400	30400	8700	40800	31400	8120	33100	86900
$AUC_{0-inf.}$	[ng.h/mL]	5980*	24700	36900*	8990	42300	31500	8770	33200	87200
$t_{½, z}$	[h]	2.6	2.1	2.1*	1.1	5.2	2.9	1.5	2.9	2.9

* unreliable

n.c. not calculated

BIA 9-1079 (metabolite):
Exposure (AUC) was less than dose-proportional, in Study Week 1 and was generally dose-proportional in HDM and HDF after Study Weeks 13 and 26.

There was little difference in exposures (AUC) between M and F; slight accumulation was observed in HDM and HDF from Study Week 1 to after Study Week 26.

Sponsor's table:

BIA 9-1079		Week 1			After Week 13			After Week 26		
Dose [mg/kg/day]		100	500	1000	100	500	1000	100	500	1000
Male										
C_{max}	[ng/mL]	592	586	546	806	480	1030	399	430	651
t_{max}	[h]	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	24
AUC_{0-t}	[ng.h/mL]	5990	6190	7730	8020	8560	20700	5220	7150	14700
Female										
C_{max}	[ng/mL]	335	350	648	250	358	1290	211	386	841
t_{max}	[h]	6.0	6.0	6.0	6.0	6.0	6.0	2.0	2.0	1.0
AUC_{0-t}	[ng.h/mL]	2980	3700	7060	2870	5710	13000	2650	4510	10800

Stability and Homogeneity
BIA 9-1067 was in the in the range of 83.5 to 109.8% The homogeneous distribution of BIA 9-1067 did not deviate more than 10.8% from the corresponding mean.

The test item was found to be stable and homogeneous at 4 hours or 8 days at room temperature.

Summary:
There were no drug-related neoplasias. Neoplastic findings, largely of the pituitary gland, were common to aged rats and without relationship to dose or drug. The FDA biostatistical review (HeiPei Chen, PhD) and the sponsor's analysis found no differences in tumor incidence from controls.

Sufficient numbers of animals were dosed for not less than 104 weeks and survived to terminal necropsy. Premature deaths or euthanasia in HDM, HDF, MDM, and MDF were largely due to regurgitation and/or inhalation of gavaged test article. Histologic evidence of foreign material (characteristic of gavage or regurgitation of test compound) was found in nose, pharynx, trachea, larynx and/or lungs. Broncho-pneumonia/aspiration pneumonia, and/or inflammation of the upper respiratory tract was evidence of inhaled test article.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development (Rat)

Study title: Fertility and Early Embryonic Development Study in the Rat

Study no.:	FLG0052
Study report location:	4.2.3.5.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	SEP 11, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Opicapone/39129-1-12/97.6%

Key Study Findings

Yellow/orange stained bedding and feces attributed to presence of yellow crystalline drug
NOEL in M and F on fertility = 1000 mg/kg/day

Methods

Doses:	0, 150, 375, 1000 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Suspension in 0.2 % w/v hydroxypropyl methylcellulose
Species/Strain:	Rat Crl:WI (Han)
Number/Sex/Group:	20
Satellite groups:	NA
Study design:	Males were dosed for 28 days prior to pairing, during pairing, and through the day before necropsy (~ 14 days post-female necropsy). Females were dosed for 14 days prior to pairing, during pairing, and through Gestation Day 6
Deviation from study protocol:	Minor, with no effect on study results.

Observations and Results

Mortality
Death in one CM and in one LDM was attributed to gavage error.

Clinical Signs:
Clinical signs were limited to yellow/orange stained feces and bedding.

Body Weight
There was a slight reduction in body weight gain in HDF prior to pairing. No adverse changes were attributed to administration of the test article.

Food Consumption
There were no differences among groups.

Toxicokinetics
NA

Stability and Homogeneity
Formulations from Study Weeks 1 and 8/9 were demonstrated to be homogenous (within 7 % of the nominal concentrations).

Necropsy
No test article-related gross findings were reported.

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)

No test-article effect on mating, fertility, or estrous cycling, the group-mean number of corpora lutea or implantations, or on early embryonic development were reported.

Sponsor's tables:

Table 13 - Time course of mating

(Page 1 of 1)

Group : 1 2 3 4

Test article : Control BIA 9-1067

Dose (mg/kg/day) : 0 150 375 1000

Group	Number paired	N	Number of females mating on day after pairing 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Mean number# of days taken to mate ± S.D
1	20	20	2	6	4	8												2.9 ± 1.1
2	20	20	2	7	7	4												2.7 ± 0.9
3	20	20	1	5	9	4								1				3.3 ± 2.2
4	20	20	2	8	6	4												2.6 ± 0.9

N = number of animals in mean
# = statistically analysed

Table 12 - Group mean number of oestrus cycles - pre-pairing

(Page 1 of 1)

Group : 1 2 3 4

Test article : Control BIA 9-1067

Dose (mg/kg/day) : 0 150 375 1000

Group	N	Mean number of complete oestrous cycles ± S.D Pre-mating dosing (11 days)
1	20	2.0 ± 0.2
2	20	2.0 ± 0.0
3	20	1.9 ± 0.4
4	20	1.8 ± 0.5

N = number of animals in mean

Table 15 - Group mean fertility and mating data

(Page 1 of 1)

Group : 1 2 3 4

Test article : Control BIA 9-1067

Dose (mg/kg/day) : 0 150 375 1000

Group	Sex	Number paired	Number mated	Number fertile	Copulation index#	Fertility index#
1	M	20	20	20	100.0	100.0
2	M	20	20	20	100.0	100.0
3	M	20	19	18	95.0	94.7
4	M	20	20	20	100.0	100.0

Group	Sex	Number paired	Number mated	Number fertile	Copulation index#	Fertility index#
1	F	20	20	20	100.0	100.0
2	F	20	20	20	100.0	100.0
3	F	20	20	19	100.0	95.0
4	F	20	20	20	100.0	100.0

= statistically analysed

Table 17 - Group mean uterine / implantation data

(Page 1 of 1)

Group : 1 2 3 4

Test article : Control BIA 9-1067

Dose (mg/kg/day) : 0 150 375 1000

	Group 1	Group 2	Group 3	Group 4
Number of females with implantations at scheduled kill	20	20	19	20
Number of corpora lutea	277	281	256	261
Mean number per female#	13.9	14.1	13.5	13.1
Standard deviation	1.1	1.4	2.1	1.6
Number of implantations	253	252	234	235
Mean number per female#	12.7	12.6	12.3	11.8
Standard deviation	2.5	2.7	3.2	2.3
Mean % pre-implantation loss#	9.1	9.7	9.6	10.0
Number of early embryo/foetal deaths	13	17	19	11
Number of dead embryos	0	0	0	0
Mean % post-implantation loss#	5.1	6.7	8.8	4.6
Number of live embryos	240	235	215	224
Mean number per female#	12.0	11.8	11.3	11.2
Standard deviation	2.6	2.6	3.7	2.3
Mean % of implantations	94.9	93.3	91.2	95.4

= statistically analysed

9.2 Developmental toxicity in the rat

9.2.1

Oral (Gavage) Developmental Toxicity Dose Range Finding Study in the Rat
Study No. FLG0044
(b) (4)

Pregnant Crl:WI (Han)rats, 5/group were dosed PO gavage with 0, 100, 300, or 1000 mg/kg/day BIA 9-1067 from GD 6 to 17. Satellite TK groups were of 3C, 9/dosed group.
The NOAEL for maternal toxicity was 1000 mg/kg/day.
The NOAEL for embryo fetal developmental effects was 1000 mg/kg/day.

Sponsor's table: mean TK for BIA 9-1067

Group	6	7	8
Dose (mg/kg/day)	100	300	1000
Day 6			
Cmax (ng/mL)	4090	9530	36200
Tmax (hr)	1	1	1
AUClast (hr*ng/mL)	10100	31200	98500
Tlast (hr)	8	12	24
Day 17			
Cmax (ng/mL)	5900	14200	25300
Tmax (hr)	1	1	1
AUClast (hr*ng/mL)	15100	33800	87600
Tlast (hr)	8	12	12

9.2.2

Study title: Oral (Gavage) Developmental Toxicity Study in the Rat

Study no:	FLG0045
Study report location:	4.2.5.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	SEP 18, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Opicapone/39129-1-12/97.6%

Key Study Findings
No significant external or internal fetal abnormalities were reported.

NOAEL (Dam): HD (1000 mg/kg/day)
NOAEL (Fetus): HD (1000 mg/kg/day)

Methods

Doses:	0 (vehicle), 150, 375, 1000 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.2 % w/v hydroxypropyl methylcellulose
Species/Strain:	Rat Crl:WI (HAN)
Number/Sex/Group:	20 timed-mated F rats
Satellite groups:	NA
Study design:	Dosed GD 6-17, Necropsy on GD 20
Deviation from study protocol:	Minor, with no impact on study results

Observations and Results

Mortality
None

Clinical Signs
Red/yellow feces and bedding, due to color of test article, was seen in all three dose groups.

Body Weight
No test article-related effects were reported. 2 HDF lost 26 and 33 g on gestation days 7-8: subsequent body weight gain in those two animals was similar to C.

Food Consumption
No test article related effects were reported.

Stability and Homogeneity
Formulations were within 98 % to 107 % of nominal and homogenous.

Necropsy
No test article-related effects were reported.
No test-article effects on corpora lutea, implantations, live fetuses, or pre-post implantation losses were reported.

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

Dose	mg/kg/day	0	150	375	1000
	N	20	20	20	20
	Live fetuses present	19	19	19	18
	Not pregnant*	1	1	1	2
	*Implantation scars	none	none	none	none

Offspring (Malformations, Variations, etc.)
No test-article effects on mean litter weight, mean fetal weigh, mean placental weight or M:F ratio were reported. No drug-related increases in major abnormalities were reported.

In HD litters, the incidence of the variant left-sided umbilical artery was 24.3% compared to 17.5% in C and the range of 13.9 to 23.3% in the laboratory historical control database.

The finding of fetuses with frontal bone uni- or bilateral uneven ossification was limited to HD (3.5%). The incidence in the laboratory control animal database was reported to be 0-5.6%.

The incidence of ossification of digital phalanges in the MD (34.5%) and HD (40%) was greater C (25.4%) but within the laboratory historical control range of 16-45%.

9.3 Embryofetal development (rabbit)

In a dose-range finding gavage study (FLG0046) in mature non-pregnant New Zealand White female rabbits administered 250, 375, 425, or 500 mg/kg/day, marked inappetence and body weight loss precluded doses in excess of 375 mg/kg/day.

Sponsor's table: TK of BIA 9-1067 at 425 mg/kg/day PO

Test Article/Metabolite	BIA 9-1067
Day 1	
Mean C_{max} (ng/mL)	738
Mean T_{max} (hr)	4
Mean AUC_{0-24} (ng.hr/mL)	5810
Metabolite to Parent ratio of AUC_{0-24}	
Day 7	
Mean C_{max} (ng/mL)	1510
Mean T_{max} (hr)	1
Mean AUC_{0-24hr} (ng.hr/mL)	14300
[a]Time Dependence of AUC_{0-24hr}	2.46
Metabolite to Parent ratio of AUC_{0-24}	

[a]Ratio of Mean AUC_{0-24hr} on Day 7 to Day 1

In a dose-range finding gavage study (FLG0047), pregnant New Zealand White rabbits were administered 150, 250, or 375 mg/kg/day from gestation day (GD) 6 through GD 18. Inappetence and body weight loss led to the death of 4 of 5 of HD and 2 of 5 MD animals. The NOEL for maternal toxicity was 150 mg/kg/day. No effect on pregnancy status was noted in surviving MD or LD animals. Based on these data, the HD for the main study was recommended to be between 150 and 250 mg/kg/day.

Study title: Oral (gavage) developmental toxicity study in the rabbit

Study no:	FLG0049
Study report location:	4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	OCT 6, 2008
GLP compliance:	Yes (OECD)
QA statement:	Yes
Drug, lot #, and % purity:	BIA 9-1067/39139-1-12/97.6%

Key Study Findings

Within the first week, one MD and 2 HD dams were euthanized for persistent and severe inappetence.

There were no test article-related effects on pregnancy parameters or embryofetal development in survivors.

Because of death at the MD, the maternal NOAEL was 100 mg/kg/day.
The fetal NOAEL was a maternal dose of 225 mg/kg/day.

Methods

Doses:	C (vehicle), 100, 175, or 225 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	8mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.2% hydroxypropyl methylcellulose
Species/Strain:	Rabbit: HsdlF:NZW
Number/Group:	20
Satellite groups:	TK: 3/group
Study design:	Dosed from GD 6 through GD 18 TK of BIA 9-1067 and metabolite BIA 9-1079 Euthanized on GD 28 and pregnancy, number of corpora lutea, and number and distribution of implantations noted. Fetuses examined for external, visceral, and skeletal abnormalities.
Deviation from study protocol:	Minor, with no impact on study outcome

Observations and Results

Mortality

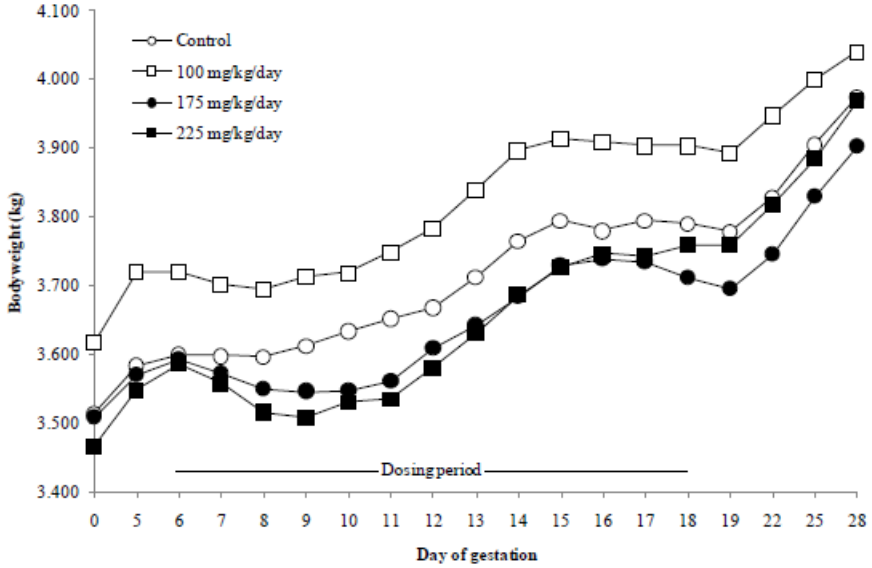
One MD and 2 HD females were euthanized for persistent and severe inappetence during the first week of dosing. One HD female was euthanized on Study Day 27 with a torsion of the right uterine horn. One HD TK female was euthanized for a fractured ischium.

Clinical Signs

Findings consisted of sporadic reduction in food consumption at the MD and HD and some reduced or liquid feces toward the end of the dosing period.

Body Weight
Significant body weight loss was noted at the HD during the first week of dosing. Subsequent body weight gain was similar among groups.

Sponsor's figure:



Food Consumption
During the first week of dosing, food consumption was reduced at the MD and HD; subsequent consumption was similar among all groups.

Toxicokinetics
Marked interindividual variability was greater for parent than for metabolite. Mean exposures increased with dose in an approximately proportional manner. Exposure to metabolite exceeded parent, up to10 fold, at the LD and MD.

Sponsor's table:

Compound	Dose	Gestation	Tmax	Cmax	AUClast	Tlast	Cmax/D	AUClast/D
	mg/kg	Day	hr	ng/mL	hr*ng/mL	hr	(ng/mL)/(mg/kg)	(hr*ng/mL)/(mg/kg)
BIA 9-1067	100	6	1	200	713	8	2.00	7.13
		18	1	367	1020	8	3.67	10.2
	175	6	1	308	1030	8	1.76	5.88
		18	1	261	789	8	1.49	4.51
	225	6	1	659	2610	12	2.93	11.6
		18	1	806	4520	24	3.58	20.1
BIA 9-1079	100	6	1	1500	7300	24	15.0	73.0
		18	1	1070	7070	24	10.7	70.7
	175	6	1	1690	10200	24	9.66	58.4
		18	1	1340	8850	24	7.66	50.6
	225	6	1	2130	14000	24	9.47	62.1
		18	1	1250	13600	24	5.56	60.4

Stability and Homogeneity
Test article formulations were reported as homogenous in the vehicle and stable for 7 days when stored at room temperature. Formulation concentrations of 10, 30 and 100 mg/mL were stable for 10 weeks when stored at -20^0 C.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)
There were no differences among groups for pregnancy, mean numbers of implantations, live fetuses, or pre- and post-implantation losses.

Offspring (Malformations, Variations, etc.)
There were no test article-related major abnormalities. One HD fetus had major abnormalities of the skull. No major abnormalities were found at the MD. Two LD fetuses had exencephaly with associated skull bone malformation. Given the lack of dose-relationships, the major abnormalities were considered to be incidental.

Minor abnormalities of runted fetuses were reported in all three dose groups. There were no runted fetuses in the concurrent C and there were 1 LD (0.5 %), 2 MD (1.0 %) and 1 HD (0.6 %) runted fetuses: the incidences were within the laboratory historical control range of 0.0 % – 2.5 %. Increased numbers of minor abnormalities, notably in the number of ribs (13) and thoracic vertebrae (13), were seen at all doses and the number of affected fetuses was significantly higher at the HD (47% of fetuses in 13/15 litters examined), compared to C (38% of fetuses in 15/20 litters examined). The number of affected fetuses was within the laboratory historical control range of 25-64% at all doses.

Sponsor's table: Statistically significant minor abnormalities and variations
Maternal dose: 0 (Group 1), 100 (Group 2), 175 (Group 3) or 225 (Group 4) mg/kg/day

Foetal Findings	Type	Background Data (%)	Group 1 number of foetuses (%)	Group 2 number of foetuses (%)	Group 3 number of foetuses (%)	Group 4 number of foetuses (%)
Visceral, Thoracic cavity – common carotid artery-uni- or bilateral: arising form innominate artery	variant	2.3 – 16.6	6 (3.9)	6 (5.5)	17* (10.7)	10* (7.8)
Skull – maxilla- uni- or bilateral: incomplete ossification	minor	1.3 – 44.7	11 (14.7)	15 (22.21)	18 (24.1)	17* (25.4)
Hyoid:cornua bent	minor	0.0 – 12.0	0 (0.0)	3 (6.7)	2 (2.6)	4* (6.1)
Vertebra – number of pre-sacral vertebra: 27	minor	3.9 – 28.7	6 (5.7)	9 (6.6)	22* (16.1)	9* (11.4)
Number of thoracic vertebra: 13	variant	25.9 – 64.3	37 (27.7)	48* (42.7)	56* (39.2)	49** (46.7)
Number of lumbar vertebra: 6	variant	16.6 – 44.8	32 (22.7)	42 (38.5)	36 (24.2)	42* (37.0)
Number of ribs:13	variant	25.9 – 64.3	37 (27.7)	48* (42.7)	56 (39.2)	49** (46.7)
One or more sternebra: misshapen or misaligned	minor	0.0 – 6.2	0 (0.0)	0 (0.0)	3* (2.1)	2* (1.9)
Phalanges: one or more digit incomplete ossification	variant	15.1 – 60.6	9 (8.4)	16* (16.0)	25* (18.0)	13*(13.0)

Statistical significance relates to the number of foetuses and not to the percentage of foetuses with an abnormality or variation. * = $p<0.05$, ** = $p<0.01$

9.4 Prenatal and Postnatal Development (Rat)

Study title: Oral (gavage) pre-and post-natal developmental toxicity study in the rat.

Study no:	FLG0053
Study report location:	eDR 4.2.3.5.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	OCT 20, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BIA 9-1067/39129-1-10/97.9%

Key Study Findings

Maternal clinical signs were limited to yellow stained feces (indicating presence of test article) and bedding.

In F1 offspring of MD and HD dams, there was a significant increase in the percentage of pups with eyes open on Lactation Day 15 compared to C.

Two F1 HDM were found moribund and euthanized on PND 34 or PND 62. A reduced clinical condition, brown peri-anal staining, and distended abdomen were causes for euthanasia of the first animal, on PND 34. Gross findings were of a distended GI tract

with green fluid in the colon and mesenteric lymphadenopathy. The second HDM was found hunched, with reduced activity and rapid breathing, and was euthanized. There were no post mortem findings upon gross examination.

In post-weaning week 1 there was a lower mean body weight gain in the F1 HDM when compared to F1 CM.
In gestation days 0-7, the pregnant F1 HDF had higher mean body weight gains when compared to F1 CF.

The maternal NOAEL was 1000 mg/kg/day.
The NOAEL for F1 development was 375 mg/kg/day (based on death in 2 F1 HDM).

Methods

Doses:	0 (vehicle), 150, 375, or 1000 mg/kg/day
Frequency of dosing:	Daily: Gestation Day (GD) 6 to Lactation Day (LD) 20
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.2 % w/v hydroxypropyl methylcellulose
Species/Strain:	Crl:WI(HAN)
Number/Sex/Group:	22 mated F - Gestation Day (GD) 4
Satellite groups:	NA
Study design:	Dosed from GD 6 to Lactation Day (LD) 20
Deviation from study protocol:	None affecting study outcome.

Observations and Results

F_0 Dams

Survival:	Necropsies on LD 21
Clinical signs:	MD, HD (and 2 LD): Yellow stained nesting material and reddened feces (attributed to presence of drug, which is yellow)
Body weight:	From GD 7 to 12, HDF had a greater weight gain than C. From GD 9 to 12, HDF had lower weight gain than C. However, over the duration of gestation there were no differences among groups. From LD 10-21 the HD gained significantly more than C.
Food consumption:	There were no differences among groups during gestation. There was increased food consumption in HDF during lactation.
Uterine content:	No treatment effect reported on the mean number of pups born compared to the number of implantation scars per female.
Necropsy observation:	Gross only: No differences among groups
Toxicokinetics:	NA
Other:	NOAEL: 1000 mg/kg/day

F_1 Generation

Survival:	Two HDM were euthanized in moribund condition at 34 days and 62 days of age. In the first, there was evidence of abnormal GI contents (green fluid in the cecum) and enlarged mesenteric lymph nodes; there were no gross or microscopic abnormalities at post mortem exam of the older HDM. There were no other survival/mortality differences or gross or microscopic differences among the groups.
Clinical signs:	No test article related effects were reported.
Body weight:	Slight or transient increased body weight in HDM. The mean body weight at balanopreputial separation in HDM and MDM tended to be higher than CM.
Food consumption:	No test article-related effects were reported.
Physical development:	At the MD and HD, there was a significant increase in the percentage of pups with eyes open on Day 15 of age when compared with the Controls
Neurological assessment:	E-maze: There were no differences from C. Auditory response: There were no differences from C. Rotarod tests: There were no differences from C.
Reproduction:	Days to balanopreputial separation was increased by 1 day (HDM) or 2 days (MDM). The delay was within historical controls for the laboratory.
Fertility:	No differences from C in copulation plugs
Pregnancy:	No differences from C in number of pregnant animals/groups.
Necropsy:	In offspring culled on LD 21, F1 M and non-pregnant F1 F at 14 days after mating, F1 M at 2-weeks post-mating, or F1 F at GD 13, there were no differences from controls.

Toxicokinetics: See table below

Sponsor's table: Exposure (AUC) margins of BIA 9-1067 (opicapone) to human at the MRHD

Table 7: Safety Margins of Opicapone AUC Exposure at NOAEL Doses in Reproductive and Developmental Toxicity Studies Relative to Human AUC at the 50 mg/day Therapeutic Dose

Study Type (species)	Dosing Duration	NOAEL Dose (mg/kg/day)	AUC_{0-t} (µg×h/mL)	Safety Margin[a]
Fertility and early embryonic development (rat)	M: 2 weeks before mating through Day 57 F: 2 weeks before mating through GD6	1000	M: 121[b] F: 122[b]	M: 59.9 F: 60.4
Embryo-fetal development (rat)	GD6 – GD17	1000	87.60[c]	43.4
Embryo-fetal development (rabbit)	GD6 – GD18	225	4.52[d]	2.2
Pre/postnatal development (rat)	GD6 – LD21	NOAEL for F1 generation: 375 mg/kg/day	39.6[e]	19.6

AUC=area under the plasma concentration=time curve; AUC_{0-t}=area under the plasma concentration-time curve from time zero to the time of the last measurable concentration; F=female; F1=offspring of the parent generation; GD=Gestation Day; M=male; NOAEL=no observed adverse effect level; TK=toxicokinetic(s).

[a] The mean AUC_{0-t} at the human oral therapeutic dose of 50 mg/day=2.02 µg×h/mL (Clinical Study NBI-OPC-1706).

[b] Supportive TK from 13-week rat repeat-dose study (Report D38007), Week 13.

[c] Supportive TK from dose ranging embryo-fetal study (Report FLG0044), GD17.

[d] AUC on GD18.

[e] Interpolated from exposures at 300 and 1000 mg/kg/day in the rat dose ranging embryo-fetal study (Report FLG0044).

10 Special Toxicology Studies

Study title: Opicapone (BIA 9-1067): 13-week oral (gavage) combination toxicity study in the Wistar rat with levodopa and carbidopa

Study no.: D55805

Study report location: 4.2.3.7.7. eDR

Conducting laboratory and location: (b) (4)

Date of study initiation: SEP 25, 2012

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: BIA 9-1067/62321-2-4/100%
Levodopa/076K1152/>99%
Carbidopa/5000338/99.6%

Groups 3, 4, and 5:

combined LD = 1000 mg/kg/day BIA 9-1067 + 20 LD + 5.0 CD mg/kg/day
combined MD = 1000 mg/kg/day BIA 9-1067 + 50 LD + 12.5 CD mg/kg/day
combined HD = 1000 mg/kg/day BIA 9-1067 + 120 LD + 30 CD mg/kg/day

Key Study Findings:

In combined HDF there was an increase in the incidence and severity of pancreatic exocrine acinar cell apoptosis and increased absolute and relative weight of the ovaries. Mammary gland hyperplasia was reported in 4/10 combined HDM.

The LD metabolite methyl-L-dopa, seen at levels that increased over time in the CD/LD group, was not found in quantifiable levels in any of the combination groups.

NOAEL: combined MD (1000 mg/kg/day of BIA 9-1067 and 50 mg/kg/day LD + 12.5 mg/kg/day CD) based on exocrine acinar apoptosis in the combined HD.The NOEL for BIA 9-1076 was 1000 mg/kg/day.

Methods

Doses:	0 (vehicle), 1000 mg/kg/day BIA 9-1067, 20 LD + 5.0 CD mg/kg/day, 1000 BIA 9-1067 + 20 LD + 5.0 CD mg/kg/day 1000 BIA 9-1067 + 50 LD + 12.5 CD mg/kg/day 1000 BIA 9-1067 + 120 LD + 30 CD mg/kg/day
Frequency of dosing:	Once daily: LD+CD administered 2 hours post-BIA 9-1067.
Route of administration:	Oral gavage
Dose volume:	20 mL/kg BIA 9-1067/ 10 mL/kg LD+CD
Formulation/Vehicle:	0.2% hydroxypropyl methylcellulose
Species/Strain:	Rat RccHan™: WIST(SPF)
Number/Sex/Group:	10
Age:	6 weeks
Weight:	118.6-152.4 g
Satellite groups:	TK: C 3/sex and dosed 9/sex
Unique study design:	Combination study
Deviation from study protocol:	Minor, with no impact on study results

Observations and Results

Mortality:
One TK combined HD male bit off and attempted to swallow the gavage tube and was euthanized in distress.

Clinical Signs:
Post-dose ruffled fur and prostration was noted in a dose-related manner in all groups treated with LD/CD, notably through Study Week 5. Prostration was noted occasionally in

the majority of combined HD males through Study Day 90. Yellow feces were noted in all groups administered BIA 9-1076.

Body Weights:
The combined HDM mean body weight gain was lower than other groups and mean absolute final body weight was 12% less than CM; combined HDF tended to have lower body weights but not significantly so.

Food Consumption:
Food consumption tended to increase in the combined HD and MD groups, most notably in M.

Ophthalmoscopy:
No test article-related effects were reported.

Hematology:
- Increased high fluorescence (immature) reticulocytes were found in LD/CD F and combined HD F.
- Increased absolute number of lymphocytes and large unstained cells were found in combined HD F.
- Methemoglobin increased over C and greater than laboratory historical data, in combined LD, MD and HD at +66.7%, +83.3% and +83.3%, respectively.

Clinical Chemistry:
Non-adverse changes related to test article were: decreased calcium (combined HDM), increased potassium (combined LDF and combined HDF), increased phosphorus (BIA M, and combined HDM and F). Decreased protein in combined MDM and combined HD M and decreased albumin levels in all groups administered BIA 9-1067 were also reported.

Urinalysis:
No test article-related effects were reported.

Gross Pathology:
Grossly visible nodular hyperplasia of brown fat adjacent to the exorbital lacrimal gland was seen in 3 combined LDM, 3 combined MDM and 1 combined MDF, and 7 combined HDM and 2 combined HDF.

Organ Weights:
Thymus weights were significantly lower in combined HDM than in CM and ovarian weights in combined HDF were greater than in CF.

Sponsor's table:

Dose Levels mg/kg bw/day of	Group 2		Group 4		Group 5		Group 6	
Opicapone	1000		1000		1000		1000	
Levodopa	0		20		50		120	
Carbidopa	0		5		12.5		30	
	Males	Females	Males	Females	Males	Females	Males	Females
Thymus abs.	-	-	-	-	-	-	-47.9**	-
to bw	-	-	-	-	-	-	-40.5**	-
to brain	-	-	-	-	-	-	-48.4**	-
Adrenals abs.	-	-14.8*	-17.4**	-	-15.9*	-16.0*	-14.5*	-17.3*
to bw	-11.8*	-	-17.6**	-	-17.6**	-15.6*	-	-
to brain	-10.7*	-	-18.7**	-	-16.7**	-17.1*	-15.2**	-17.2*
Epididymides/ Ovaries abs.	-	-	-	-	-	-	-16.6**	+29.6**
to bw	-	-	-	-	-	-	-5.3#	+37.2**
to brain weight	-	-	-	-	-	-	-16.9**	+31.5**

*: p<0.05; **: p<0.01; #: not significant; Dunnett-test

Histopathology:
Adequate Battery: Yes
Pathology report: Yes
Peer Review: Yes, by an external peer reviewing pathologist
Histological Findings:

No test article-related effects were reported in rats dosed only with BIA 9-1067. Pancreatic acinar cell vacuolation with dense bodies (interpreted as apoptosis) was seen in 5/10 combined HDF. This change was found to be adverse.

Sponsor's table:

Finding / Groups	1		2		3		4		5		6	
Total Affected / Mean Severity	(10) M	(10) F	(10) M	(10) F	(10) M	(10) F	(10) M	(10) F	(10) M	(10) F	(10) M	(10) F
Acinar cell vacuolation	0	0	1/2.0	0	0	1/1.0	0	0	0	1/1.0	0	5/1.8

Mammary gland hyperplasia was reported in 4/10 combined HDM.

Sponsor's table:

Finding / Groups	1		2		3		4		5		6	
Total Affected / Mean Severity	(10) M	(10) F	(10) M	(10) F	(10) M	(10) F	(10) M	(10) F	(10) M	(10) F	(10) M	(10) F
Glandular hyperplasia	0	0	0	0	0	0	0	0	0	0	4/2.0	0

Hypertrophy of acinar cells of the parotid and mandibular salivary glands and of the exorbital lacrimal glands was seen in males at increased incidence with increasing doses of LD/CD. A similar incidence of hypertrophy of brown fat near the exorbital glands was also reported. These changes were found to be minor and not adverse.

Toxicokinetics:
LD levels increased with dose in a relatively proportional manner. Two groups were dosed with 20 mg/kg/day of levodopa and levodopa exposures were incrementally greater in the group also administered BIA 9-1067. The levodopa metabolite, methyl-L-dopa, was quantifiable only in the CD/LD group (20 LD + 5.0 CD mg/kg/day).

Sponsor's table: Levodopa levels in M and F rats

Levodopa TK Parameters Obtained from Mean Concentrations in Plasma on Days 1/2 and 92/93

Oral administration of Opicapone, Levodopa and Carbidopa in Male Wistar Rats

Period	Parameters	*Units*	GROUPS 3	4	5	6
Target dose		*mg/kg/day*	20	20	50	120
Day 1/2	AUC_{0-t}	*ng·h/mL*	4514	6238	14040	37816
Day 92/93	AUC_{0-t}	*ng·h/mL*	6085	9224	29078	31173
	R_{ac}	-	1.3	1.5	2.1	0.8

Not enough data (1 or 2 quantifiable levels in plasma) were obtained to calculate all the pharmacokinetic parameters.

Levodopa TK Parameters Obtained from Mean Concentrations in Plasma on Days 1/2 and 92/93

Oral administration of Opicapone, Levodopa and Carbidopa in Female Wistar Rats

Period	Parameters	*Units*	GROUPS 3	4	5	6
Target dose		*mg/kg/day*	20	20	50	120
Day 1/2	AUC_{0-t}	*ng·h/mL*	--	3255	12554	36379
Day 92/93	AUC_{0-t}	*ng·h/mL*	3092	5372	16594	24802
	R_{ac}	-	--	1.7	1.3	0.7

Not enough data (1 or 2 quantifiable levels in plasma) were obtained to calculate all the pharmacokinetic parameters.

--: Not reported given that only one Levodopa concentration was obtained

Sponsor's table: exposure (AUC) to Methyl-L-dopa

Molar Exposure Relationship of Methyl-L-dopa *vs* Levodopa
Oral administration of Opicapone, Levodopa and Carbidopa in Male Wistar Rats

Sex	Period	Group	Parent Dose	Levodopa (P) AUC_{0-t}	Methyl-L-dopa (M) AUC_{0-t}	(M)/(P) Ratio
			mg/kg/day	*nM·h*	*nM·h*	
Males	1/2	3	20	22889	145334	6.3
		4	20	31633	-	-
		5	50	71203	-	-
		6	120	191774	-	-
	92/93	3	20	30859	344584	11.2
		4	20	46776	-	-
		5	50	147462	-	-
		6	120	158084	-	-
Females	1/2	3	20	--	121470	-
		4	20	16509	-	-
		5	50	63663	-	-
		6	120	184488	-	-
	92/93	3	20	15679	309619	19.7
		4	20	27243	-	-
		5	50	84150	-	-
		6	120	125778	-	-

--: Not reported given that only one Levodopa concentration was obtained
No concentrations of the metabolite (Methyl-L-dopa) were observed in groups 4, 5 or 6

The half-life of BIA 9-1067 ranged from 1.7 to 7.1 hours, and no accumulation was reported.

Sponsor's table:

Opicapone TK Parameters Obtained from Mean Concentrations in Plasma on Days 1/2 and 92/93
Oral administration of Opicapone, Levodopa and Carbidopa in Male Wistar Rats

Period	Parameters	*Units*	GROUPS 2	4	5	6
Target dose		*mg/kg/day*	1000	1000	1000	1000
Day 1/2	C_{max}	*ng/mL*	30667	26500	35700	28433
	t_{max}	*h*	0.5	1	1	0.5
	λ_z	h^{-1}	0.198	0.228	0.286	0.243
	$t_{½,z}$	*h*	3.5	3.0	2.4	2.9
	AUC_{0-t}	*ng·h/mL*	103950	91598	140827	138857
	$AUC_{\%extr}$	%	0.8	22.3	0.1	0.3
	AUC_{0-inf}	*ng·h/mL*	104789	--	140985	139334
Day 92/93	C_{max}	*ng/mL*	30400	37833	28800	22467
	t_{max}	*h*	1	0.5	0.5	0.5
	λ_z	h^{-1}	0.258	0.172	0.098	0.148
	$t_{½,z}$	*h*	2.7	4.0	7.1	4.7
	AUC_{0-t}	*ng·h/mL*	104658	121190	122300	98061
	$AUC_{\%extr}$	%	0.2	1.4	8.0	2.6
	AUC_{0-inf}	*ng·h/mL*	104859	122885	132987	100720
	R_{ac}	-	1.0	1.3	0.9	0.7

-- : Value not reported due to AUC%extr > 20%

Opicapone TK Parameters Obtained from Mean Concentrations in Plasma on Days 1/2 and 92/93
Oral administration of Opicapone, Levodopa and Carbidopa in Female Wistar Rats

Period	Parameters	*Units*	GROUPS 2	4	5	6
Target dose		*mg/kg/day*	1000	1000	1000	1000
Day 1/2	C_{max}	*ng/mL*	33733	44400	32833	34533
	t_{max}	*h*	0.5	0.5	0.5	0.5
	λ_z	h^{-1}	0.405	0.216	0.163	0.100
	$t_{½,z}$	*h*	1.7	3.2	4.3	6.9
	AUC_{0-t}	*ng·h/mL*	85657	113272	94902	158696
	$AUC_{\%extr}$	*%*	6.6	0.4	1.6	7.8
	AUC_{0-inf}	*ng·h/mL*	91670	113733	96493	172086
Day 92/93	C_{max}	*ng/mL*	35133	38467	34500	36900
	t_{max}	*h*	0.5	0.5	0.5	0.5
	λ_z	h^{-1}	0.256	0.303	--	0.164
	$t_{½,z}$	*h*	2.7	2.3	--	4.2
	AUC_{0-t}	*ng·h/mL*	108043	109216	124306	81023
	$AUC_{\%extr}$	*%*	0.2	0.1	--	30.9
	AUC_{0-inf}	*ng·h/mL*	108242	109295	--	--
	R_{ac}	-	1.3	1.0	1.3	0.5

-- : Value not reported due to $r^2 < 0.900$ or AUC%extr > 20%

Stability and Homogeneity:

The formulations were homogenous and within nominal stability.

11 Integrated Summary and Safety Evaluation

Summary:

BIA 9-1067 is a selective, reversible inhibitor of catechol-O-methyltransferase (COMT) in vitro. When administered in vivo to monkey and rat, BIA 09-1076 consistently inhibited metabolism of co-administered levodopa to 3-o-methyl-dopa.

In a primate model of Parkinson's disease, BIA 9-1067 alone did not affect Parkinson-like behavior but, when administered with levodopa and benserazide, BIA 9-1067 had a beneficial effect when compared to levodopa/benserazide alone.

Oral absorption and systemic distribution were rapid, with an average T_{max} of 1-4 hours, in rat, mouse, dog, and monkey and tissue distribution (based on radioactivity) was maximal at 4 hours post-oral administration. Minimal-to-no drug was detected in the CNS; catechol levels in the cerebrum of monkeys administered oral BIA 9-1067 were not significantly different from controls administered only vehicle. Metabolism was largely through reduction in nonclinical species and through sulfation in human. BIA 9-1079, the major metabolite in animal species, was found at very low concentrations in human subjects. The main metabolite found in human (BIA 9-1103) was found in rat, mouse, and monkey and exceeded clinical concentrations in rats when dosed with BIA 9-1067 at

the NOAEL of 1000 mg/kg/day. CYP2C8 and CYP2C9 were inhibited by BIA 9-1067 and by metabolites BIA 9-1079 and BIA 9-1103.

Although metabolites were detected in urine, excretion of drug-related material was mainly fecal: in all tested species dose-related orange- or yellow-stained feces were reported, and this was attributed to the presence of the yellow-colored test article.

BIA 9-1067 had no relevant effects on hERG-mediated currents in HEK cells or on action potential of isolated Purkinje fibers. In vivo administration in rat and monkey resulted in no test-article effects on electrocardiograms, respiratory function, or behavior.

In toxicity studies in mice, rats, dogs, monkeys, and minipigs crystalline BIA 9-1067 was administered orally in 0.2% hydroxypropyl methylcellulose. Due to the physicochemical properties of the formulation, the maximum feasible dose was 1000 mg/kg and, in most species, was the NOAEL. Non-adverse findings were largely limited to transient inactivity, sporadic emesis, diarrhea or pasty stools, and transient decreases in body weight gains. In the 6-month study in rat, the MD (500 mg/kg/day) was the NOAEL due to increased bilirubin levels (in the absence of histologic changes) at the HD of 1000 mg/kg/day. Notable inappetence with marked body weight loss was seen in rabbit at doses ≥ 175 mg/kg/day; an MTD was determined to be 100 mg/kg/day for pregnant rabbits. When administered intravenously to rats at single doses of 1, 2, 5, 8, or 10 mg/kg in DMSO, the formulation was acutely fatal at 8 and 10 mg/kg, adverse at 5 mg/kg, and non-adverse at 1 and 2 mg/kg.

When BIA 9-1067 was administered in combination with levodopa/carbidopa (LD/CD), adverse findings of pancreatic acinar apoptosis in F rats and mammary hypertrophy in M were limited to the highest dose (120/50 mg/kg/day) of LD/CD. L-Dopa has been shown to be converted to dopamine in isolated acinar cells, and dopamine has been shown to increase the cyclic AMP content of pancreatic acini in vitro [1,2]. Cyclic AMP regulates apoptosis [3], thus at the high dose of levodopa, dopaminergic increases in cyclic AMP may then upregulate acinar cell apoptosis.

No adverse effects on reproductive function were seen. Minor variations in rat and rabbit fetuses were seen in embryofetal development studies at incidences within the testing laboratory's historical control database. BIA 9-1076 administration did not affect male fertility and, when administered to female rats from conception through weaning, had no impact on behavior, learning and memory, or fertility in F1 offspring. The NOEL for male and female fertility and for early embryonic development was 1000 mg/kg/day. In a pre- and postnatal development study in pregnant Crl:WI (HAN) rats, BIA 9-1067 was well tolerated in the pregnant rats; the HD (1000mg/kg/day) was found to be the maternal NOEL. A finding of earlier eye-opening in the HD was non-adverse and the NOAEL for pups through weaning on LD 21 was a maternal dose of 1000 mg/kg/day. There was no effect on pregnancy parameters in F1 females (corpora lutea, implantations, and live embryos) compared to C F-1 females. Because of two F1 post-weaning deaths at the HD of 1000 mg/kg/day, the NOAEL for F1 development was found to be the MD of 375 mg/kg/day.

BIA 9-1067 and metabolite BIA 9-1079 were negative in genetic toxicology (Ames, in vitro chromosomal aberration in human peripheral blood lymphocytes, and in vivo mouse micronucleus) studies. Lifetime (104-week) bioassays of BIA 9-1067 were conducted in Wistar Han rats and CD-1 mice. Both were adequately designed and conducted; however, the high dose volume and relative viscosity of the dose made the formulation difficult to gavage and resulted in increased mortality at the MD and HD in both studies due to regurgitation, aspiration pneumonia, and/or esophageal perforations. Sufficient numbers of animals survived through 104 weeks in both studies, and there were no drug-related neoplasms in either study. Under the conditions of these studies, BIA 9-1067 was found to be non-carcinogenic.

Evaluation:

The sponsor has submitted a comprehensive nonclinical safety package that includes safety pharmacology, toxicology, reproductive/developmental, genetic toxicology, and carcinogenicity studies. The pivotal studies were well conducted and GLP compliant, either under US FDA or OECD guidelines.

With the exceptions of 6-month oral and acute intravenous administration to rat and oral administration to rabbit, the no adverse effect level (NOAEL) in the pivotal nonclinical studies was at the oral dose of 1000 mg/kg/day. On a body surface area (BSA) basis, the NOAEL in 28-day toxicity studies, the 6-month study in rat, and the MTD in rabbit exceeded the recommended clinical dose by not less than 100-fold. In the embryofetal study in rabbit, the fetal NOAEL (at the highest maternal dose of 225 mg/kg/day) was 90-fold greater than the clinical maximum recommended human dose (MRHD).

Exposure (AUC) at the NOAEL in 28-day studies and the MTD in rabbit exceeded exposures at the MRHD by not less than 7-fold and in the 6-month rat study by not less than 21-fold. Exposure at the NOAEL for fetal development in rabbits (at the highest maternal dose of 225 mg/kg/day) was 2.2-fold the clinical exposure.

Table: Comparison of nonclinical dose and exposure (AUC) to the MRHD of 50 mg/day ($AUC_{(0\text{-last})}$ of 2021 ng*h/mL)

Species and duration of dosing	**NOAEL mg/kg/day**	**NOAEL BSA mg/m2**	**Margin (fold > MRHD)**	**AUC (ng*h/mL)**		**Margin (fold > MRHD)**
Mouse 28 days	1000	3000	100	Male	35066	17
				Female	62764	31
Rat 28 days	1000	6000	200	Male	24561	12
				Female	16831	8
Monkey 28 days	1000	12000	400	Male	27965	31
				Female	22490	11
Rabbit 7 days	425 (MTD)	5100	170	Female	14300	7
Rabbit 13 days (GD 18)	225 (fetal NOAEL)	2700	90	Female	4250	2.2
Rat 6 months	500	3000	100	Male	59182	29
				Female	41607	21

References:

1 Vayssette, J, N, Vaysse, and A. Ribet, 1986, Dopamine receptors in pancreatic acinar cells from dog, Erur J Pharmacol, 122(3):321-8

2 Vayesse, N, J Laval, C Senarens, JP Esteve and A Ribet, 1982, Dopamine-stimulated cyclic AMP and binding of [3H] dopamine in acini from dog pancreas, Biochem Biophys Acta, 720(4):378-83

3 Insel, PA, L Zhang, F Murray, H Hokuchi, and AC Zambon, 2012, Cyclic AMP Is both a pro-apoptotic and anti-apoptotic second messenger, Acta Physiol 204(2) 277-287

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