CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

207500Orig1s000 / 207501Orig1s000

PHARMACOLOGY REVIEW(S)

Comments on NDA 207500 and 207501 Isavuconazonium Sulfate capsules

From A. Jacobs, AD

Date : January 29. 2015

1. I concur that there are no pharm-tox issues that preclude approval and that the pregnancy labeling conveys the concern.

2. A case could be made for waiving the PMR request for carcinogenicity studies in 2 species, based on the severity and rarity of the disease for which chronic use is proposed.

3. I have discussed these and other comments with the supervisor and they will be addressed as appropriate.

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/s/

ABIGAIL C JACOBS
01/29/2015

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application numbers:	207500 207501
Supporting document number:	1
Applicant's letter date:	July 8, 2014
CDER stamp date:	July 8, 2014
Product:	CRESEMBA (isavuconazonium sulfate) capsules for oral administration CRESEMBA (isavuconazonium sulfate) injection, lyophilized powder for solution (final name yet to be determined)
Indication:	Invasive aspergillosis and invasive mucormycosis
Applicant:	Astellas Pharma Global Development, Inc. 1 Astellas Way Northbrook, IL 60062
Review Division:	Division of Anti-infective Products
Reviewer:	Owen McMaster, Ph.D.
Supervisor:	Wendelyn Schmidt, Ph.D.
Division Director:	Sumathi Nambiar, MD
Project Manager:	Alison Rodgers

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1 Executive Summary

1.1 Introduction

Astellas Pharma Global Development, Inc. is seeking to market CRESEMBA (isavuconazonium sulfate) for the treatment of patients with invasive aspergillosis and invasive mucormycosis who are at least 18 years old. CRESEMBA is available as a 100 mg capsule or as a lyophilized powder for intravenous administration. CRESEMBA contains isavuconazonium sulfate which is the pro-drug of the active moiety, isavuconazole. The active moiety, isavuconazole is a triazole antifungal agent, which inhibits the conversion of lanosterol to ergosterol via its inhibition of the cytochrome P-450 dependent enzyme lanosterol 14-alpha-demethylase. The presence of isavuconazole in the fungal cell is believed to result in the depletion of ergosterol within the fungal cell membrane (thereby damaging the fungal cell membrane) and the accumulation of toxic methylated sterol precursors which also compromise fungal cell functions. In early development the chloride salt was used for nonclinical toxicology. (b) (4)

Recommendations

1.1.1 Approvability

There are no nonclinical pharmacology or toxicology data that preclude the approval of CRESEMBA.

1.1.2 Additional Non Clinical Recommendations

The applicant should conduct carcinogenicity studies to evaluate the carcinogenic risk of this drug product.

Labeling

The following text is to be included in the prescribing information:

HIGHLIGHTS OF PRESCRIBING INFORMATION

HIGHLIGHTS OF PRESCRIBING INFORMATION

----------WARNINGS AND PRECAUTIONS----------

- Embryo-Fetal Toxicity: Do not administer to pregnant women unless the benefit to the mother outweighs the risk to the fetus. Inform pregnant patient of hazard (5.5).

----------USE IN SPECIFIC POPULATIONS----------

- **Pregnancy: CRESEMBA should only be used if the benefits to the mother outweigh the risk to the fetus. Inform pregnant woman of risk (8.1).**
- **Mothers should not breast feed while taking CRESEMBA (8.3)**

5 WARNINGS AND PRECAUTIONS

5.5 Embryo-Fetal and Neonatal Toxicity

CRESEMBA may cause fetal harm when administered to a pregnant woman. CRESEMBA should be used during pregnancy only if the potential benefit to the patient outweighs the risk to the fetus. Women who

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become pregnant while receiving CRESEMBA are encouraged to contact their physician [see *Use in Specific Populations* (8.1)]

Perinatal mortality was significantly increased in the offspring of pregnant rats dosed orally with (b) (4) at 90 mg/kg/day (b) (4) the maintenance human dose based on (b) (4) during pregnancy through the weaning period.

(b) (4) administration was associated with dose-related increases in the incidences of (b) (4) rudimentary cervical ribs), in rats and rabbits at 30 and 45 mg/kg, respectively, doses equivalent to about one fifth and one tenth of the clinical exposures based on AUC comparisons. In rats, dose-related increases in the incidences of zygomatic arch fusion and supernumerary ribs/rudimentary supernumerary ribs were also noted at 30 mg/kg and above, equivalent to one fifth the clinical dose based on AUC comparisons.*[see Nonclinical Toxicology (13.1)]*.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C

There are no adequate and well-controlled clinical studies of CRESEMBA in pregnant women. CRESEMBA should be used during pregnancy only if the potential benefit to the patient outweighs the risk to the fetus. Women who become pregnant during CRESEMBA treatment are encouraged to contact their physician.

Risk Summary

Based on animal data, CRESEMBA is predicted to have the potential for increasing the risk of adverse developmental outcomes above background risk.

Animal Data

Perinatal mortality was significantly increased in the offspring of pregnant rats dosed orally with CRESEMBA at 90 mg/kg/day (less than half the maintenance human dose based AUC comparisons) during pregnancy through the weaning period.

Isavuconazonium administration was associated with dose-related increases in the incidences of (b) (4) rudimentary cervical ribs), in rats and rabbits at 30 and 45 mg/kg, respectively, doses equivalent to about one fifth and one tenth of the clinical exposures based on AUC comparisons. In rats, dose-related increases in the incidences of zygomatic arch fusion and supernumerary ribs/rudimentary supernumerary ribs were also noted at 30 mg/kg and above, equivalent to one fifth the clinical dose based on AUC comparisons. (b) (4)

8.3 Nursing Mothers

(b) (4) is excreted in the milk of lactating rats following intravenous administration. (b) (4) Mothers should not breast-feed children while taking CRESEMBA.

13 NON-CLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, and Impairment of Fertility

(b) (4)

No mutagenic or clastogenic effects were detected in the *in vitro* bacterial reverse mutation assay and the *in vivo* bone marrow micronucleus assay in rats.

Oral administration of isavuconazonium did not affect the fertility in male or female rats treated at doses up to 90 mg/kg/day (less than a half the clinical dose based on AUC comparisons)

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1.2 Brief Discussion of Nonclinical Findings

The nonclinical toxicology program was extensive, and adequate to support the marketing of CRESEMBA. Studies included *in vitro* and *in vivo* studies using mice, rats, monkeys, rabbits, hamsters and guinea pigs. The applicant evaluated the effects of oral CRESEMBA administration for up to 39 weeks in monkeys, 26 weeks in rats and 13 weeks in mice. Intravenous CRESEMBA was evaluated in rats and monkeys for up to 6 weeks. The median treatment duration for mucormycosis in patients was 102 days but at least one patient has been treated for up to 882 days. Adverse effects were related to dose, not duration of dosing, since no new toxicities developed as drug was administered for longer durations. Intravenous dosing was associated with hemorrhage, vasculitis/perivasculitis, necrosis and/or thrombosis at the injection site but there were no other new toxicities compared to oral dosing. This is consistent with the 67 % bioavailability of isavuconazonium.

In addition to general toxicology, there were evaluations of genotoxic potential, fertility, embryofetal toxicity, pre- and postnatal development, local tolerance, immunotoxicity and mechanistic studies. Study designs, including species selection, dose selection and study durations were appropriate and pivotal studies were conducted according to GLP.

The nonclinical data provide support for the marketing of isavuconazonium for aspergillosis and mucormycosis. The risk of adverse events can be addressed by the appropriate use of the drug. The following were identified as adverse effects are of special interest:

Reduction of the QT interval
Isavuconazonium administration is associated with shortened QT interval in some patients. This has been ascribed to isavuconazole's ability to inhibit both hERG-mediated potassium currents and the L-type calcium channels, I_{CaL} at the same time. In clinical trials, CRESEMBA shortened the QTc interval in a concentration-related manner. CRESEMBA is therefore contraindicated in patients with Familial Short QT syndrome.

Liver toxicity
In repeat dose toxicology studies, isavuconazonium administration was associated with reversible increases in liver weights and/or hepatocellular hypertrophy in mice and rats. Since isavuconazole induces CYP3A and/or CYP2B, the hepatocellular hypertrophy is considered an adaptive response accompanying the increase in activity of the hepatic microsomal drug metabolizing enzymes. There was no evidence of any hepatocellular damage and these liver findings were reversible at the end of a four week treatment-free period.

The CRESEMBA prescribing information instructs physicians to evaluate liver-related laboratory tests at the start and during the course of CRESEMBA therapy and to monitor patients who develop abnormal liver-related laboratory tests during CRESEMBA therapy for the development of more severe hepatic injury. They are also advised to discontinue CRESEMBA if clinical signs and symptoms consistent with liver disease develop that may be attributable to CRESEMBA.

Thyroid toxicity
In rats, repeated administration of isavuconazonium was associated with an increase in thyroid weights and thyroid follicular cell hypertrophy/hyperplasia, but these effects were not observed in monkeys. There is evidence that humans and rats handle thyroid hormones differently and that these rat thyroid findings do not indicate a risk to humans. See Wu, K-M et al, (2006) Preclinical development of new drugs that enhance thyroid hormone metabolism and clearance: Inadequacy of using rats as an animal model for predicting human risks in an IND and NDA. American Journal of Therapeutics 13, 141-144. (2006).

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Adrenal findings
Repeated administration of isavuconazonium to monkeys resulted in increases in adrenal weights and/or vacuolation/hypertrophy of adrenocortical cells. There were no atrophic or necrotic lesions and these adrenal changes were reversible. The clinical significance of these adrenal findings is unclear.

Embryo-fetal / peri-natal toxicity
When administered to pregnant animals during early embryo-fetal development, isavuconazonium induced skeletal abnormalities and/or variations in the rat and rabbit at doses as low as one tenth of the systemic exposure at the maintenance dose of 200 mg/day, based on AUC comparisons. In dams dosed orally during pregnancy and through the weaning period, there was increased perinatal mortality among rat pups. Isavuconazole was detected in the milk of lactating dams at levels up to 17 times higher than plasma levels. The prescribing information describes the nonclinical findings and indicates that CRESEMBA may cause fetal harm when administered to a pregnant woman. Women who become pregnant while on CRESEMBA are encouraged to contact their doctor. CRESEMBA should be used during pregnancy only if the potential benefit to the patient outweighs the risk to the fetus. Bone abnormalities have also been associated with the administration of other azole antifungals such as ketoconazole and fluconazole to pregnant dams.

Bone fractures
Bone fractures were increased in (3/8) juvenile monkeys dosed with isavuconazonium for 39 weeks at doses similar to clinical exposures. Although one high dose female rat showed a diaphysis fracture of the femur in the 26 week study in adult rats, it is not clear that this fracture was drug-related. This finding may relate to the effects of isavuconazonium on calcium homeostasis. The drug is not indicated for patients below 18 years of age.

Carcinogenicity
The applicant has not conducted carcinogenicity studies and indicated that they have no plans to conduct carcinogenicity evaluations. By way of justifying this decision, they quoted the ICHS1A guideline which says that Carcinogenicity testing is required for pharmaceutical products that will be continuously administered for periods longer than 6 months. However, they neglected to quote the section of the document which states that *"It is expected that most pharmaceuticals indicated for 3 months treatment would also likely be used for 6 months. In an inquiry to a number of pharmaceutical research and regulatory groups no cases were identified in which a pharmaceutical would be used only for 3 months."*

The applicant maintains that since other azoles have shown to be carcinogenic, they should be allowed to add a statement to their prescribing information in lieu of conducting carcinogenicity studies. They proposed the following statement:

Two-year carcinogenicity studies of (b) (4) *have not been performed. Hepatocellular adenomas and carcinomas have been reported in mice and rats in carcinogenicity studies for other drugs in the azole class.*

This reviewer recommends that the applicant conduct one two year carcinogenicity study in rats and one other abbreviated carcinogenicity evaluation (such as the Tg-rasH2 mouse). This can be conducted post-approval as a PMR.

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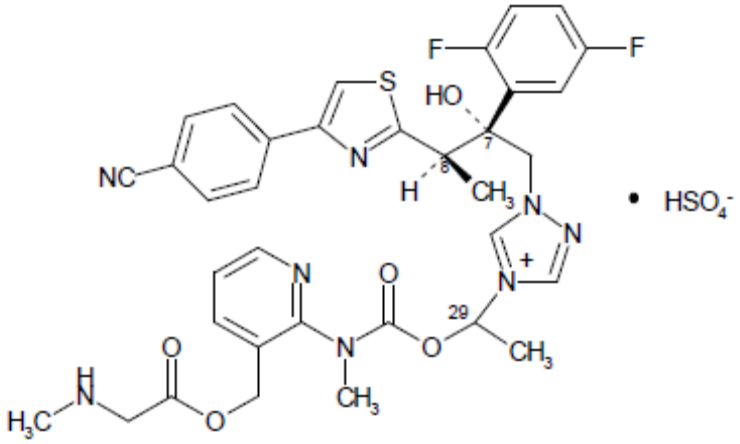
2 Drug Information
2.1 Drug
2.1.1 CAS Registry Number: 946075-13-4
2.1.2 Generic Name: Isavuconazonium sulfate
2.1.3 Code Names: BAL8557
BAL8557-002
(b) (4)

2.1.4 Chemical Name (USAN):
(b) (4)

2.1.5 Molecular Formula: $C_{35}H_{35}F_2N_8O_5S \cdot HSO_4$
Molecular Weight: 814.84
2.1.6 Structure



2.1.7 Pharmacologic class: Azole antifungal agent.
2.2 Relevant INDs: IND 72,593 (intravenous BAL8557),
IND 119,307 (BAL 8557 capsule)
Relevant NDA: NDA 207501 (intravenous BAL8557)
Relevant DMF: None

2.3.1 Drug Formulation

Isavuconazonium sulfate hard capsules are hard capsules containing 186.3 mg isavuconazonium sulfate corresponding to 100 mg isavuconazole (active moiety BAL4815). The capsules have a Swedish Orange (reddish brown color) body and a white cap. The capsules are imprinted with “ISA” (cap) and the Astellas logo (body) in black ink.

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Table 1: Composition of the Isavuconazonium sulfate hard capsules

Component	Function	Amount per tablet (mg)
Isavuconazonium sulfate	Drug substance	186.3 (corresponding to 100 mg isavuconazole)
Magnesium citrate Microcrystalline Cellulose Talc Colloidal Silicon dioxide Stearic acid (b) (4) Capsule (size #0 elongated) Ink (b) (4) Black)	(b) (4)	(b) (4)
Total capsule weight		695.9

Table 2: Composition of the Isavuconazonium sulfate for injection

Component	Function	Amount per tablet (mg)
Isavuconazonium sulfate	Drug substance	372.6 (corresponding to 200 mg isavuconazole)
Mannitol	(b) (4)	96.0
Sulfuric acid	pH adjusting agent	(b) (4)
Water for Injection (b) (4)	(b) (4) (b) (4)	
Type 1 glass vial	Container	one
(b) (4) rubber, (b) (4)	Stopper	one
Aluminum flip-off caps)	Seal	one

The amount of isavuconazonium sulfate per vial will be adjusted based on (b) (4) sulfuric acid is added as necessary for pH adjustment. (b) (4)

Solubility
The solubility of drug substance was >1.0 g/mL in any of the pH conditions tested (pH 1, 3, 5 and 7).

2.4 Comments on Novel Excipients
No novel excipients were used in this drug product

2.5 Comments on Impurities/Degradants of Concern
Several impurities (b) (4) but were adequately qualified since they were present at appropriate levels in drug batches used for toxicology studies.

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2.6 Proposed Clinical Population and Dosing Regimen

Patients 18 years old and above with Invasive aspergillosis and Invasive mucormycosis. A 200 mg loading dose is to be administered every 8 hours, for 48 hours (6 total doses), via oral or IV administration. The intravenous formulation must be administered via a slow infusion over a minimum of one hour, set with an in-line filter pore size 0.2 µm to 1.2 µm. The maintenance dose (200 mg, once per day, oral or i.v.) should be initiated 12 to 24 hours after the last loading dose.
Patients have been dosed for a median of about 100 days for mucormycosis, but individual patients have been dosed for over two years.

2.7 Regulatory Background

This is the first NDA submitted for this new molecular entity. It has never been approved for sale by any other agency.

3 Studies Submitted
3.1 Pivotal Studies Reviewed

Pharmacology
Primary Pharmacology

J-147*000 Aoki Y, Kokado M, Arisawa M. 2000. An inhibitory activity of (b) (4) against P450 lanosterol C-14 demethylase, Roche Research Report No. J-147*000. Internal Report, Roche, Kamakura, Japan.

Secondary Pharmacodynamics
See Microbiologist's Review by Dr. Shukal Bala

Safety Pharmacology

9755-PT-0001 BAL4815: Effect on HERG Tail Currents Recorded from Stably Transfected HEK 293 Cells in Comparison with Ketoconazole, Voriconazole and Fluconazole (b) (4) Protocol Nos. BAP00371 and BAP000372)

9755-PT-0002 General Pharmacology of (b) (4)
9755-PT-0003 Effect of Isavuconazole (BAL4815) on Conductance through Human Cardiomyoctye Membrane Ion Channels Expressed in Mammalian Cells

Pharmacokinetics/ADME

9766-ME00017 (b) (4) Pharmacokinetics of (b) (4) (prodrug), (b) (4) (active metabolite) and (b) (4) (pro-moiety) following single intravenous and oral administration of (b) (4) to cynomolgus monkeys and food effect in monkeys
9766-ME-0004 *In vitro* conversion of (b) (4) in rat, monkey and human plasma
9766-ME-1020 Quantitative Whole Body Autoradiography of Rats Following a 21 Day Repeat Oral Administration of [cyano-^{14}C]ASP9766 Sulfate
9766-ME-0005 *In vitro* conversion of two diastereomers of (b) (4) in rat, monkey and human plasma
9766-ME-1019 Placental transfer and excretion into milk after a single intravenous administration of [^{14}C] ASP9766 sulfate to rats
9766-ME-1017 Blood and plasma concentrations and excretion of radioactivity after a single oral or intravenous administration of [^{14}C] ASP9766 sulfate to monkeys

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9766-ME-0049 Plasma and brain concentrations of isavuconazole and voriconazole in rats after single oral administration.
9766-ME-0022 Biliary excretion and metabolic profile in bile and urine after i.v. administration of $^{14}C/^{3}H$ (b) (4) (the water soluble azole prodrug) to bile duct cannulated rats
9766-ME-1029 Blood and plasma concentrations and excretion of radioactivity after a single oral or intravenous administration of [^{14}C] ASP9766 sulfate to rats
9766-ME-0048 Investigation of the potential DDI potential between Maalox and isavuconazole *in vivo* in the mouse after oral administration as BAL8557

General Toxicology

Single dose studies

9766-TX-0001 Single oral and intravenous administration toxicity study of (b) (4) in rats
9766-TX-0002 A single dose toxicity study of (b) (4) in monkeys (Oral, i.v. bolus and i.v. infusion)

Repeat dose studies

Oral studies
9766-TX-0017 A Repeated Dose Toxicity Study of (b) (4) administered Orally to Cynomolgus Monkeys for 4 Weeks Followed by a 4 Week Recovery Period
9766-TX-0005 13-week preliminary toxicity study by the oral route (gavage) in mice
9766-TX-0015 13-Week Toxicity Study by Oral Route (Gavage) in Rats Followed by a 4-Week Treatment-Free Period
9766-TX-0018 13-Week Toxicity Study by Oral Route (Gavage) in Cynomolgus Monkeys Followed by a 4-Week Treatment-Free Period
9766-TX-0016 BAL8557: 26-Week Toxicity Study by the Oral Route (Gavage) in Rats Followed by a 4-Week Treatment-Free Period
9766-TX-0019 BAL8557: 39-week toxicity study by the oral route (gavage) in cynomolgus monkeys followed by a 4-week treatment-free period

Intravenous studies
9766-TX-0011 Two-week infusion toxicity study of (b) (4) in monkeys (including recovery study)
9766-TX-0009 6-Week Intravenous (Infusion) Toxicity Study in the Rat with a 4-Week Recovery
9766-TX-0012 6-Week Toxicity Study by Daily 2-Hour Intravenous Infusion in Cynomolgus Monkeys with a 4-Week Treatment-Free Period

Genotoxicity

9766-TX-0023 Mutagenicity Evaluation of (b) (4) in the Ames Test
9766-tx-0027 (b) (4) Mouse Lymphoma Cell Mutation Test (ML/TK)
9766-TX-030 Micronucleus Assay in Bone Marrow Cells of the Rat with BAL8557 (Water Soluble Azole).

Reproductive and Developmental Toxicity
9766-TX-0049 BAL8557: Study of Fertility and Early Embryonic Development to Implantation in the Rat

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9766-TX-0052	BAL8557: Study for Effects on Embryo-Fetal Development in the Han Wistar Rat
BAP00412	BAL8557: Study for Effects on Embryo-Fetal Development in the Himalayan Rabbit
9766-TX-0056	BAL8557: Study for Effects on Pre- and Postnatal Development Including Maternal Function in the Han Wistar Rat

Studies not reviewed:

Dose range finding studies did not get written reviews neither did studies which were duplicative. For example, if a study was conducted with the chloride (the original salt that was developed) and then repeated using the sulfate salt (the current product), then the chloride study did not get a written review. Since toxicities were similar within a species as duration of dosing increased and since the median duration for mucormycosis was 102 days, some oral studies of intermediate durations were not reviewed.

Previous Reviews Referenced: None

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4 Pharmacology

4.1 Primary Pharmacology

Studies conducted to investigate the primary pharmacology of CRESEMBA are discussed in the Microbiologist's Review by Dr. Shukal Bala. One study is described briefly below.

Aoki Y, Kokado M, Arisawa M. 2000. An inhibitory activity of (b) (4) against P450 lanosterol C-14 demethylase, Roche Research Report No. J-147*000. Internal Report, Roche, Kamakura, Japan.

The applicant provided a summary of this internal report from Roche.

This study compared the effects of isavuconazole and ketoconazole on *in vitro* cholesterol biosynthesis using a cell-free rat liver extract in which P450 lanosterol C-14 demethylase (P45014DM) was induced by cholestyramine treatment.

In controls, only 4, 4-desmethylsterols were detected, indicating that the three methyl groups at C4alpha, C4beta, and C14 were removed. In the presence of isavuconazole (10 µM) or ketoconazole (1µM), 4, 4-dimethylsterols were prevalent, indicating that P45014DM was inhibited by both compounds in rat liver.

The study also compared the P45014DM extracted from rat liver vs P45014DM extracted from *C. albicans*. Isavuconazole exhibited similar inhibitory activity for the rat liver and for *C. albicans* as Voriconazole. The selectivity ratio (rat/*C. albicans)* was 93 for isavuconazole compared to 85 for Voriconazole. In contrast, the selectivity ratio was 17600 for fluconazole.

Table P1: Effect of isavuconazole and azole reference drugs on P45014DM activity of rat liver and C. albicans.

Compound	**IC_{50} (mg/L)**		**Selectivity (ratio)**
	Rat liver	***C. albicans***	**(rat/*C. albicans*)**
Isavuconazole	4.0	0.043	93
Ketoconazole	0.39	0.030	13
Fluconazole	300	0.017	17600
Itraconazole	1.1	0.042	26
Voriconazole	4.4	0.052	85
BMS207147 (Ravuconazole)	2.8	0.020	140

Isavuconazole inhibited *in vitro* cholesterol biosynthesis via inhibition of P450 lanosterol C-14 demethylase. Compared to other azoles tested, the inhibitory activity of isavuconazole is expected to have moderate inhibitory activity against the human P450 enzyme. Inhibition of P45014DM resulted in accumulation of methylated intermediates. It is believed that these toxic intermediates could cause damage to the fungal cells. In addition, the depletion of ergosterol is believed to contribute to the disruption of the structure and function of the cell membrane. Isavuconazole is comparable to voriconazole in its selectivity for rat liver $P450_{14DM}$ over C. albicans $P450_{14DM}$

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4.2 Secondary Pharmacology

4.3 Safety Pharmacology Studies

Study title: BAL4815: Effect on hERG Tail Currents Recorded from Stably Transfected HEK 293 Cells in Comparison with Ketoconazole, Voriconazole and Fluconazole ([redacted] (b) (4) Protocol Nos. BAP00371 and BAP000372)

Study # 9755-PT-0001

Study report location: EDR

Conducting laboratory [redacted] (b) (4)

Date of study initiation: 23 December 2004

GLP compliance: Yes

QA statement: Yes

Drug, lot #: MUE00002

Purity (%): 98 %

Key Study Findings: BAL4815 inhibited hERG-mediated potassium current with an IC50 of 5.82 µM

The whole-cell voltage clamp technique was used to determine the effects of isavuconazole on the IKr-like membrane potassium current in a human embryonic kidney cell line (HEK293) transfected with the human *ether-à-go-go*-related gene (hERG). Isavuconazole was tested at concentrations of 30 µM, 10 µM, 3 µM and 1 µM (at least n=3 cells). E-4031, a selective IKr blocker was used as the positive reference compound.

Results

BAL4815 inhibited hERG-mediated potassium current with an IC_{50} value of 5.82 µM. The test system was validated using the selective IKr blocker E-4031 which resulted in 94% inhibition at 100 nM.

Table P2: Inhibition of hERG-mediated K+ current in HEK293 cells (%) by increasing concentrations of cumulatively-applied isavuconazonium or E-4031

Isavuconazonium concentration	Remaining tail current (%)	Dunnett *P < 0,05 (vs vehicle)
Vehicle	100	
30 µM	8.6	*
10 µM	33	*
3 µM	71	*
1 µM	93	NS

Discussion and Conclusions

BAL4815 inhibited hERG-mediated potassium current with an IC_{50} value of 5.82 µM. This was similar to ketoconazole which inhibited HERG-mediated potassium current with an IC_{50} of 5.46 µM, respectively. No inhibition was found with Voriconazole and Fluconazole (data not shown).

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Study title: General Pharmacology of (b) (4)

Study # 9755-PT-0002

Study report location: EDR

Conducting laboratory and location: (b) (4)

Date of study initiation: September 20, 1999

GLP compliance: No

QA statement: Yes

Drug, lot #: JO-10179

Purity (%): 98.99 %

In this general pharmacological study, (b) (4) at 10, 30 and 90 mg/kg was administered orally to mice and rats, in order to investigate its effects on general behavior, the central nervous system, water and electrolyte metabolism, and the gastrointestinal system. (b) (4) at 2.5, 7.5 and 22.5 mg/kg was administered intravenously to anesthetized cynomolgus monkeys in order to investigate its effects on the respiratory and cardiovascular systems. In the in vitro experiments, (b) (4) at 10^{-7}, 10^{-6}, 10^{-5}M was added directly to the bathing fluid.

Evaluations included

1. Observation of general behavior in mice (Irwin method'), prior to, and 1, 2, 4, 6 and 8 hours after the vehicle and test article administrations.
2. Effects on spontaneous locomotor activity in mice
3. Anticonvulsive effects on maximal electric shock seizures in mice
4. Anticonvulsive effects on pentylenetetrazol-induced convulsion in mice
5. Convulsive effects on pentylenetetrazol-induced convulsion in mice
6. Effects on pentobarbital-induced sleeping time in mice
7. Effects on acetic acid-induced writhing in mice
8. Effects on normal body temperature in rats
9. Effects on respiration, blood pressure, heart rate, femoral artery blood flow and ECG in anesthetized monkeys
10. Effects on spontaneous motility and agonist-induced contractions of the isolated guinea pig ileum.
11. Effects on urinary volume and urinary electrolytes in rats
12. Effects on the intestinal transport of charcoal meal in mice

Results

At 30 and 90 mg/kg, the pentobarbital-induced sleeping time was significantly prolonged compared to the control group.

Exposing the isolated guinea pig ileum to 10^{-5}M, isavuconazonium resulted in the disappearance of spontaneous motility immediately after the addition of the test article. Spontaneous motility reappeared 1 to 2 minutes after the addition of test article in all preparations, although, the frequency of spontaneous motility was suppressed.

Discussion

Besides two specific effects, isavuconazonium showed very little impact on general behavior, the central nervous system, water and electrolyte metabolism, and the gastrointestinal system.

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Pentobarbital-induced sleeping time is likely to have increased due to the P450-inhibiting property of isavuconazole. The inhibition of pentobarbital metabolism potentiated the CNS depressant effect resulting in the increase in pentobarbital-induced sleeping time.

The isavuconazole-induced suppression of spontaneous motility of the isolated guinea pig ileum is related to its pharmacological blockade of the L-type calcium channels. Published studies (Grasa, L., et al. (2004). *The role of calcium in the contractility of rabbit small intestines in vitro. Journal of Physiology and Pharmacology 55 :(3) 639-650*) have documented the critical role of calcium homeostasis in intestinal motility. Other calcium channel blockers such as nifedipine also decrease motility in intestinal segments and spontaneous activity in small intestines was eliminated in calcium-free solutions.

Cases of serious gastrointestinal obstruction have been identified in patients on calcium channel blockers such as nifedipine, with no known gastrointestinal disease, including the need for hospitalization and surgical intervention. In clinical trials, isavuconazonium side effects included abdominal disorders such as constipation and abdominal pain, but the incidence is generally comparable to that observed with voriconazole.

Reference ID: 3689714

Study title:	Effect of Isavuconazole (BAL4815) on Conductance through Human Cardiomyoctye Membrane Ion Channels Expressed in Mammalian Cells
Study #	9755-PT-0003
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	02 August 2013
GLP compliance:	No
QA statement:	Yes

The *in vitro* effects of isavuconazole were evaluated at room temperature using the QPatch HT® (Sophion Bioscience A/S, Denmark), an automatic parallel patch clamp system. The test article was evaluated at concentrations of 1, 3, 10, and 30 µM (n ≥3) against the following ion channels:

1. Cloned L-type calcium channels (hCav1.2, encoded by the human CACNA1C gene and coexpressed with the β2 subunit, encoded by the human CACNB2 gene and the α2δ1 subunit encoded by the human CACNA2D1 gene in CHO cells), responsible for ICa,L, high threshold calcium current.
2. Cloned hERG potassium channels (encoded by the KCNH2 gene and expressed in HEK293 cells), responsible for the IKr, rapid delayed rectifier potassium current.
3. Cloned hKir2.1 potassium channels (encoded by the human KCNJ2 gene and expressed in HEK293 cells), responsible for IK1, inwardly rectifying potassium current.
4. Cloned hKir3.1/hKir3.4 potassium channels (expressed by the human KCNJ3 and KCNJ5 genes and coexpressed in HEK293 cells), responsible for IACh, inwardly rectifying potassium current.
5. Cloned Kir6.2/SUR2A potassium channels (expressed by the human KCNJ11 and SUR2A genes and coexpressed in HEK293 cells), responsible for the ATPsensitive current, IKATP.
6. Cloned hKv1.5 potassium channels (encoded by the human KCNA5 gene and expressed in CHO cells), responsible for IKur, ultra-rapid delayed rectifier potassium current.
7. Cloned hKv4.3/KChiP2.2 potassium channels (encoded by the human KCND3 and KCNIP2 genes coexpressed in HEK293 cells), responsible for the transient outward current.
8. Cloned hKvLQT1/hminK potassium channels (encoded by the human KCNQ1 and KCNE1 genes and coexpressed in CHO cells), responsible for IKs, slow delayed rectifier potassium current.
9. Cloned hNav1.5 sodium channels (SCN5A gene expressed in CHO cells), responsible for the inward sodium current.

Results

Isavuconazole had most impact on the L-type calcium channels (hCav1.2), Sodium channels (hNav1.5, tonic), Sodium channels (hNav1.5, phasic), Potassium channels (hERG) and Potassium channels (hKvLQT1/hminK)

Reference ID: 3689714

but had very little effect in the ion channels hKir2.1, hKir3.1/hKir3.4, Kir6.2/SUR2A, hKv1.5 and hKv4.3/KChiP2.2, (IC_{50} values were > 30 µM.

Table P3: IC_{50} (µM) values for ion channels most impacted by isavuconazole

IC_{50} (µM)	Channel	Responsible for:
6.6	L-type calcium channels (hCav1.2)	High threshold calcium current I_{CaL}
14.8	Sodium channels (hNav1.5, tonic)	inward sodium current.
20.4	Sodium channels (hNav1.5, phasic)	inward sodium current
19.4	Potassium channels (hERG)	IKr rapid delayed rectifier potassium current.
24.0	Potassium channels (hKvLQT1/hminK)	IKs, slow delayed rectifier potassium current.

The positive controls performed as expected, confirming the sensitivity of the test systems to ion channel inhibition or potentiation (data not shown).

Discussion

The strongest effects were seen against the L-type calcium channel, I_{CaL} which is responsible for the high threshold calcium current. Also strong were the effects against I_{Kr}, the rapid delayed rectifier potassium current. I_{CaL} and I_{Kr} blocks have opposite effects on the QT interval of the ECG: I_{CaL} reduces the QT interval whereas I_{Kr} prolongs it. QT interval is more sensitive to I_{CaL} block, therefore a drug which blocks both channels results in QT shortening.(Zemzemi, N. et al (2014) Effects of L-type calcium channel and human ether-a-go-go related gene blockers on the electrical activity of the human heart: a simulation study. Europace. 2014, Sep 15. pii: euu122). The toxicological implications of the other potassium and sodium channels are unclear

Conclusion

Isavuconazole inhibits several channels including L-type calcium channels and human ether-a-go-go related gene (IKr) channels, which have opposing effects on the QT interval. Simulation models predict that compounds that inhibit both channels would be associated with QT shortening. In clinical trials, CRESEMBA shortened the QTc interval in a concentration-related manner. CRESEMBA is therefore contraindicated in patients with Familial Short QT syndrome.

Reference ID: 3689714

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption

When isavuconazonium was administered orally to rats, 68- 87 % was converted to isavuconazole in the plasma and Tmax occurred between 3-5 hours (Study 9766-ME-0017). Fasted monkeys convert more isavuconazonium (87 %) and achieve Tmax faster (3 hours) compared to fed monkeys (F_{oral} 68 % and Tmax 5 hours). After isavuconazonium administration to monkeys, isavuconazonium was not measureable (see Table PK1, below) in plasma from blood samples were taken at 0.5, 1, 2, 4, 7, 24, 31, 48 and 72 hours post dose, despite the fact that isavuconazole was detected during that same time, (with a T max of 3 or 5 hours, see Table PK2). Isavuconazonium was detected after intravenous dosing, when blood samples were taken starting at 5 minutes (at 0.1, 0.25, 0.5, 1, 2, 4, 7, 24, 31, 48 and 72 hours after dosing). See Table PK1

Table PK1: Mean pharmacokinetic parameters of [redacted] (prodrug) following single intravenous bolus and oral administration of [redacted] to female monkeys. Study 9766-ME-0017

b(4)

Compound		[redacted]		
Route		I.V.	P.O. without food	P.O. with food
Dose (mg/kg) [1]		3	3	3
PK parameters for [redacted]	Units			
Dose as [redacted]	(mg/kg)	2.73	2.73	2.73
Cmax	(µg/mL)	-		
Co	(µg/mL)	3.06±1.76		
Tmax	(hr)	-		
Lambda_z	(1/hr)	6.73±4.33		
T1/2	(min)	7.98±4.40		
AUC(0-last)	(µg.hr/mL)	0.328±0.221		
AUC(0-inf)	(µg.hr/mL)	0.343±0.228	NM	NM
AUMC(0-inf)	(µg.h^2/mL)	0.0368±0.0282		
MRT	(hr)	0.0974±0.0252		
CL	(mL/min/kg)	202±168		
Vss	(L/kg)	1.01±0.55		
Vz	(L/kg)	1.78±0.77		

b(4)

[redacted]

b(4)

NM: Not measurable

- : Not calculated

Reference ID: 3689714

Table PK2: Mean pharmacokinetic parameters of [redacted] (active metabolite) following single intravenous bolus and oral administration of [redacted] to female monkeys and following single intravenous bolus administration of [redacted] to female monkeys. Study 9766-ME-0017

b(4)

Compound		[redacted]	[redacted]	[redacted]	[redacted]
Route		I.V.	P.O. without food	P.O. with food	I.V.
Dose (mg/kg)		3	3	3	1
PK parameters for [redacted]	Units				
Dose as [redacted]	(mg/kg)	1.66	1.66	1.66	1
Co	(µg/kg)	-	-	-	0.525±0.077 4)
Cmax	(µg/mL)	1.03±0.23	0.360±0.067	0.175±0.079	0.972±0.489
Tmax	(hr)	0.083±0.00	3.33±1.15	5.00±1.73	0.194±0.096
Lambda_z	(1/hr)	0.0708±0.0054	0.0569±0.0162	0.0772±0.0206	0.0692±0.0355
T1/2	(hr)	9.84±0.72	12.8±3.6	9.40±2.35	11.7±5.3
AUC(0-last)	(µg.hr/mL)	4.94±0.81	4.25±0.91	3.40±1.57	3.53±0.73
AUC(0-inf)	(µg.hr/mL)	5.08±0.81	4.39±0.81	3.54±1.54	3.66±0.62
AUMC(0-inf)	(µg.h²/mL)	52.9±5.3	69.5±17.3	55.6±18.9	36.5±7.8
MRT	(hr)	10.5±1.2	16.0±4.1	16.1±1.5	10.4±3.7
CL	(mL/min/kg)	-	-	-	4.64±0.74
CL/F 5)	(mL/min/kg)	5.54±0.87	6.43±1.13	8.71±3.08	-
Vss	(L/kg)	-	-	-	3.01±1.46
Vz	(L/kg)	-	-	-	4.95±2.83
Vz/F 5)	(L/kg)	4.74±1.02	7.28±2.69	7.42±3.74	-
$F_{conversion}$	(%)	84.2±11.3	-	-	-
F_{oral}	(%)	-	86.9±12.8	67.9±19.0	-

[redacted]

b(4)

4) C_0 = C 5min

5) When [redacted] was administered intravenously, F means the conversion ratio of [redacted] to [redacted] in the body.
When [redacted] was administered orally, F means a combination of the conversion ratio of [redacted] to [redacted] in the body and oral bioavailability.

- : Not calculated

Despite being measureable after intravenous administration of isavuconazonium, overall exposure to isavuconazonium in plasma was very low (< 1% of total isavuconazole exposure) suggesting a rapid conversion of [redacted]. These data are consistent with clinical data, where intact isavuconazonium was also not detected in plasma or urine after oral administration in healthy volunteers.

These data are supported by study 9766-ME-0004, an *in vitro* study in plasma which showed that isavuconazonium was converted to isavuconazole, with a $t_{1/2}$ of less than 2 min in rat, rabbit, cynomolgus monkey, and human plasma.

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Distribution

Tissue distribution of isavuconazonium was assessed by quantitative whole body autoradiography (QWBA) for ^{14}C. Two types of ^{14} C-radiolabeled isavuconazonium sulfate were used to study the disposition of drug after administration. [cyano-^{14} C] isavuconazonium sulfate with ^{14} C-labeled at the benzonitrile substructure of the active moiety was used for following the homeostasis of the active moiety. [pyridinylmethyl-^{14}C] isavuconazonium sulfate with ^{14} C-labeled at the methyl group of the pyridine ring was used for monitoring the inactive cleavage product.

In the late stage of development, two types of ^{14}C -radiolabeled isavuconazonium sulfate, [cyano-^{14}C] isavuconazonium sulfate for the active moiety and [pyridinylmethyl-^{14}C] isavuconazonium sulfate for the inactive cleavage product were synthesized for full assessment of ADME in rats and cynomolgus monkeys as well as in humans.

In study 9766-ME-1020, after oral dosing, the highest concentrations of ^{14} C-radioactivity were measured in the bile, urine, liver, adrenals and intestinal mucosa. Tissue distribution patterns of [cyano-^{14} C]*-radioactivity in male rats after 21 consecutive daily doses were generally similar to those after a single dose, or after 7, 14, and 20 consecutive doses. Steady state had been achieved for most tissues with measurable levels of radioactivity through 7 and 14 days after the initial dose. Bone was the only tissue devoid of radioactivity throughout the course of this study.

Table PK3. Tissues levels of ^{14} C-radioactivity (mcg eq. of isavuconazonium/g tissue) one hour after dosing with [cyano-^{14} C] isavuconazonium sulfate after 14 days of repeated dosing. Study 9766-ME-1020

Tissue	^{14} C-Radioactivity concentrations (mcg eq. of isavuconazonium) in tissue (g)
bile	157
urine	58
liver	58
adrenal gland	23
small intestinal mucosa	22
cecum mucosa	18
brown fat	10
kidney	9.3
stomach mucosa	9
exorbital lacrimal gland	6.6
Harderian gland	6.6
intra-orbital lacrimal gland	6.8
pancreas	6.3
thyroid	5.2

[Cyano-^{14} C]-radioactivity in blood and plasma (LSC analysis) reached the Cmax at 4 h after the last dose, and then decreased with t1/2 of 319 h in blood and 48.2 h in plasma. By 168 hours after the last dose, radioactivity concentrations had declined to below measurable levels in most tissues except in the adrenals, brown fat, kidney, liver, myocardium, preputial gland, thyroid, blood and plasma. At 672 h after the last dose, radioactivity remained quantifiable in only the adrenal gland and adrenal cortex. Overall, repeat dosing of [cyano-^{14}C] isavuconazonium sulfate did not lead to accumulation of radioactivity in any tissues of male albino rats.

Reference ID: 3689714

Table PK4 ^{14}C -Radioactivity Concentrations in Tissue at 1 and 24 Hours after Repeated Oral Administration of 30 mg/kg of [Cyano-^{14}C] Isavuconazonium Sulfate to Male Albino Rats[Study 9766-ME-1020]

Tissue	^{14}C-radioactivity concentrations in tissue (mcg eq/g)							
	Day 1		Day 7		Day 14		Day 21	
	1 h	24 h	1 h	24 h	1 h	24 h	1 h	24 h
Adrenal cortex	10.1†	6.54†	20.9†	14.3†	23.1†	16.9†	22.8†	12.7†
Adrenal gland	9.51†	5.83†	19.3†	12.4†	21.2†	14.0†	20.7†	11.4†
Adrenal medulla	8.04†	3.47†	11.7†	6.37†	19.2†	5.12†	8.12†	7.21†
Aorta	2.00	ND	2.75	1.51	4.53	2.35	4.22	ND
Arterial wall	ND	ND	1.88	ND	3.43	ND	ND	ND
Bile	101	27.3	472	27.7	157	ND	186	25.3
Blood	0.783	ND	1.70	1.34	3.04	1.41	2.60	1.01
Bone	BLQ	ND	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Bone marrow	2.35	ND	1.90	0.979	4.03	BLQ	3.24	BLQ
Brain cerebellum	1.55	ND	1.81	BLQ	2.74	BLQ	2.59	BLQ
Brain cerebrum	1.51	ND	1.86	BLQ	2.72	BLQ	2.66	BLQ
Brain medulla	1.60	ND	1.79	BLQ	2.93	BLQ	2.79	BLQ
Brain olfactory lobe	1.46	ND	1.44	BLQ	2.46	BLQ	2.34	BLQ
Bulbo-urethral gland	2.21	ND	3.08	0.933	4.60	1.27	4.27	0.788
Cecum	BLQ	7.13	6.41	6.57	13.5	13.6	9.97	ND
Cecum mucosa	4.12	7.42	16.6	11.4	18.2	10.9	16.6	ND
Diaphragm	2.86	BLQ	3.52	0.920	5.01	1.27	3.84	0.753
Epididymis	1.00†	ND	1.57	0.819†	2.26†	0.925†	2.22	BLQ†
Esophagus	3.00	0.896	2.10	1.19	2.89	BLQ	2.52	BLQ
Exorbital lacrimal gland	3.62	0.881	3.94	1.37	6.64	1.21	5.60	1.20
Eye lens	BLQ	ND	BLQ	BLQ	BLQ	BLQ	0.794	BLQ
Eye uveal tract	1.21	ND	1.39	1.06	2.16	0.948	2.22	1.17
Eye	BLQ	ND	BLQ	BLQ	0.787	BLQ	BLQ	BLQ
Fat (brown)	5.34	0.939	7.00	1.69	9.92	2.20	9.33	1.50
Fat (mesenteric)	0.802	1.80	2.96	2.21	1.17	2.67	3.43	2.33
Fat (reproductive)	BLQ	2.27	3.76	3.66	2.03	3.73	3.62	2.99
Fat (white)	1.74	1.59	2.53	2.20	2.39	1.42	2.75	1.04
Harderian gland	3.63	1.03	4.55	1.73	6.58	1.38	6.58	1.06
Intra-orbital lacrimal gland	3.34	ND	3.38	1.39	6.77	1.33	5.89	1.32
Kidney cortex	4.67	ND	6.60	2.42	9.24	2.73	8.76	2.33
Kidney medulla	4.64	ND	6.10	2.43	9.31	2.33	8.49	1.97
Kidney	4.65	1.42	6.46	2.43	9.29	2.58	8.59	2.16

Reference ID: 3689714

Table PK4 (cont'd) ^{14}C –Radioactivity Concentrations in Tissue at 1 and 24 Hours after Repeated Oral Administration of 30 mg/kg of [Cyano-^{14}C] Isavuconazonium Sulfate to Male Albino Rats [Study 9766-ME-1020]

Large intestine	BLQ	1.91	3.49	1.90	4.00	1.46	3.65	1.92
Large intestine mucosa	2.55	1.92	3.21	4.06	5.32	2.31	4.37	5.15
Liver	35.8	7.53	40.1	15.6	58.8	18.4	54.4	14.2
Lung	2.03	0.757	3.13	1.41	3.14	1.16	3.38	0.814
Lymph node	1.22	ND	2.06	0.987	2.80	1.12	2.44	ND
Lymph node mandibular	1.35	ND	1.82	ND	2.96	ND	2.87	ND
Muscle	1.32	BLQ	1.59	BLQ	1.84	BLQ	1.27	BLQ
Myocardium	3.10	0.964	3.99	1.49	5.60	1.83	5.77	1.47
Nasal mucosa	1.36	0.895	1.98	1.19	3.17	1.76	3.04	0.931
Pancreas	3.99	0.749	4.20	1.05	6.34	1.17	5.91	BLQ
Pineal body	1.84	ND	4.13	ND	4.47	ND	5.49	ND
Pituitary gland	2.50	ND	ND	ND	4.85	ND	2.88	ND
Preputial gland	ND	ND	4.00†	3.12†	4.52†	2.20†	4.85†	4.65†
Prostate	2.78	BLQ	3.24	1.10	3.23	1.17	4.33	0.839
Rectum mucosa	0.989	0.821	1.75	0.778	1.55	1.30	4.54	BLQ
Salivary gland	2.91	BLQ	3.56	0.868	5.03	0.941	4.52	BLQ
Seminal vesicle	BLQ	BLQ	1.97	0.783	1.63	0.938	1.92†	BLQ
Skin (non-pigmented)	1.06	BLQ	1.87	0.988	2.16	1.01	2.25	BLQ
Small intestine	1.74	3.65	9.95	3.07	10.0	2.57	12.1	2.38
Small intestine mucosa	3.15	7.81	16.7	7.26	21.9	9.43	32.5	13.6
Spinal cord	1.41	ND	1.80	ND	2.92	BLQ	2.68	ND
Spleen	2.12	ND	2.47	1.00	4.02	1.11	3.50	1.09
Stomach	4.31	BLQ	4.74	1.56	5.93	1.11	3.61	1.14
Stomach mucosa	5.48	ND	6.27	ND	9.00	ND	7.38	ND
Stomach wall	1.57	ND	3.19	ND	2.43	ND	2.27	ND
Testis	1.01	BLQ	1.50	0.882	2.26	1.00	2.21	0.752
Thymus	1.42	BLQ	1.53	BLQ	2.26	BLQ	2.43	BLQ
Thyroid	2.15	ND	4.88	2.08	5.23	2.52	5.74	3.23
Tongue	2.43	BLQ	3.22	0.927	4.27	0.970	4.04	0.809
Tooth pulp	1.23	ND	1.67	1.07	3.07	0.884	2.79	ND
Urinary bladder	5.39	3.77	4.79	1.71	2.67	12.8	3.60	ND
Urine	8.85	9.75	16.6	16.4	58.0	20.4	21.5	24.0
Blood (by LSC)	1.08	1.03	2.16	1.41	3.36	1.75	2.76	1.31
Plasma (by LSC)	1.59	1.57	2.73	1.53	4.57	1.60	3.47	1.05

BLQ: Below limit of quantitation; LSC: Liquid scintillation counter; ND: Not detectable (sample shape was not discernible from the background or surrounding tissue) † Tissue appeared to be fat soaked. Radioactivity concentration is shown as isavuconazonium (free form) equivalent.

Source: Study [9766-ME-1020]

Blood Brain Barrier penetration

In Study.9766-ME-0049, oral administration of 25 mg/kg isavuconazonium sulfate to rats resulted in isavuconazole brain at levels about twice those detected in the plasma, based on C_{max} and AUC_{inf} comparisons.

Table PK5: Pharmacokinetics of Isavuconazole in brain tissue after oral administration of 25 mg/kg isavuconazonium to rats. Study.9766-ME-0049

Compound	Matrix	$T_{1/2}$ (h)	T_{max} (h)	C_{max} (ng/mL)	AUC_{last} (ng.h/mL)	AUC_{inf} (ng.h/mL)	% ext
Isavuconazole	Brain	3.7	3	6947	53678	54309	1.2
Isavuconazole	Plasma	3.8	3	3666	30275	30678	1.3

Placental transfer

In Study 9766-ME-1019, intravenous administration of [cyano-^{14}C] isavuconazonium sulfate at 5 mg/kg to female rats on day 14 of pregnancy resulted in radioactivity levels in the fetus and fetal tissues were comparable to those in the maternal blood. Radioactivity levels decreased with time after dosing.

Reference ID: 3689714

Table PK5: Radioactivity Concentrations in Tissue after Single Intravenous Administration of 5 mg/kg of [Cyano-^{14}C] Isavuconazonium Sulfate to Female Rats on Day 14 of Pregnancy. Study 9766-ME-1019

Tissue	^{14}C-radioactivity concentrations in tissue (mcg eq./mL or mcg eq./g)			
	0.5 h	8 h	24 h	48 h
Blood	0.89 ± 0.10	0.34 ± 0.02	0.12 ± 0.01	0.05 ± 0.02
Plasma	0.82 ± 0.11	0.42 ± 0.04	0.15 ± 0.02	0.05 ± 0.01
Brain	2.02 ± 0.19	0.76 ± 0.06	0.17 ± 0.03	0.03 ± 0.01
Heart	4.35 ± 0.18	1.66 ± 0.22	0.37 ± 0.05	0.06 ± 0.02
Lung	3.84 ± 0.32	1.54 ± 0.22	0.41 ± 0.01	0.11 ± 0.02
Liver	17.93 ± 0.81	5.51 ± 0.60	1.98 ± 0.21	0.62 ± 0.09
Kidney	5.70 ± 0.68	2.34 ± 0.21	0.67 ± 0.06	0.16 ± 0.03
Spleen	2.74 ± 0.15	1.00 ± 0.12	0.28 ± 0.02	0.09 ± 0.00
Pancreas	7.51 ± 0.24	2.79 ± 0.19	0.71 ± 0.14	0.13 ± 0.02
Mammary gland	7.21 ± 0.99	7.43 ± 1.35	1.44 ± 0.22	0.23 ± 0.13
Ovary	5.58 ± 0.41	2.64 ± 0.24	0.81 ± 0.17	0.15 ± 0.02
Uterus	1.50 ± 0.03	0.72 ± 0.04	0.20 ± 0.05	0.05 ± 0.01
Amniotic fluid	0.08 ± 0.00	0.04 ± 0.01	0.01 ± 0.01	ND
Placenta	2.46 ± 0.10	0.89 ± 0.11	0.24 ± 0.02	0.06 ± 0.02
Fetus	0.77 ± 0.10	0.34 ± 0.02	0.12 ± 0.03	0.03 ± 0.01

ND: Not detected (below detection limit)

Mean ± standard deviation in 3 animals.

Radioactivity concentration is shown as isavuconazonium (free form) equivalent.

Source: [Study 9766-ME-1019]

Plasma Protein binding

In study 9677-ME-1011, the plasma protein binding of isavuconazole was evaluated over a range of isavuconazole concentrations (0.2-20 µg/mL). Binding was above 96 % in all the species examined (ICR mice, (98.7%-99.1%), Wistar rats (97.3%-97.9%), SD rats (97.3%-97.8%), Hartley guinea pigs, (96.4%-97.2%), Himalayan rabbits (97.3%-98.0%) cynomolgus monkeys, (99.0%) and humans, (99.2%-99.4%).

Metabolism

After a single oral 10 mg/kg dose of isavuconazonium chloride to male SD rats, isavuconazonium was not detected in plasma, suggesting a rapid conversion of isavuconazonium. . In vitro studies confirmed that isavuconazonium was rapidly converted to the active moiety, isavuconazole, with a $t_{1/2} < 2$ min in rat, rabbit, cynomolgus monkey, and human plasma (Study 9766-ME-0004, see Figure PK7). Isavuconazonium was minimally (less than 20% in 5 min) converted in dog plasma (data not shown). The conversion of isavuconazonium to isavuconazole was completely inhibited by the addition of an esterase inhibitor (1 mmol/L paraoxon) in rat, cynomolgus monkey, and human plasma, suggesting the involvement of plasma esterases in the conversion of isavuconazonium to isavuconazole (Table PK6).

Table PK6 $t_{1/2}$ values (and % inhibition) of isavuconazonium in the presence and absence of the esterase inhibitor paraoxon

	$t_{1/2}$ (minutes)		% inhibition
	control	+ paraoxon	
Rat	0.25	36	99
Monkey	0.85	45	98
Human	0.75	73	99

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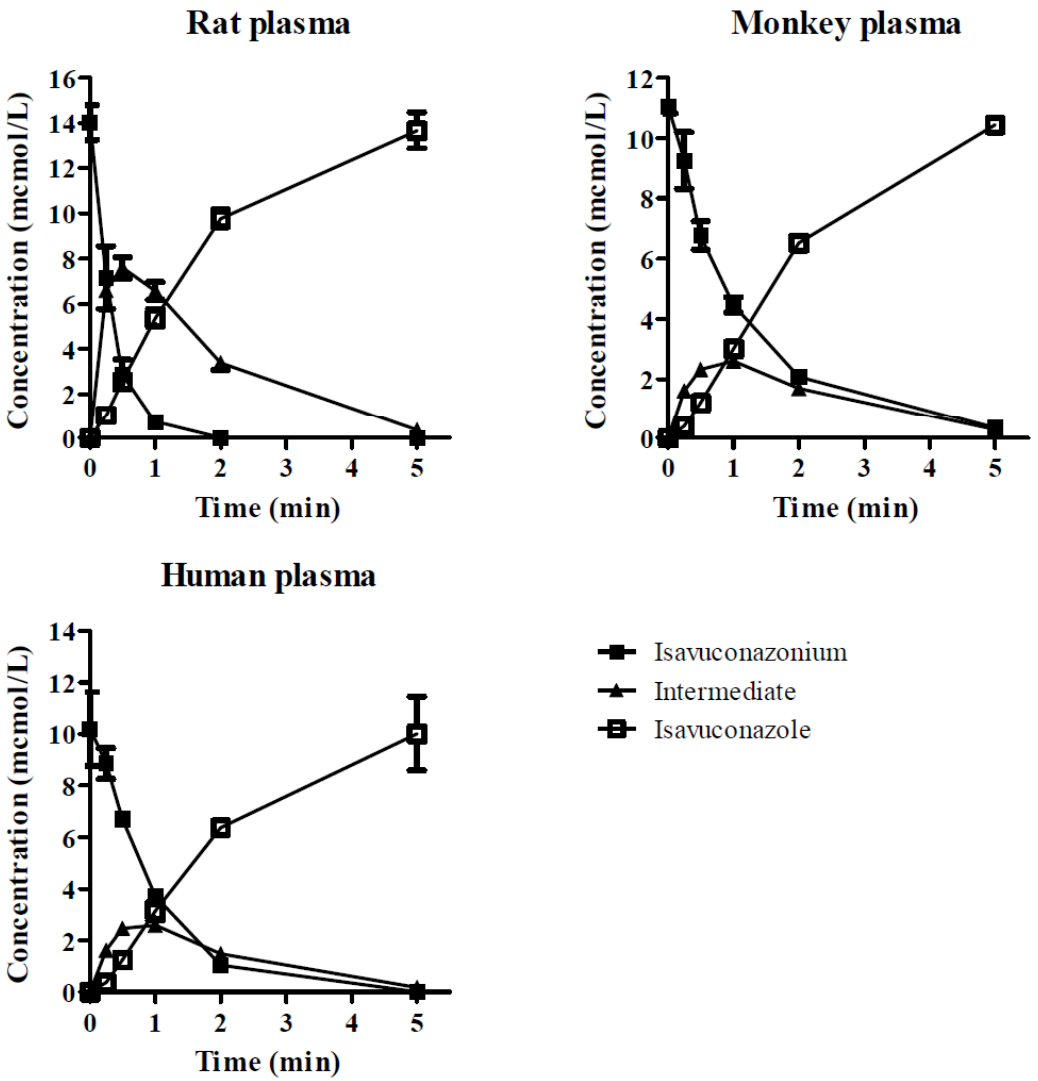
Figure PK7: Isavuconazole concentration/times curves showing the *In Vitro* Conversion of 10 µmol/mL isavuconazonium in Rat, Monkey and Human Plasma



Isavuconazonium concentration: 10 mcmol/L
Mean ± standard deviation of the triplicate experiments.
Source: [Study 9766-ME-0004]

Isavuconazonium sulfate contains 3 chiral centers [redacted] (b) (4) In study 9766-ME-0005, plasma concentrations of isavuconazonium, isavuconazole, and BAL8728 were simultaneously analyzed using liquid chromatography in combination with tandem mass spectrometry (LC-MS/MS). [redacted] (b) (4) before converting to isavuconazole and the inactive cleavage product, BAL8728. See Figure PK7. See Figure PK8.

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Figure PK8 Isavuconazonium metabolism

(b) (4)

Reference ID: 3689714

Table PK9: Pharmacokinetic Parameters after Single Intravenous and Oral Administrations of Isavuconazonium Chloride and after a Single Intravenous Administration of Isavuconazole to Female Monkeys Study 0766-ME-1017

Administered compound	Isavuconazonium chloride					Isavuconazole
Dosing route	IV			PO without food	PO with food	IV
Dose (mg/kg)	3			3	3	1
Dose as isavuconazole (mg/kg)	1.66			1.66	1.66	1
Dose as BAL8728 (mg/kg)	0.624			0.624	0.624	
Analyte	Isavuconazonium	Isavuconazole	BAL8728	Isavuconazole	Isavuconazole	Isavuconazole
Pharmacokinetic parameters						
t_{max} (h)	NC	0.083 ± 0.000	0.083 ± 0.000	3.33 ± 1.15	5.00 ± 1.73	0.194 ± 0.096
C_0 (mcg/mL)	3.06 ± 1.76	NC	NC	NC	NC	0.525 ± 0.077
C_{max} (mcg/mL)	NC	1.03 ± 0.23	0.388 ± 0.044	0.360 ± 0.067	0.175 ± 0.079	0.972 ± 0.489
AUC_{inf} (mcg·h/mL)	0.343 ± 0.228	5.08 ± 0.81	0.218 ± 0.06	4.39 ± 0.81	3.54 ± 1.54	3.66 ± 0.62
$t_{1/2}$ (h)	0.133 ± 0.073	9.84 ± 0.72	0.395 ± 0.015	12.8 ± 3.6	9.40 ± 2.35	11.7 ± 5.3
F_{con} (%)	NC	84.2 ± 11.3	NC	NC	NC	NC
F_{oral} (%)	NC	NC	NC	86.9 ± 12.8	67.9 ± 19.0	NC
CL (mL/min/kg)	202 ± 168	NC	NC	NC	NC	4.64 ± 0.74
CL/F_{con} (mL/min/kg)†	NC	5.54 ± 0.87	50.5 ± 15.9	6.43 ± 1.13	8.71 ± 3.08	NC
V_{ss} (L/kg)	1.01 ± 0.55	NC	NC	NC	NC	3.01 ± 1.46
V_z/F_{con} (L/kg)†	NC	4.74 ± 1.02	1.74 ± 0.62	7.28 ± 2.69	7.42 ± 3.74	NC

NC: Not calculated

Mean ± standard deviation of 3 animals.

† When isavuconazonium was administered intravenously, F_{con} means the conversion ratio of isavuconazonium to isavuconazole and BAL8728 in the body. When isavuconazonium was administered orally, F_{con} means a combination of the conversion ratio of isavuconazonium to isavuconazole in the body and oral bioavailability.

Source: [Study 9766-ME-0017]

Study 0766-ME-1017 shows that after intravenous administration of isavuconazonium to rats and cynomolgus monkeys, isavuconazonium was rapidly converted to isavuconazole and BAL8728. The Fcon of isavuconazonium to isavuconazole reached 84.2% in cynomolgus monkeys.

Postulated metabolic Pathway

Major metabolic pathways of isavuconazonium in rats and cynomolgus monkeys were cleavage of isavuconazonium, followed by generation of isavuconazole and BAL8728 (M5). After the thiazole ring of isavuconazole was cleaved, metabolites were formed by oxidation and subsequent glucuronidation, acetylcysteine conjugation, hydrolysis of cyano group, oxidation of the carbamoyl form (in rats and cynomolgus monkeys).

Isavuconazole was also metabolized by oxidation and subsequent glucuronidation or glutathione conjugation, and then cysteinylglycine conjugation through hydrolysis (in rats), and by glucuronidation, oxidation and subsequent glucuronidation, hydrolysis of cyano group, oxidation of the carbamoyl form or cysteinylglycine conjugation, and then cysteine conjugation through hydrolysis (in cynomolgus monkeys). The carbamate group of BAL8728 was cleaved and metabolized by oxidation or glucuronide conjugation in rats and cynomolgus monkeys. BAL8728 was also metabolized by glutathione conjugation, and then cysteine conjugation through hydrolysis in rats, and by cysteine and acetylcysteine conjugations in cynomolgus monkeys.

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Table PK 10: Cross-Species Comparison of Metabolites in Plasma

Sample	Rats	Cynomolgus Monkeys	Humans
Plasma	**Isavuconazole** **M3** **M4** **BAL8728 (M5)** **M6** **M7** **M28** **M29** M46 M47	**Isavuconazole** **M1** **M4** **BAL8728 (M5)** **M6** M61	**Isavuconazole** **M1** **M2** **M3** **M4** **BAL8728 (M5)** **M6** **M7**

Text in bold: Metabolites observed in humans.

Source: [Studies 9766-ME-1007, 9766-ME-1018, 9766-ME-1021, 9766-ME-1027, 9766-ME-1030].

All metabolites including isavuconazole in human plasma, except for M2, were found in rats and monkeys Since M2 accounted for approximately 1/10000 ion intensity of isavuconazole by LC-MS measurement it was considered to be negligible compared to isavuconazole.

Excretion

In Study No. 9766-ME-1017, oral administration of [cyano-^{14}C] isavuconazonium sulfate or [pyridinylmethyl-^{14}C] isavuconazonium sulfate to cynomolgus monkeys resulted in isavuconazole-related materials in both the feces (58 %) and urine (38 %), while BAL8728-related materials predominantly (87 %) in the urine (See Table PK11). The overall recovery was 95.9% or more for [cyano-^{14}C]-radioactivity, and 96.0% or more for [pyridinylmethyl-^{14}C]-radioactivity.

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Table PK11: Urinary and Fecal Excretion of Radioactivity (% of dose) after Single Oral Administration of 3 mg/kg of [Cyano-^{14}C] Isavuconazonium Sulfate or [Pyridinylmethyl-^{14}C] Isavuconazonium Sulfate to Male Monkeys

Sampling periods (h)	Cumulative excretion of [cyano-^{14}C]-radioactivity (% of dose)		Cumulative excretion of [pyridinylmethyl-^{14}C]-radioactivity (% of dose)	
	Urine	Feces	Urine	Feces
0 to 6 h	10.0 ± 3.4	–	58.3 ± 28.2	–
0 to 12 h	12.9 ± 4.0	–	64.2 ± 23.4	–
0 to 24 h	25.2 ± 3.8	17.6 ± 10.1	82.3 ± 5.1	5.1 ± 1.6
0 to 48 h	33.2 ± 2.4	39.0 ± 11.0	85.4 ± 3.3	8.4 ± 1.6
0 to 72 h	35.6 ± 1.4	48.7 ± 6.5	86.0 ± 3.2	8.9 ± 1.8
0 to 96 h	36.7 ± 0.8	53.8 ± 3.9	86.2 ± 3.1	9.2 ± 1.8
0 to 120 h	37.2 ± 0.5	56.4 ± 1.7	86.3 ± 3.2	9.4 ± 1.9
0 to 144 h	37.5 ± 0.4	57.5 ± 0.7	86.5 ± 3.1	9.5 ± 1.8
0 to 168 h	37.7 ± 0.3	58.2 ± 0.6	86.5 ± 3.2	9.6 ± 1.9
Cage wash (% of dose)	0.8 ± 0.3		1.6 ± 0.8	
Total recovery (% of dose)	95.9 ± 0.3		96.0 ± 3.2	

–: Not determined

Mean ± standard deviation of 3 animals.

Source: [Study 9766-ME-1017]

In study No. 9766-ME-0022, a single intravenous dose of 2.5 mg/kg of [^{14}C/^{3} H] isavuconazonium chloride to bile duct-cannulated rats, resulted in cumulative excretion of radioactivity of 7.8% in urine and 81.4% in bile for ^{14} C-radioactivity, and 63.6% in urine and 18.8% in bile for 3H-radioactivity. The percentage of ^{14}C radioactivity excreted into bile suggested isavuconazole-related materials are mainly eliminated into the bile and M5 is mainly eliminated via the urine.

Table PK12 Urinary and Biliary Excretion of Radioactivity (% of dose) after Single Intravenous Administration of 2.5 mg/kg of [^{14}C /3H] Isavuconazonium Chloride to Male Rats

Sampling periods (h)	Cumulative excretion of ^{14}C-radioactivity (% of dose)		Cumulative excretion of ^{3}H-radioactivity (% of dose)	
	Urine	Bile	Urine	Bile
0 to 7 h	4.57 ± 0.94	69.62 ± 2.03	52.83 ± 1.53	17.87 ± 5.00
0 to 24 h	7.48 ± 0.61	80.14 ± 0.44	62.31 ± 4.86	18.28 ± 4.91
0 to 48 h	7.75 ± 0.67	81.43 ± 0.64	63.64 ± 5.49	18.75 ± 4.92

Mean ± standard deviation of 3 animals.

Source: [Study 9766-ME-0022]

Enterohepatic Circulation

In Study No. 9766-ME-1029, bile samples collected from bile duct-cannulated rats (0 to 6 h) after a single intravenous administration of 5 mg/kg of [cyano-^{14}C] isavuconazonium sulfate or [pyridinylmethyl-^{14}C] isavuconazonium sulfate were administered intraduodenally to a separate group of bile duct cannulated

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rats. The cumulative excretion of radioactivity over 48 h post-dose was 9.9% in urine and 56.2% in bile for [cyano-^{14}C]-radioactivity, and 70.2% in urine and 12.9% in bile for [pyridinylmethyl-^{14}C]-radioactivity These results showed that at least 66.1% of the administered [cyano-^{14}C]-radioactivity, and 83.1% of [pyridinylmethyl-^{14}C]-radioactivity were reabsorbed, indicating that constituents derived from [cyano-^{14}C] isavuconazonium sulfate and [pyridinylmethyl-^{14}C] isavuconazonium sulfate undergo enterohepatic circulation in rats.

Table PK13: Urinary and Biliary Excretion of Radioactivity (% of dose) after Single Intraduodenal Administration of Bile (0.5 mL) Collected from Other Rats Intravenously Administered 5 mg/kg of [Cyano-^{14}C] Isavuconazonium Sulfate or [Pyridinylmethyl-^{14}C] Isavuconazonium Sulfate

Sampling periods (h)	Cumulative excretion of [cyano-^{14}C]-radioactivity (% of dose)		Cumulative excretion of [pyridinylmethyl-^{14}C]-radioactivity (% of dose)	
	Urine	Bile	Urine	Bile
0 to 7 h	3.7 ± 0.8	32.2 ± 1.8	55.4 ± 9.8	10.1 ± 1.2
0 to 24 h	9.4 ± 0.5	54.9 ± 4.3	68.0 ± 9.5	12.9 ± 1.9
0 to 48 h	9.9 ± 0.5	56.2 ± 4.7	70.2 ± 7.2	12.9 ± 1.9

Mean ± standard deviation of 4 animals.
Source: [Study 9766-ME-1029]

Lactating rats

In study 9766-ME-1019, pregnant dams were intravenously administered a single 5 mg/kg dose of [cyano-^{14}C] isavuconazonium sulfate or [pyridinylmethyl-^{14}C] isavuconazonium sulfate on day 14 after parturition) o. 9766-ME-1019. Cyano-^{14} C isavuconazonium sulfate, radioactivity concentrations in the milk were 8.14 and 4.53 times as high as those in the maternal plasma at 0.5 and 8 h post-dose, respectively. See Table PK14

Table PK14 Radioactivity Concentrations in Tissue µg eq/mL or µg eq/g after Single Intravenous Administration of 5 mg/kg of [Cyano-^{14}C] Isavuconazonium Sulfate to Lactating Rats (Day 14 after Parturition)

Tissue	^{14}C-radioactivity concentrations in tissue (mcg eq./mL or mcg eq./g)			
	0.5 h	8 h	24 h	48 h
Dam				
Blood	0.86 ± 0.09	0.53 ± 0.16	0.35 ± 0.20	0.13 ± 0.08
Plasma	0.81 ± 0.09	0.35 ± 0.09	0.22 ± 0.11	0.09 ± 0.04
Milk	7.00 ± 0.67	2.40 ± 0.25	0.43 ± 0.13	0.25 ± 0.16
Nursing pup				
Blood	ND	0.05 ± 0.02	0.15 ± 0.06	0.19 ± 0.11
Plasma	ND	0.06 ± 0.03	0.17 ± 0.11	0.25 ± 0.14
Brain	0.01 ± 0.02	0.03 ± 0.02	0.02 ± 0.01	0.01 ± 0.01
Heart	0.02 ± 0.02	0.05 ± 0.02	0.05 ± 0.03	0.04 ± 0.02
Lung	0.03 ± 0.05	0.05 ± 0.03	0.06 ± 0.03	0.06 ± 0.03
Liver	0.11 ± 0.14	0.31 ± 0.13	0.37 ± 0.17	0.34 ± 0.12
Kidney	0.02 ± 0.02	0.11 ± 0.04	0.16 ± 0.08	0.16 ± 0.09
Intragastric milk lump	3.29 ± 2.97	5.20 ± 0.69	0.93 ± 0.20	0.33 ± 0.12

ND: Not detected (below detection limit)
Mean ± standard deviation of 3 animals.
Radioactivity level is shown as isavuconazonium (free form) equivalent. Source: [Study 9766-ME-1019]

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Interaction with antacid

In Study 9766-ME-0048, pharmacokinetic drug interactions with Maalox®, were investigated in non-fasted and fasted mice after oral administrations of isavuconazonium sulfate. In non-fasted mice, the Cmax of isavuconazole was slightly decreased when administered together with Maalox®, however, the area under concentration-time curve from zero time until the last measurable concentration (AUClast) was comparable when isavuconazonium was administered alone or together with Maalox®, or sequentially (10 min and 30 min) after Maalox®.

Summary

There was good absorption of CRESEMBA after oral administration, with bioavailability between 62 % (rats) and 87 % (monkeys) after a single oral dose. Isavuconazole was eliminated with a $t_{½}$ of 5 h in rats and 10 h in monkeys. Except for the active moiety isavuconazole, no individual metabolite was observed with an AUC > 10 % of drug related material. In some rat and monkey studies, induction of CYP3A and/or CYP2B was observed.

CRESEMBA is extensively bound to proteins, with plasma protein binding above 96% for all species, including humans (99%). Quantitative whole body autoradiography data showed that, the ^{14}C -radioactivity concentrations were highest in the adrenal cortex and liver, followed by the small intestinal mucosa, brown fat, Harderian gland, pancreas, intra-orbital lacrimal gland, kidney cortex, adrenal medulla, stomach mucosa and the thyroid. After intravenous administration to pregnant rats, isavuconazole-related radioactivity was detected in the fetuses, in some instances at levels comparable to that in the maternal plasma.

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6. General Toxicology

6.1 Single-Dose Toxicity

Study title:	Single oral and intravenous administration toxicity study of [redacted] (b) (4) in rats
Sponsor reference # :	9766-TX-0001
Study report location:	EDR
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	January 2000
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #:	JO-10179
Purity (%):	98.99 %
Frequency of dosing:	Single
Route of administration:	Oral and intravenous
Doses	500, 1000, 2000 mg/kg (oral) 5, 10, 20 mg/kg (i.v. bolus) 10, 20, 40 mg/kg (intravenous, 0.1 mL/min
Dose volume:	2 mL/100g body weight
Formulation/Vehicle:	Distilled water
Species/Strain:	Rat, Sprague Dawley
Number/Sex/Group:	5
Age:	4 weeks old
Weight:	Male 110-160 g, Female 90-140 g

Key study findings: The maximum nonlethal (MNL) single oral dose was 500 mg/kg. The MNL intravenous dose was higher at slower infusion rates. The MNL intravenous dose was 5 mg/kg at 1 mL/min and 20 mg/kg at 0.1 mL/min.

In this study rats were given a single dose of isavuconazonium and observed for 14 days. The maximum nonlethal single oral dose was 500 mg/kg. Significant findings included deaths and clinical signs which are shown below on Table T1.

Table T1: Mortality and clinical signs in rats dosed with single oral doses of isavuconazonium chloride

Dose	Mortality	Clinical signs
500	0/10	Decreased spontaneous activity and prone position
1000	7/10	Decreased spontaneous activity and prone position, staggering gait, jumping, labored respiration, cyanosis, hypothermia, diarrhea and lacrimation, decreased body weight, Pulmonary congestion, gastric hemorrhage and yellowish content inside the lower alimentary tract.
2000	10/10	

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Intravenous administration of (b) (4) to rats, at administration speed of 1 mL/min resulted in a maximum nonlethal intravenous dose of 5 mg/kg. Deaths and clinical signs are shown on Table AT3.

Table T2: Mortality and clinical signs in rats dosed with single intravenous doses of isavuconazonium chloride at administration speed of 1 mL/min

Dose	Mortality	Clinical signs
5	0/10	Decreased spontaneous activity and prone position
10	5/10	Decreased spontaneous activity and prone position, staggering gait, irregular respiration (dyspnea), shivering/tremor
20	10/10	Decreased spontaneous activity and prone position, irregular respiration (dyspnea), shivering/tremor

Table T3: Mortality and clinical signs in rats dosed with intravenous doses of isavuconazonium chloride at administration speed of 0.1 mL/min

Dose	Mortality	Clinical signs
0	0/10	No significant changes
10	0/10	No significant changes
20	0/10	No significant changes
40	1/10	Decreased spontaneous activity, lying on the belly, tremor and irregular respiration staggering gait, jumping,

After oral administration, the maximum tolerated dose was 500 mg/kg. Above 500 mg/kg, drug was not well tolerated and animals were in poor condition and/or died.

Bolus injections of 20 mg/kg resulted in 100 % mortality in rats, with no mortality at 5 mg/kg. When the rate of infusion was decreased to 0.1 mL/min, no mortality occurred at 20 mg/kg. (See Table T2 and T3). When the rate of infusion was reduced to 0.1 mL/min the maximum nonlethal intravenous dose increased to 20 mg/kg. Deaths and clinical signs are shown on Table T3.

Body weights:
At 1000 and 2000 mg/kg, body weights were slightly reduced by up to 15 % up to day 3 but body weights were similar to controls in surviving animals by day 7.

Gross pathology:
Findings in 2000 mg/kg animals included lung congestion and watery, yellow/white content in the stomach/duodenum/ileum/jejunum. Animals dosed intravenously at 40 mg/kg showed blackness and crust formation at the injection site and loss of tail

Discussion:

In rats, the maximum nonlethal oral dose was 500 mg/kg. The toxic effects after an intravenous dose were different depending on the rate of infusion. After i.v. bolus injection (1mL/min), severe adverse findings (including deaths) were observed above 5 mg/kg for rats. When the rate of infusion was decreased, animals were much more tolerant to the effects of the drug and the maximum nonlethal dose was increased to 20 mg/kg. Future toxicity studies were designed for based on these data. Repeat dose toxicity studies were conducted at doses of up to 150 mg/kg/day.

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Study title:	A single dose toxicity study of [redacted] (b) (4) in monkeys (Oral, i.v. bolus and i.v. infusion)
Sponsor reference	9766-TX-0002
Study report location:	EDR
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	October 1999
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #:	MTS2111
Purity (%):	98.93 %
Frequency of dosing:	Single
Route of administration:	Oral and intravenous
Doses	500, 1000, 2000 mg/kg (oral) 4, 8, 16, 32 and 64 mg/kg (i.v. bolus) 90 and 120 mg/kg (i.v, 0.1 mL/min)
Dose volume:	2.5-10 mL/kg body weight
Formulation/Vehicle:	Distilled water
Species/Strain:	Cynomolgus monkeys
Number of males /Group:	5
Age:	4-6 years old
Weight:	3.7 to 4.8 kg
Key study findings:	The maximum nonlethal oral dose in cynomolgus monkeys was 1000 mg/kg

Key study findings: The maximum nonlethal (MNL) single oral dose was 500 mg/kg. The MNL intravenous dose was higher at slower infusion rates. The MNL intravenous dose was 5 mg/kg at 1 mL/min and 20 mg/kg at 0.1 mL/min

Results

The maximum nonlethal oral dose in cynomolgus monkeys was 1000 mg/kg. Mortality and clinical signs are shown on Table T4.

Table T4: Mortality and clinical signs in cynomolgus monkeys dosed with oral doses of isavuconazonium chloride

Dose	Mortality	Clinical signs
500	0/2	Vomiting, decreased food intake
1000	0/2	Salivation
2000	1/2	Red face, loss of strength, prone position or sitting, incomplete eyelid opening, decreased spontaneous activity and dyspnea.

The toxic effects of intravenous dosing resulted in varying degrees of toxicity depending on the dose and infusion rates. Slower infusion rates (administration speed 0.1 mL/min) resulted in better tolerability (See Table AT5) compared to higher infusion speeds (administration speed 1 mL/min). The maximum nonlethal bolus (1 ml/min) intravenous dose in monkeys was 32 mg/kg. Mortality and clinical signs are shown on Table T5

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Table T5: Mortality and clinical signs in monkeys dosed with intravenous doses of isavuconazonium chloride at administration speed of 1 mL/min

Dose	Mortality	Clinical signs
4	0/2	Incomplete eye opening, red face
8	0/2	Loss of strength, lying on belly, red face
16	0/2	Dyspnea, respiration stop, Incomplete eye opening, red face, loss of strength, lying on belly, decreased spontaneous motor activity
32	0/2	Dyspnea, incomplete eye opening, red face, loss of strength, lying on belly, decreased spontaneous motor activity
64	2/2	Loss of strength, red face, lying on side, respiration stop, death

At the slower infusion rate of 0.1 mL/min, the maximum nonlethal intravenous (infusion) dose in cynomolgus monkeys was 90 mg/kg. Mortality and clinical signs are shown on Table T6

Table T6: Mortality and clinical signs in monkeys dosed with intravenous doses of isavuconazonium chloride at administration speed of 0.1 mL/min

Dose	Mortality	Clinical signs
90	0/2	Incomplete eye opening, decreased spontaneous motor activity
120	1/2	Incomplete eye opening, decreased spontaneous motor activity, sitting, loss of strength, lying on belly, vomiting, death

Body weights:
There were no biologically significant weight changes in monkeys.

Gross pathology:
There were no abnormal gross pathology findings.

Blood Chemistry

One animal receiving the 32 mg/kg (bolus) dose showed marked but transient increases in AST (24x controls) and ALT (4.5x controls) without accompanying increases in ALP. Values were comparable to controls by 6 days post dose.

Summary of individual study findings:

The maximum nonlethal oral dose of isavuconazonium in monkeys was 1000 mg/kg. The toxic effects of intravenous dosing resulted in varying degrees of toxicity depending on the dose and infusion rates. Slower infusion rates (administration speed 0.1 mL/min) resulted in better tolerability (See Table AT5) compared to higher infusion speeds (administration speed 1 mL/min). While bolus doses resulted in markedly increased liver enzymes and/or death at doses ≥32 mg/kg, doses up to 90 mg/kg were tolerated at infusion rates of 0.1 ml/min. Reducing the rate of infusion reduced the toxic effects of this drug.

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6.2 Repeat-Dose Toxicity

Study title:	A Repeated Dose Toxicity Study of (b) (4) administered Orally to Cynomolgus Monkeys for 4 Weeks Followed by a 4 Week Recovery Period
Key study findings:	NOAEL was 10 mg/kg/day. Increased liver and adrenal weights and decreased cholesterol and increased triglycerides were observed at 30 and 90 mg/kg. Mortality was observed at 90 mg/kg.
Study no.:	9766-TX-0017
Document location	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 24, 2006
GLP compliance:	(b) (4)
QA report :	Yes
Drug, lot #,	MTS2113
% purity:	98.35%

Methods

Doses:	0, 10, 30 or 90 mg/kg
Species/strain:	Cynomolgus monkey
Number/sex/dose (main study):	3
Age	3-6 years old.
Weight	Males: mean 3.5-4.6 kg Females: mean 2.5-3.6 kg
Route	Oral gavage
Formulation	Purified water
Volume	2 mL/kg
Number/sex/dose (recovery group):	1 (control and high dose)

Results**:**

Mortality:
Two females from the 90 mg/kg group died (one on Day 16 and one on Day 25). These animals showed sporadic salivation, vomiting, diarrhea, decrease in spontaneous activity, emaciation, decreases in food consumption and body weight, an accentuated lobular pattern in the liver, increase liver weight, fatty change in the hepatocytes, enlarged adrenal , thickening of the zona fasciculata, increases in BUN and creatinine, necrotic changes in the colon and esophagus, atrophy in the lymphoid tissues and glands.

Clinical signs:
No clinical signs were recorded at 10 mg/kg/day. At 30 mg/kg/day, monkeys showed excessive salivation. At 90 mg/kg, signs of toxicity included vomiting (transiently observed at Weeks 1 and 2 of dosing), salivation, soft stool/diarrhea, decrease in spontaneous activity, and emaciation late in the dosing period. All animals were observed at least three times daily.

Body weights:

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Animals dosed at 90 mg/kg showed body weight decreases of 12 to 18 % beginning 2 weeks after dosing began. At the end of dosing mean body weights were reduced by about 15 % compared to predose weights, although some individual animals that died showed body weight decreases of 30 % compared to predose values. At the end of the reversibility period, the bodyweights were comparable to predose values. The body weight for each animal was measured once prior to the initiation of dosing and once a week thereafter and on the day of necropsy.

Feed consumption
At 90 mg/kg feed consumption was significantly reduced by 14 to 71 % of predose values beginning in Week 1. Feed consumption was comparable to predose levels by the end of the reversibility period. The number of solid food pieces supplied and the number left over were recorded daily for each animal, and food consumption was calculated. The average daily consumption was calculated each week.

EKG
In Week 4, disappearance of the P wave was noted 4 hours after dosing in one high dose male and one high dose female. There were no other biologically significant effects on the EKG. Electrocardiogram examinations were performed on all animals using an electrocardiograph system for animals, taking a standard limb lead (I; II, III and aVR, aVL, aVF) without anesthesia. ECG was recorded once prior to the initiation of dosing between 13:18 and 14:25; 4 and 24 hours following dosing at Weeks 2 (Days 10 and 11) and 4 (Days 24 and 25) of dosing and Week 4 of recovery between 13:22 and 13:50. The ECG was evaluated for heart rate, PR interval, QRS interval, QT interval and QTc of the wave of II lead.

Ophthalmology
No test article-related findings were noted at the end of the treatment period. Slit-lamp examinations were performed on all animals once prior to the initiation of dosing, once on Day 18 of dosing, and once on Day 18 of recovery. Ocular fundus examinations were performed on all animals once prior to the initiation of dosing, once on Day 18 of dosing and once on Day18 of recovery.

Hematology/Clinical chemistry
Total cholesterol was reduced by 22 % at 30 mg/kg (Day 13 and Day 27, males only) and by up to 39 % at 90 mg/kg (Day 13 and Day 27, males and females). Triglycerides were increased at 30 mg/kg (by 50 to 70 % beginning at Day 13) and at 90 mg/kg (by 85 to 370 % beginning on Day 13) compared to predose values. These effects were reversible since values were comparable to control or predosing values by the end of the 4 week recovery period. Blood was drawn from the femoral vein of each animal and placed in vials precoated with an anticoagulant (EDTA-2K) once prior to the initiation of dosing, once on Days 13 and 27 of dosing (prior -to dosing) and once on Day 27 of recovery.

Urinalysis:
There were no biologically relevant quantitative or qualitative differences noted between control and treated animals at the end of the treatment period or at the end of the reversibility period. Urinalysis was performed on all animals once prior to the initiation of dosing, once on Day 26 of dosing and once on Day 26 of recovery. Fresh urine was collected within 2 hours prior to dosing using a metabolic cage.

Gross pathology:
At 90 mg/kg, an accentuated lobular pattern and/or enlargement of the liver were noted in two males and one female at 90 mg/kg. Enlargement of the adrenals was observed in one female. Emaciation was observed in two males and one female. No abnormal gross pathology findings were recorded at 10 or 30 mg/kg.

Organ weights
Isavuconazonium was associated with increases in absolute liver weights in males and females at 30 mg/kg (+ 25 to +40 %) and 90 mg/kg (+ 70% to +106 %). Adrenal weights were also increased in both sexes at 30 mg/kg (+27 to 29 %) and 90 mg/kg (+ 42 to +94 %). Relative organ were also similarly increased, but interpretation was

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confounded by the drug's significant effects on bodyweights. Changes to other organs were small or inconsistent or not dose dependent.
Reversibility
At the end of the recovery period the mean adrenal weight remained increased at 30 mg/kg (by +109 % in females) and at 90 mg/kg (by about 60 % in males and females compared to controls). Changes to other organs were small or inconsistent or not dose dependent.
Organ weights were obtained for the brain, pituitary, thyroids, submandibular glands, thymus, heart, lungs (including bronchi), liver, adrenals, kidneys, spleen, pancreas, testes, epididymides, seminal vesicle, prostate, ovaries and uterus of all animals.

Histopathology:

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

In the 90 mg/kg group, slight hypertrophy of the hepatocytes was seen in the livers of all males and one female. Very slight or slight fatty change of the centrilobular hepatocytes was noted in one male and one female. In the adrenal, slight thickening of the zona fasciculata was noted in two males and one female.

In the 30 mg/kg group, very slight or slight hypertrophy of the hepatocytes was seen in the livers of all males and two females. No test article-related changes were noted in the 10 mg/kg group.

Reversibility
No abnormal histopathological findings were detected at the end of the reversibility period.

For all animals the heart, aorta, spleen, thymus, bone and bone marrow, submandibular lymph nodes, mesenteric lymph nodes, lungs, bronchi, trachea, tongue, esophagus, stomach (fundus, pylorus), small intestine (duodenum, jejunum, ileum), large intestine (cecum, colon, rectum), pancreas, liver, gallbladder, kidneys, urinary bladder, epididymides, seminal vesicle, prostate, ovaries, uterus, vagina, pituitary, thyroids, parathyroids, adrenals, cerebrum, cerebellum, brain stem, spinal cord, sciatic nerve, lacrimal gland, skin (gluteal area), mammary gland (female), submandibular glands, skeletal muscle (quadriceps femoris) and face skin (gross lesion) were fixed in 10% neutral buffered formalin. The eye balls and optic nerves were fixed in a mixture of formaldehyde and glutaraldehyde, and the testes were fixed in Bouins' solution. All organs and tissues were sectioned, stained with Hematoxyline-Eosin and examined. Photographs were taken of abnormal sites.

Electron Microscopy
The liver and left kidney were fixed in 3% glutaraldehyde and 1% osmium tetroxide, and epoxy resin (Quetol 812)-embedded block specimens were prepared for electron microscopy.

Toxicokinetics
Exposure to isavuconazole was approximately dose-proportional when male and female cynomolgus monkeys were dosed with isavuconazonium at doses between 10 and 90 mg/kg/day for 4 weeks. There was evidence of slight accumulation, (Day 28 AUC ~1.5x Day 1 AUC) and slight differences in pharmacokinetics between the sexes (see Table T7, Gender Factor as low as 0.4 at 90 mg/kg).

Monkeys dosed with isavuconazonium at 90 mg/kg showed isavuconazole AUC values of 172 µg*h/mL on Day 1 and 259 µg*h/mL on Day 28. These exposures are higher than those seen in patients receiving the clinical dose of CRESEMBA, which have AUC values of 121 µg*h/mL. Tmax appears to increase with increasing dose.

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Table T7 Mean pharmacokinetic parameters of isavuconazole in male and female cynomolgus monkeys during a 4-week oral toxicity study of isavuconazonium chloride

Study No.	Species-Strain-Number/Sex/Group-Weight-Age	Study Design	Dose(mg/kg/day)-Route-Schedule-Duration	REF
09-53-10	Monkey-Cynomolgus-4 M,4 F-2.64-5.00 kg- 3-6 years	Parallel groups. Plasma was collected at 0, 1, 2, 4, 7, and 24 hours after dose on days 1 and 28, and 0 and 4 hours after dose on day 14.	10-PO-Daily-4 Weeks 30-PO-Daily-4 Weeks 90-PO-Daily-4 Weeks	

Study No.	Study Design	Concentration-Formulation	Drug-Source-Batch Number-Labelled Form	REF
09-53-10	Parallel groups. Plasma was collected at 0, 1, 2, 4, 7, and 24 hours after dose on days 1 and 28, and 0 and 4 hours after dose on day 14.	5-mg/mL-Solution 15-mg/mL-Solution 45-mg/mL-Solution	[redacted] Lot No. MTSZ113 - Unlabelled	

b(4)

Study No.	Dose(mg/kg/day)-Route-Frequency-Duration	Study Day	Analyte	C_{max} (μg/mL)	AUC T_1-T_2 (μg·hours/mL)	T_1-T_2	T_{max} (hours)	$T_{1/2}$ (hours)	AF	GF	EF
09-53-10	10-PO-Daily-4 Weeks	1	Ro 09-4815	1.76(34)	18.4(22)	0-24	3.50	NC		1.0	NC
		28		1.80(19)	19.5(17)	0-24	2.50	NC	1.1	1.0	
09-53-10	30-PO-Daily-4 Weeks	1	Ro 09-4815	4.02(28)	55.5(31)	0-24	5.13	NC		1.0	NC
		28		5.00(15)	65.7(20)	0-24	5.50	NC	1.2	0.77	
09-53-10	90-PO-Daily-4 Weeks	1	Ro 09-4815	10.6(65)	172(65)	0-24	8.50	NC		0.43	NC
		28		16.2(26)	259(26)	0-24	6.50	NC	1.5	0.75	

NC: Not calculated
AF: Accumulation Factor - Ratio of AUC after repeated administration to the AUC on the first study day.
GF: Gender Factor - Ratio of AUC in Female Animals to AUC in Male Animals
EF: Exposure Factor - Ratio of Observed AUC to the AUC Associated with Therapeutic Effect in Humans
( ): CV%

Discussion

In this four-week oral gavage toxicity study of isavuconazonium chloride in monkeys, 90 mg/kg was clearly above the maximum tolerated dose as evidenced by the mortality, reduction in feed consumption, reduction in body weight, emaciation, EKG changes (disappearance of the P wave), liver and adrenal enlargement. These changes occurred at an isavuconazole AUC of 259 µg*h/mL, about two times that seen in patients receiving the clinical dose of CRESEMBA, which have AUC values of 121 µg*h/mL.

The next lower dose, 30 mg/kg, was associated with decreased cholesterol and increased triglycerides, increased liver weights and increased adrenal weights. These changes may be related to CRESEMBA's effects on P450 enzymes and have been seen in other toxicology studies.

The NOAEL for this 4 week study was 10 mg/kg, which results in an isavuconazole exposure (AUC) of about 20 121 µg*h/mL, about one sixth of the clinical exposure.

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Study title:	13-week preliminary toxicity study by the oral route (gavage) in mice
Key study findings:	Mortality, enlarged liver, increased liver weights, centrilobular hepatocellular hypertrophy, and hepatocellular vacuolation. NOAEL less than 30 mg/kg.
Study no.:	9766-TX-0005
Document location	EDR
Conducting laboratory and location:	(b) (4) (b) (4)
Date of study initiation:	1 August 2006
GLP compliance:	Yes
QA report :	Yes
Drug, batch #,	17CH03SD.HQ00002.02
% purity:	85.2 %

Methods

Doses:	0, 30, 90 or 300 mg/kg/day
Species/strain:	Mouse, CD-1
Number/sex/group (main study):	10/sex/dose
Age	8 weeks old
Weight	Males (mean 36g); females (mean 27g)
Route	Oral gavage
Formulation	Purified water
Volume	10 mL/kg/day
Number/sex (satellite group):	2 (control) or 12 /sex/dose

Methods and Results:

Mortality:

Mortality was observed at doses above 30 mg/kg and deaths were distributed throughout the duration of the study. One male died at 300 mg/kg (study day 11) and three 90 mg/kg mice died on Days 21, 81, 83. No unscheduled deaths or premature sacrifices occurred in the control or low-dose groups during the study. Each animal was checked for mortality or signs of morbidity at least twice a day during the treatment and treatment-free periods, including weekends and public holidays.

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T9: Clinical signs in mice treated for 13 weeks with oral isavuconazonium:

Clinical signs observed in surviving animals during the study are summarized below:

Sex	Male				Female			
Dose-level (mg/kg/day)	0	30	90	300	0	30	90	300
Abdominal breathing		1	4	3			1	1
Loud breathing			2	1				1
Dyspnea			2	3				2
Increased in size/abdomen			3	4				
Thin appearance		1	3	3			1	1
Hunched posture			2	1			1	1
Piloerection		1	3	2			1	1
Cold to touch			1	0			1	
Hypoactivity			1	1			1	
Pallor of extremities				1			1	
Reflux at dosing		1	3	1				2
Half-closed eyes			2					
Total	*0/12*	*3/22*	*10/20*	*7/21*	*0/12*	*3/22*	*4/21*	*6/22*

Clinical signs:

Clinical signs (see Table T9) were observed transiently and with low frequency but were most often observed at 90 and 300 mg/kg. Each animal was observed once a day, at approximately the same time, for the recording of clinical signs. In addition, detailed clinical examinations were made once a week until the end of the study.

Body weights:

There were no toxicologically significant effects of isavuconazonium on body weights in any animals. The body weight of each animal was recorded once before group allocation, on the first day of treatment, and then once a week until the end of the study.

Food consumption:

There were no drug-related differences in food consumption between treated and control animals. The quantity of food consumed by each animal was recorded once a week during the treatment period.

Hematology:

There were no toxicologically significant effects on hematology parameters. Prior to blood sampling and during urine collection, the animals were deprived of food for an overnight period of at least 14 hours. Water was provided throughout the food deprivation period. Blood samples were taken from the orbital sinus of the animals (before the daily treatment) under isoflurane anesthesia and collected into tubes containing the appropriate anticoagulant

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Although bone marrow smears were prepared from the femoral bone of all the animals at necropsy, the bone marrow differential cell count was not determined since no relevant abnormalities were observed during the hematological investigations.

Clinical chemistry:

Slightly increased alanine aminotransferase activity (1.7 x controls) was noted in females treated at 300 mg/kg/day. Cholesterol levels were decreased in animals treated at 300 mg/kg/day (males: -45 %, females: -14) well and higher mean glucose values were observed in females (+47 %).

Table T10. Cholesterol levels (mmol/L) in mice after 13 week of oral isavuconazonium

Isavuconazonium dose	0	30	90	300
Males (n=5)				
mean	3.1	2.8	2.6	1.7*
S.D.	0.77	0.48	0.62	0.65
Females				
mean	2.1	2.2	1.8	1.8
S.D.	0.44	0.55	0.63	0.28

* P< 0.05 Dunn test.

Gross pathology:

Liver enlargement was noted in three males and four females treated at 300 mg/kg/day. All surviving animals were asphyxiated by carbon dioxide or anesthetized by an intraperitoneal injection of pentobarbital sodium and sacrificed by exsanguination.

Organ weights

Table T11, Isavuconazonium-related increases in absolute and relative liver weights (% control) at 90 and 300 mg/kg.

Sex	Male			Female		
Group	2	3	4	2	3	4
Dose-level (mg/kg/day)	30	90	300	30	90	300
Body weight	0	+1	-5	+4	0	+1
- Liver						
. absolute	+6	+23*	+66**	+11	+25**	+79**
. relative	+6	+22**	+75**	+7	+24**	+75**

Statistically significant from controls: *: p<0.05, **: p<0.01.
The significance concerned the organ weight values and not the percentages.

The body weight of each animal was recorded for all animals before sacrifice at the end of the treatment and treatment-free periods. The organs specified in the Tissue Procedure Table were weighed wet as soon as possible after dissection.

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TISSUE PROCEDURE TABLE

Organs	Organ weights	Preservation of tissues	Microscopic examination
Macroscopic lesions		X	X
Adrenals	X	X	
Aorta		X	
Brain (including medulla/pons cerebellar and cerebral cortex)	X	X	
Cecum		X	
Colon		X	
Duodenum		X	
Epididymides	X	X	
Esophagus		X	
Eyes with Harderian glands		X	
Femoral bone with articulation		X	
Gall bladder		X	
Heart	X	X	X
Ileum (with Peyers patches)		X	
Jejunum		X	
Kidneys	X	X	X
Liver	X	X	X
Lungs with bronchi		X	X
Lymph nodes (mandibular and mesenteric)		X	
Mammary glands/area		X	
Ovaries	X	X	
Pancreas		X	
Pituitary gland		X	
Prostate		X	
Rectum		X	
Salivary glands (sublingual and submaxillary)		X	
Sciatic nerve		X	
Seminal vesicles		X	
Skeletal muscle		X	
Skin		X	
Spinal cord (cervical, thoracic and lumbar)		X	
Spleen	X	X	
Sternum with bone marrow		X	
Stomach with forestomach		X	
Testes	X	X	
Thymus	X	X	
Thyroids with parathyroids		X	
Tongue		X	
Trachea		X	
Urinary bladder		X	
Uterus (horns and cervix)		X	
Vagina		X	

Histopathology: Adequate Battery: yes (√), no ( )
Peer review: yes (√), no ( )

Results

Slight to marked centrilobular hepatocellular hypertrophy and hepatocellular vacuolation were noted in the animals treated at 90 or 300 mg/kg/day. Although only two males and 3 females were evaluated at 90 mg/kg, the incidence

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and severity of the two findings appear to be dose related. No histopathology evaluations were conducted at 30 mg/kg. See Table T12.

Table T12: Incidence of hepatocellular hypertrophy and vacuolation in mice.

Incidence of hepatocellular hypertrophy and vacuolation

Sex	Male				Female			
Group	1	2	3	4	1	2	3	4
Dose-level (mg/kg/day)	0	30	90	300	0	30	90	300
Number examined	10	-	2*	10	10	-	3*	10
Centrilobular hepatocellular hypertrophy	0	-	1 (3.0)	10 (3.0)	0	-	1 (2.0)	10 (3.0)
Vacuolated hepatocellular micro/macrovacuolation	2 (1.0)	-	1 (1.0)	8 (2.1)	3 (1.0)	-	2 (1.5)	6 (2.3)

-: not examined; *: examined for necropsy abnormalities; 0: not found.

Toxicokinetics:

Exposure to isavuconazole (as measured by AUC values) was supraproportional to dose-related between 30 and 300 mg/kg. There was no indication of drug accumulation. See Table T13, below.

Table T13 Mean pharmacokinetic parameters of BAL4815 in male and female mice during a 13-week oral toxicity study with BAL8557

Dose (mg/kg)	Gender	Day 1			Week 13		
		C_{max} (ng/mL)	t_{max} (h)	AUC_{0-24h} (ng.h/mL)	C_{max} (ng/mL)	t_{max} (h)	AUC_{0-24h} (ng.h/mL)
30	Female	2050	2	14300	2030	1	13600
	Male	1340	2	8580	2350	1	10300
90	Female	9130	2	92000	6990	1	50400
	Male	5800	4	80600	5560	1	35700
300	Female	23800	2	175000	14200	2	111000
	Male	13800	4	170000	9340	4	151000

Blood samples were collected on day 1 and during week 13 at 0, 1, 2, 4, 8 and 24 h after administration, from 2 animals/sex/group at each sampling time, and on the same days at 2 hours after application in the vehicle control group. Each animal was sampled once on each occasion.

Discussion and Conclusion
This study was designed to support dose selection for a planned carcinogenicity study. When administered orally to CD-1 mice for 13 weeks at 90 mg/kg/day and above, isavuconazonium was associated with enlarged liver, increased liver weights, centrilobular hepatocellular hypertrophy and hepatocellular vacuolation. Decreased cholesterol levels at 300 mg/kg were consistent with the pharmacology of the isavuconazonium. In light of the deaths and liver findings that occurred at 90 mg/kg, this dose clearly exceeds the Maximum tolerated dose (MTD). Doses for the carcinogenicity study should be below 90 mg/kg/day. The NOAEL was less than 30 mg/kg because slight increases in liver weight were seen at 30 mg/kg.

Reference ID: 3689714

Study title:	13-Week Toxicity Study by Oral Route (Gavage) in Rats Followed by a 4-Week Treatment-Free Period
Key study findings:	At 90 mg/kg/day: reversible increases liver and thyroid weight, moderate hepatocellular hypertrophy, increased activity of the thyroid.
Study no.:	9766-TX-0015
Document location	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	14 April 2005
GLP compliance:	Yes
QA report :	Yes
Drug, batch #,	17CH03.NJ00001
% purity:	91.5%

Methods

Doses:	0, 10, 30, 90 mg/kg/day
Species/strain:	Rat, Sprague Dawley
Number/sex/group (main study):	14/sex/dose
Age	6 weeks old
Weight	Males (mean 207g); females (mean 162g)
Route	Oral gavage
Formulation	Purified water
Volume	5 mL/kg/day
Number/sex (Recovery group):	2/sex/dose 6/sex/dose

Methods

Results:

Mortality:
Three deaths occurred at 30 mg/kg but no deaths occurred at 90 mg/kg. One death was due to a gavage accident, one animal was sacrificed due to poor condition and one death was not preceded by any clinical signs. While no deaths occurred at the higher dose, attribution to drug cannot be ruled out. Each animal was checked for mortality or signs of morbidity at least twice a day during the treatment and treatment-free periods, including weekends and public holidays.

Clinical signs:
Each animal was observed once a day, at approximately the same time, for the recording of clinical signs. In addition, detailed clinical examinations were made once a week until the end of the study.

Body weights:
There were no toxicologically significant effects of isavuconazonium on bodyweights in any animals. The body weight of each animal was recorded once before group allocation, on the first day of treatment, and then once a week until the end of the study.

Food consumption:
There were no biologically significant drug-related differences in food consumption between treated and control animals. The quantity of food consumed by the animals in each cage was recorded once a week during the

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study. Food consumption was calculated per animal and per day.
Ophthalmoscopy:
There were no drug-related differences in ophthalmoscopy findings between treated and control animals. Ophthalmological examinations were performed on all animals before the beginning of the treatment period and on surviving animals from the control and high-dose groups on one occasion at the end of the treatment period.

Hematology:
There were no toxicologically significant effects on hematology parameters. A few parameters showed statistically significant differences between treated animals and controls but changes were either slight or not dose-related or within the normal range. For example, statistically significant, lower (-18 %) lymphocyte count in males given 90 mg/kg/day and lower (~ 15%) fibrinogen level in males given 30 and 90 mg/kg/day were within the range of control values.

Prior to blood sampling and during urine collection, the animals were deprived of food for an overnight period of at least 14 hours. Water was provided throughout the food deprivation period. Blood samples were taken from the orbital sinus of the animals (before the daily treatment) under isoflurane anesthesia and collected into tubes containing the appropriate anticoagulant.

Clinical chemistry:
No toxicologically important blood chemistry findings were recorded. Statistically significant decreases in alkaline phosphatase (-20 %), and higher urea (+22 %) observed at the end of treatment were of no toxicological significance. Other changes were slight, not dose-related, related to a few animals only and generally within the normal physiological range.

Blood chemistry and urinalysis parameters were determined for all surviving animals at the end of the treatment period.

Urinalysis:
No toxicologically relevant differences in qualitative or quantitative urinary parameters were observed in any test article-treated groups. For urine collection, the animals were individually placed in metabolic cages. The urine was collected in the presence of thymol crystals.

Postmortem Evaluations:
On completion of the treatment or treatment-free period, after at least 14 hours fasting, all surviving animals were asphyxiated by carbon dioxide or anesthetized by an intraperitoneal injection of pentobarbital sodium and sacrificed by exsanguination.

Gross pathology
Thyroid enlargement was noted in one female given 90 mg/kg/day. There were no other toxicologically significant differences in gross pathology findings between treated and control animals.

Organ weights
At the end of the treatment period, statistically significant increases in absolute liver (+12-35 %) and thyroid (+15-19 %) weights were recorded in males and females at 90 mg/kg/day. Relative organ weights were also increased. No toxicologically significant differences were observed between treated and control animals at the end of the recovery period. The body weight of each animal was recorded for all animals before sacrifice at the end of the treatment and treatment-free periods. The organs specified in the Tissue Procedure Table were weighed wet as soon as possible after dissection.

<u>Histopathology</u>: Adequate Battery: yes (√), no ( )—explain
Peer review: yes ( ), no ( )

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Slight to moderate centrilobular hepatocellular hypertrophy were noted in 8/10 males and 10/10 females given 90 mg/kg/day.

Changes indicative of increased thyroid activity, (follicular cell hypertrophy and small follicles, together with a decreased eosinophilic colloidal content), were found in control and treated animals at all dose-levels but were more severe (slight to moderate)) in animals given 90 mg/kg/day. By the end of the recovery period, these findings had resolved (the incidence and severity of morphological changes indicative of thyroid activity were similar between treated and control groups).

Minimal foamy alveolar macrophages were noted in the lungs of three males and four females given 90 mg/kg/day *versus* one control male rat.

Toxicokinetics:

Exposure to isavuconazole (as measured by AUC values) was approximately dose-proportional between 10 and 90 mg/kg. There was a slight accumulation of BAL4815 (< 2-fold) in male and in female rats between Day 1 to week 13. Plasma isavuconazole AUC values in females were about twice as high compared to male rats given the same dose of isavuconazonium. AUC values at 90 mg/kg were half (females) to a quarter (males) of the clinical exposure of 121 µg*h/mL See Table T14, below.

Table T14 Mean pharmacokinetic parameters of isavuconazole in male and female rats during a 13-week oral toxicity study with BAL8557

Dose	Timing	Isavuconazonium			
		Male		Female	
		C_{max} (ng/mL)	AUC_{24} (ng*h/mL)	C_{max} (ng/mL)	AUC_{24} (ng*h/m)L
10	Day 1	245	2081	465	5643
	Day23	314	2419	762	6769
	Day 85	396	3366	939	9606
30	Day 1	762	7121	1999	2173
	Day23	1605	7954	2155	20903
	Day 85	1814	10786	2615	23426
90	Day 1	5169	49078	5700	83064
	Day23	3807	25915	7236	54876
	Day 85	3579	26606	8376	63724

Blood samples for the determination of plasma levels were collected from test article-treated animals on day 1, in week 4 and at the end of the treatment period at 0, 1, 2, 4, 8 and 24 hours after dosing. Each animal was sampled twice on each occasion. Control animals were sampled on day 1 and in weeks 4 and 13 at time-points 0 and 2 hours after dosing.

Summary
Oral gavage dosing with isavuconazonium in Sprague-Dawley rats, at 90 mg/kg/day, (exposures one half to one quarter of clinical exposures) resulted slightly increased liver and thyroid weights and enlarged thyroids. There was a slight accumulation of BAL4815 (< 2-fold) in male and in female rats between Day 1 to week 13.These effects were reversible since they were no longer noted at the end of the treatment-free period. The No Observed Adverse Effect Level (NOAEL) of BAL8557 was 30 mg/kg/day.

Reference ID: 3689714

TISSUE PROCEDURE TABLE

Organs	Organ weights	Preservation of tissues	Microscopic examination
Macroscopic lesions		X	X
Adrenals	X	X	X
Aorta		X	X
Brain (including medulla/pons cerebellar and cerebral cortex)	X	X	X
Cecum		X	X
Colon		X	X
Duodenum		X	X
Epididymides	X	X	X
Esophagus		X	X
Eyes		X	X
Femoral bone with articulation		X	
Gall bladder		X	X
Heart	X	X	X
Ileum		X	X
Infusion sites (3 levels)		X	X
Jejunum		X	X
Kidneys	X	X	X
Larynx		X	X
Liver	X	X	X
Lungs with bronchi		X	X
Lymph nodes (mandibular and mesenteric)		X	X
Mammary gland area		X	X
Optic nerve		X	X
Ovaries	X	X	X
Oviducts		X	X
Pancreas		X	X
Pituitary gland		X	X
Prostate		X	X
Rectum		X	X
Salivary glands (parotid and submandibular)		X	X
Sciatic nerve		X	X
Seminal vesicles		X	X
Skeletal muscle		X	X
Skin		X	X
Spinal cord (cervical, thoracic and lumbar)		X	X
Spleen	X	X	X
Sternum with bone marrow		X	X
Stomach		X	X
Testes	X	X	X
Thymus	X	X	X
Thyroids with parathyroids	X	X	X
Tongue		X	X
Trachea		X	X
Ureters		X	X
Urinary bladder		X	X
Uterus (corpus and cervix)		X	X
Vagina		X	X

Reference ID: 3689714

Study title:	13-Week Toxicity Study by Oral Route (Gavage) in Cynomolgus Monkeys Followed by a 4-Week Treatment-Free Period
Key study findings:	Increased liver and adrenal weights, hepatocellular hypertrophy, hypertrophy of the *Zona fasciculata* of the adrenals. NOAEL was <10 mg/kg/day.
Study no.:	9766-TX-0018
Document location	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 4, 2006
GLP compliance:	Yes
QA report :	Yes
Drug, batch #,	17CH03.NJ00001
% purity:	91.5%

Methods

Doses:	0, 10, 20 or 40 mg/kg
Species/strain:	Cynomolgus monkey
Number/sex/dose (main study):	3
Age	At least 24 months old.
Weight	Males: mean 2.8 kg Females: mean 2.2 kg
Route	oral
Formulation	Purified water
Volume	5 mL/kg
Number/sex/dose (recovery group):	1 (control and high dose)

Results:

Mortality:
There were no treatment-related deaths during the study. Each animal was checked for mortality or signs of morbidity at least twice a day during the treatment and treatment-free periods, including weekends and public holidays.

Clinical signs:
Regurgitation (during dosing or within 30 minutes afterwards), was observed at doses at and above 20 mg/kg/day at a higher rate (on more than two occasions) compared to controls. Vomiting (food) and salivation were also observed at low frequency at the two higher doses. These findings may be due to the bitter taste of the test article.

Body weights:
There were no toxicologically significant differences in body weights or body weight gain between treated animals and controls. The body weight of each animal was recorded at least twice before the beginning of the treatment period, on the first day of treatment and then once a week until the end of the study.

EKG:
No biologically significant abnormalities were observed in the electrocardiogram traces of the animals. Electrocardiographic examinations were performed on all the animals before the beginning of the treatment period

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and at least 2 hours after dosing on one occasion at the end of the treatment period. These examinations were performed using Cardiovit AT-6 Schiller standard leads I, II and III. In the first instance, the heart rate, PQ and QT intervals and the QRS-complex duration were determined on lead II. The QT interval corrected for heart rate was calculated using Bazett's formula.

Ophthalmology

No test article-related findings were noted at the end of the treatment period. Ophthalmological examinations were performed on all animals before the beginning of the treatment period and on one occasion at the end of the treatment period. Pupillary light reflexes were evaluated first. The pupils of the animals were then dilated with tropicamide and the appendages, optic media and fundus were examined by indirect ophthalmoscopy. A slit-lamp biomicroscope was used to investigate abnormalities of the anterior segment and the lens. For the electrocardiography and ophthalmological examinations, the animals were lightly anesthetized by an intramuscular injection of ketamine hydrochloride.

Hematology/Clinical chemistry

There were no toxicologically relevant effects on hematological parameters in treated animals when compared with controls or pre-dose examinations. Blood samples were taken without anesthesia (before the daily treatment) and collected into tubes containing the appropriate anticoagulant and hematology parameters determined for all animals, once before the beginning of the treatment period and during weeks 12.

Urinalysis:

No relevant quantitative or qualitative differences were noted between control and treated animals at the end of the treatment period. Urine was evaluated once before the beginning of the treatment period and at the end of the treatment period.

Gross pathology:

At the end of the treatment period, slightly increased absolute and relative adrenal and liver weights were recorded in animals given 40 mg/kg/day and were considered to be treatment-related.

Organ weights

Isavuconazonium was associated with toxicologically significant increases in absolute liver (10-29%) and adrenal weights (15-25 %) in males and females at 40 mg/kg. In males, adrenal weights were also increased at the 10 and 20 mg/kg dose (by10-17 %) and in females, absolute liver weight was also increased (+19 %) at 20 mg/kg. Relative liver were also similarly increased, but interpretation was confounded by differences in bodyweights between the groups, which were significant even predosing. Changes to other organs were small or inconsistent or not dose dependent.

At the end of the recovery period the mean adrenal weight remained increased (by +10 and +22%) in males and females respectively, compared to controls. Since the n (=1) was so small, this may be an artifact. Changes to other organs were small or inconsistent or not dose dependent. The organs weighed are shown on the Tissue Procedure Table, below.

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

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At 40 mg/kg/day, the adrenals showed minimal to moderate hypertrophy of *Zona fasciculata* in all males and females. Also at this dose, slight to moderate hepatocellular hypertrophy was noted in all males and females. Slight to moderate microvacuolation of hepatocytes (without degenerative/necrotic changes) was observed in 2 male monkeys.

Reversibility
No abnormal histopathological findings were detected at the end of the reversibility period.

Toxicokinetics

Exposure to isavuconazole was approximately dose-proportional when male and female cynomolgus monkeys were dosed with isavuconazonium at doses between 10 and 40 mg/kg/day for 13 weeks. There was no evidence of accumulation, and no differences in pharmacokinetics between the sexes.

Discussion

Isavuconazonium was associated with increased liver and/or adrenal weights at all doses. This is consistent with the organs targeted in other repeat dose studies of isavuconazonium. Slight to moderate microvacuolation of hepatocytes (without degenerative/necrotic changes) were also observed and this may reflect phospholipidosis, which has been reported with other azoles. Without electron microscopy phospholipidosis cannot be definitively confirmed, but the finding is of no toxicological consequence as it is reversible upon the cessation of dosing

The NOAEL was estimated to be less than 10 mg/kg/day. The monkeys were described as at least 24 months old. Immature epididymides indicated 11/12 male monkeys were prepubertal making this essentially a juvenile animal study. Despite the young age isavuconazonium dosing resulted in the same liver and adrenal changes as observed with more mature animals.

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Table T15: Mean pharmacokinetic parameters of BAL4815 in male and female cynomolgus monkeys during a 13-week oral toxicity study with
BAL8557

BAL4815		Male cynomolgus monkeys			Female cynomolgus monkeys		
Dose (mg/kg)	Parameters	Day 1	Day 25	Day 85	Day 1	Day 25	Day 85
10	C_{max} (ng/mL)	1270	938	1160	1830	872	1380
	T_{max} * (h)	3	2	4	4	8	4
	AUC_{last} (ng.h/mL)	11430	9940	12990	15030	9180	10140
	$AUC_{0-\infty}$ (ng.h/mL)	12040	10260	13240	15220	9380	13000
	$T_{1/2}$ (h)	5.9	4.5	4.0	3.7	3.8	3.1
20	C_{max} (ng/mL)	2630	1940	3070	3720	1850	2170
	T_{max} * (h)	4	4	4	4	4	4
	AUC_{last} (ng.h/mL)	26140	19340	24310	30430	21070	24110
	$AUC_{0-\infty}$ (ng.h/mL)	28710	20250	34870	31590	22070	24580
	$T_{1/2}$ (h)	6.7	4.7	3.9	5.1	4.6	3.6
40	C_{max} (ng/mL)	6980	5670	7360	6890	5100	7570
	T_{max} * (h)	4	4	4	4	8	8
	AUC_{last} (ng.h/mL)	88980	68360	94640	82730	66040	89870
	$AUC_{0-\infty}$ (ng.h/mL)	105900	76100	95870	96210	69610	92930
	$T_{1/2}$ (h)	8.8	5.8	3.1	8.2	4.0	3.6

* median for T_{max}

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TISSUE PROCEDURE TABLE

Organs	Organ weights	Preservation of tissues	Microscopic examination
Macroscopic lesions		X	X
Adrenals	X	X	X
Aorta		X	X
Brain (including medulla/pons cerebellar and cerebral cortex)	X	X	X
Cecum		X	X
Colon		X	X
Duodenum		X	X
Epididymides	X	X	X
Esophagus		X	X
Eyes		X	X
Femoral bone with articulation		X	
Gall bladder		X	X
Heart	X	X	X
Ileum		X	X
Infusion sites (3 levels)		X	X
Jejunum		X	X
Kidneys	X	X	X
Larynx		X	X
Liver	X	X	X
Lungs with bronchi		X	X
Lymph nodes (mandibular and mesenteric)		X	X
Mammary gland area		X	X
Optic nerve		X	X
Ovaries	X	X	X
Oviducts		X	X
Pancreas		X	X
Pituitary gland		X	X
Prostate		X	X
Rectum		X	X
Salivary glands (parotid and submandibular)		X	X
Sciatic nerve		X	X
Seminal vesicles		X	X
Skeletal muscle		X	X
Skin		X	X
Spinal cord (cervical, thoracic and lumbar)		X	X
Spleen	X	X	X
Sternum with bone marrow		X	X
Stomach		X	X
Testes	X	X	X
Thymus	X	X	X
Thyroids with parathyroids	X	X	X
Tongue		X	X
Trachea		X	X
Ureters		X	X
Urinary bladder		X	X
Uterus (corpus and cervix)		X	X
Vagina		X	X

Reference ID: 3689714

Study title:	BAL8557: 26-Week Toxicity Study by the Oral Route (Gavage) in Rats Followed by a 4-Week Treatment-Free Period
Key study findings:	Increased liver and thyroid weights, centrilobular hepatocellular hypertrophy, follicular cell hypertrophy/ hyperplasia in the thyroids. Liver and thyroid weights still slightly increased at the end of the recovery period. Thyroid adenoma was observed in one high dose male.
Study no.:	9766-TX-0016
Document location	EDR
Conducting laboratory :	(b) (4)
Date of study initiation:	June 2, 2006
GLP compliance:	Yes
QA report :	Yes
Drug, batch #,	17CH03SD.HQ00002.02
% purity:	85.2 %

Methods

Doses	0, 10, 30, 90 mg/kg/day
Species/strain:	Rat, Sprague Dawley
Number/sex/group (main study):	14/sex/dose
Age	6 weeks old
Weight	Males (mean 207g); females (mean 162g)
Route	Oral gavage
Formulation	Purified water
Volume	5 mL/kg/day
Number/sex (Recovery group):	6/sex/dose

Results:

Mortality:
There were no drug-related increases in mortality. Each animal was checked for mortality or signs of morbidity at least twice a day during the treatment and treatment-free periods, including weekends and public holidays.

Clinical signs:
Excessive salivation was observed at 30 and 90 mg/kg in both male and female rats. Each animal was observed once a day, at approximately the same time, for the recording of clinical signs. In addition, detailed clinical examinations were made once a week until the end of the study.

Body weights:
There were no toxicologically significant effects of isavuconazonium on bodyweights in any animals. The body weight of each animal was recorded once before group allocation, on the first day of treatment, once a week during the first 13 weeks of treatment period and then once every 4 weeks until the end of the study.

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Food consumption:
There were no drug-related differences in food consumption between treated and control animals. The quantity of food consumed by the animals in each cage was recorded once a week during the first 13 weeks of the treatment period and then once every 4 weeks until the end of the study. Food consumption was calculated per animal and per day.

Ophthalmoscopy:
There were no drug-related differences in ophthalmoscopy findings between treated and control animals. Ophthalmological examinations were performed on all animals before the beginning of the treatment period and on 10 animals/sex from the control and high-dose groups on one occasion at the end of the treatment period.

Hematology:
There were no toxicologically significant effects on hematology parameters. Several parameters showed statistically significant differences between treated animals and controls but changes were either slight or not dose-related or within the normal range. Prior to blood sampling and during urine collection, the animals were deprived of food for an overnight period of at least 14 hours. Water was provided throughout the food deprivation period. Blood samples were taken from the orbital sinus of the animals (before the daily treatment) under isoflurane anesthesia and collected into tubes containing the appropriate anticoagulant

Clinical chemistry:
No toxicologically important blood chemistry findings were recorded. Statistically significant decreases in alkaline phosphatase, aspartate and alanine aminotransferase observed at the end of treatment were of no toxicological significance. Blood chemistry and urinalysis parameters were determined for the last ten surviving animals/sex from each group at the end of the treatment period and for the surviving animals at the end of the treatment-free period.

Urinalysis:
Slightly elevated urine protein levels were observed in the urine of all drug treated groups. This slight change was not dose-related and was not associated with any kidney findings. For urine collection, the animals were individually placed in metabolic cages. The urine was collected in the presence of thymol crystals.

Gross pathology:
Gross pathology findings included enlarged liver and/or thyroids in males and females treated at 90 mg/kg/day. These findings correlated with increased organ weights and morphological changes noted at microscopic examination. Small thymus was observed in high dose females, but this finding had no histopathological correlates and the observed absolute decrease in the thymus weight was not statistically significant. One high dose female showed a diaphysis fracture of the femur, but it is not clear that this was drug-related. On completion of the treatment or treatment-free period, after at least 14 hours fasting, all surviving animals were asphyxiated by carbon dioxide or anesthetized by an intraperitoneal injection of pentobarbital sodium and sacrificed by exsanguination.

Organ weights
At 90 mg/kg, isavuconazonium was associated with increased liver, adrenal and thyroid weights. Decreased thymus weights were observed at all doses in female rats only. At the end of the 4 week reversibility period, liver and thyroid weights were 11 and 32 % increased compared to control animals. The body weight of each animal was recorded for all animals before sacrifice at the end of the treatment and treatment-free periods. The organs specified in the Tissue Procedure Table were weighed wet as soon as possible after dissection.

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Table T16: Differences (%) between treated and control rats in the absolute and relative organ weights measured after 26 weeks of dosing with isavuconazonium.

Treatment period

Sex	Male			Female		
Group	2	3	4	2	3	4
Dose-level (mg/kg/day)	10	30	90	10	30	90
Body weight	+2	-4	-3	+4	0	0
- *Adrenals*						
. absolute	+1	-3	-3	+2	+3	+13
. relative	+1	+2	+1	-2	+3	+13
- *Liver*						
. absolute	+3	+6	+25**	+1	0	+31**
. relative	+1	+10**	+29**	-3	0	+30**
- *Thymus*						
. absolute	+3	+4	-9	-10	-13	-22
. relative	0	+8	-5	-14	-12	-22*
- *Thyroids*						
. absolute	+9	+4	+28**	+6	0	+26**
. relative	+9	+8	+32**	+1	0	+26**

Statistically significant from controls: *: $p<0.05$, **: $p<0.01$.

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Table T17: Differences (%) between treated and control rats in the absolute and relative organ weights measured after 26 weeks of dosing with isavuconazonium, followed by a 4 week reversibility period.

Treatment-free period

Sex	Male	Female
Group	4	4
Dose-level (mg/kg/day)	90	90
Body weight	-4	+12
- *Adrenals*		
. absolute	-10	+8
. relative	-9	-3
- *Liver*		
. absolute	-7	+11
. relative	-3	+0
- *Thymus*		
. absolute	-9	+8
. relative	-10	-3
- *Thyroids*		
. absolute	+6	+32
. relative	+11	+22

Statistically significant from controls: **: p<0.01.

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

Treatment-related changes were noted in the liver of males at doses ≥ 30 mg/kg/day and females at 90 mg/kg/day, and in the thyroids of both males and females treated at 90 mg/kg/day. Findings in the liver consisted of minimal to slight centrilobular hepatocellular hypertrophy. In the thyroids at 90 mg/kg, minimal to slight follicular cell hypertrophy /hyperplasia was observed in 12 of 14 rats and follicular cell adenoma was observed in one high dose animal. At the end of the reversibility period, liver findings were less severe but liver weight was still 11 % higher than controls while preneoplastic lesions (focal hyperplasia) persisted in the thyroids of 2/6 high-dose males.

Toxicokinetics:
Exposure to isavuconazole (as measured by AUC values) was dose-related between 10 and 90 mg/kg. Plasma isavuconazole AUC values were higher in females compared to male rats given the same dose of isavuconazonium. Mean exposures (AUC values) at week 26 in the high dose were about 40 % of the exposures observed at the clinical dose, based on AUC comparisons. See Table T18, below.

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Table T18 Mean pharmacokinetic parameters of isavuconazole in male and female rats during a 26-week oral toxicity study with BAL8557

Dose (mg/kg)	Gender	Week 13		Week 26	
		C_{max} (ng/mL)	AUC_{0-24h} (ng.h/mL)	C_{max} (ng/mL)	AUC_{0-24h} (ng.h/mL)
10	Female	1130	8730	1300	11500
	Male	746	3330	497	2770
30	Female	2720	38300	7890	35400
	Male	2100	15200	1780	12600
90	Female	6600	64900	5430	59400
	Male	4830	32700	5040	37100

Blood samples for the determination of plasma levels were collected in weeks 13 and 25 at 0, 1, 2,4, 8 and 24 hours after dosing: Two animals/sex and group were sampled at each time-point. Control animals were sampled at 2 hours after dosing on each occasion.

Discussion

Isavuconazonium dosing at 90 mg/kg for 26 weeks was associated with increased liver and thyroid weights as well as hepatocellular hypertrophy, thyroid hyperplasia and follicular cell adenoma of the thyroid. A reduction in thymus weights was not clearly drug related because changes were not consistent. For example, thymus weights were slightly increased in females but slightly decreased in males during the recovery period.

In the 90 mg/kg group, at the end of the reversibility period, increased liver weights and thyroid hypertrophy persisted. The increased incidence of proliferative lesions in the thyroid after prolonged duration of treatment of rats at 90 mg/kg/day is cause for concern. However, humans handle thyroid hormones differently from rats and so these changes to the rat thyroid gland my not indicate any risk to humans. See *Wu, K-M et al, (2006) Preclinical development of new drugs that enhance thyroid hormone metabolism and clearance: Inadequacy of using rats as an animal model for predicting human risks in an IND and NDA. American Journal of Therapeutics 13, 141-144. (2006)* The NOAEL was 10 mg/kg which had a mean AUC of 7.1 10 ng/mL or about 6 % of the clinical dose.

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Study title:	BAL8557: 39-week toxicity study by the oral route (gavage) in cynomolgus monkeys followed by a 4-week treatment-free period
Key study findings:	Mortality, bone fractures, increased adrenal, liver and heart weights. Increase in vacuolated cells in the *Zona fasciculata* of the adrenal cortex. Hepatocellular hypertrophy.
Study no.:	9766-TX-0019
Document location	EDR
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	June 2, 2006
GLP compliance:	Yes
QA report :	Yes
Drug, batch #,	17CH03SD.HQ00002.02
% purity:	85.2

Methods

Doses:	0, 10, 20 or 40 mg/kg
Species/strain:	Cynomolgus monkey
Number/sex/dose (main study):	4
Age	At least 24 months old (immature)
Weight	Males: mean 3.4 kg Females: mean 3.0 kg
Route	oral
Formulation	Purified water
Volume	5 mL/kg
Number/sex/dose (recovery group):	2 (control and high dose)

Results:

Mortality
One male and one female high dose monkeys were humanely sacrificed during this study. One showed soft to markedly liquid feces, sporadically and for extended periods with vomiting and hypothermia and dehydration with reddish anal discharge between days 99 and the day of sacrifice (Day 164). Postmortem examination showed enlarged brown adrenals, enlarged liver, and a small thymus consistent with other animal studies of isavuconazonium. The second animal was sacrificed on Day 64 with an untreatable elbow fracture. Each animal was checked for mortality or signs of morbidity at least twice a day during the treatment and treatment-free periods, including weekends and public holidays.

Clinical signs:
Limb fracture was observed in three (of eight) high dose animals. One high dose female was sacrificed moribund on day 64 due to severe palpable trauma of the elbow, corresponding to a fractured elbow with subcutaneous hematoma. One male showed signs of a fractured left forelimb (radius) on days 163 to day 199, and another had fractured right crus (tibia and fibula) from days 177 to day 188. Red or reddish liquid nose discharge was seen in

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three high-dose males, and this persisted for at most 7 consecutive days. Excessive salivation was noted in animals of all treated groups.

Body weights:
There were no relevant differences in body weights or body weight gain between treated animals and controls, during the treatment and treatment-free periods. The body weight of each animal was recorded at least twice before the beginning of the treatment period, on the first day of treatment and then once a week until the end of the study.

EKG:
No biologically significant abnormalities were observed in the electrocardiogram traces of the animals in week 26 and at the end of the treatment and treatment-free periods. Electrocardiographic examinations were performed on all animals before the beginning of the treatment period and at least 2 hours after dosing on one occasion in week 26 and at the end of the treatment period. Based on the results observed at the end of the treatment period, examinations were also carried out at the end of the treatment-free period.

Ophthalmology
No test article-related findings were noted at the end of the treatment period. Since there were no adverse findings at the end of the dosing period, no evaluations were carried out at the end of the treatment-free period. Ophthalmological examinations were performed on all animals before the beginning of the treatment period and on one occasion in week 26.

Hematology/Clinical chemistry
There were no toxicologically relevant effects on hematological parameters in treated animals when compared with controls or pre-dose examinations in weeks 12, 26 and at the end of the treatment period. Blood samples were taken without anesthesia (before the daily treatment) and collected into tubes containing the appropriate anticoagulant and hematology parameters determined for all animals, once before the beginning of the treatment period, during weeks 12 and 26 and at the end of the treatment period (week 38). Statistically significant differences were either small and/or not dose related and/or changed in different directions for the sexes

Urinalysis:
There were no toxicologically relevant quantitative or qualitative differences in treated animals when compared with controls. Urine was evaluated once before the beginning of the treatment period, during weeks 12 and 26 and at the end of the treatment period (week 38).

Gross pathology:
Three of the eight high dose animals showed fractures. One was one the elbow of one of the females that was humanely sacrificed, another was of the forelimb (radius) and the third was of the right crus (tibia and fibula).

Organ weights
Isavuconazole was associated with significant increases in absolute liver (+31-41%) and adrenal weights (22-43 %) in males and females at 40 mg/kg. Relative liver and adrenal weights were also increased but the increases were not dose dependent and large within-group variations confounded the data. Liver weights were slightly increased at 20 mg/kg. Heart weight was increased in high dose male monkeys only. At the end of a four week treatment-free period, mean adrenal weight remained increased (+35%) in females compared to controls, and heart weights remained marginally increased (+10 %) in males. The organs weighed are shown on Table T19, below.

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Table T16: Differences (%) between treated and control monkeys in the absolute and relative organ weights measured after 39 weeks of dosing with isavuconazonium.

Treatment period

Sex	Male			Female		
Group	2	3	4	2	3	4
Dose level (mg/kg/day)	10	20	40	10	20	40
Final	-11	-5	+7	-15	-10	-11
Adrenal						
. absolute	+7	+4	+43	+39	+7	+22
. relative *(% body)*	+24	+12	+34	+61	+16	+37

Sex	Male			Female		
Group	2	3	4	2	3	4
Dose level (mg/kg/day)	10	20	40	10	20	40
Heart						
. absolute	-3	0	+43	-10	-8	-2
. relative *(% body)*	+10	+4	+33*	+6	+1	+10
Liver						
. absolute	+10	+11	+41	-3	+11	+31
. relative *(% body)*	+24	+18	+34	+15	+24	+49**

Statistically significant from controls: *: $p<0.05$, **: $p<0.01$.
The significance concerned the organ weights values and not the percentages.

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

In males treated at 40 mg/kg/day there was a minimal increase in the incidence and severity of focal to multifocal clusters of enlarged vacuolated cells in the *Zona fasciculata* of the adrenal cortex of all males and one of four females. This finding correlated with the increased adrenal weights. This finding was also present in 1/2 males and 1/2 females treated at 40 mg/kg/day at the end of the treatment-free period. This is likely evidence of phospholipidosis, which is a common reversible feature with azoles.

Toxicokinetics:
Table T20: Pharmacokinetics of isavuconazole in 39-Week Toxicity Study by the Oral Route (Gavage) in Cynomolgus Monkeys (Week 12/13)

Dose (mg/kg/day)	AUC (µg*h/mL)		(µg*h/mL)
	Male	Female	Mean
10	11	14	13
20	33	46	39
40	100	81	91

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There was no accumulation of isavuconazole between weeks 12 and 39 based on relatively constant AUC-values and there no difference in exposure between the sexes.

Table T21: Pharmacokinetics of isavuconazole in 39-Week Toxicity Study by the Oral Route (Gavage) in Cynomolgus Monkeys (Week 39)

Dose (mg/kg/day)	AUC (µg*h/mL)		(µg*h/mL)
	Male	Female	Mean
10	18	12	15
20	36	30	33
40	89	98	94

Discussion
The isavuconazonium dose of 90 mg/kg for 39 weeks was clearly above the maximum tolerated dose monkeys. This is evidenced by the mortality, broken bones, increased organ weights and persistent increases in adrenal and heart weights at the end of a 4 week treatment-free period.
Three of the eight high dose animals showed fractures. It is not clear if this is an extension of the pharmacology of the drug. . Isavuconazole blocks calcium channels and may also bind other molecules which impact calcium homeostasis. Bone defects are seen in fetuses exposed to isavuconazole in utero and the male monkeys appear to have been prepubertal as evidenced by the immature testes and epididymides observed in 12 of 16 animals at necropsy. In as much as they were only seen at the high dose, the fractures appear to be dose related.

Increased heart weight was also observed in high dose males. Heart failure often leads to the adaptive response of compensatory hypertrophy and a decrease in calcium uptake is a central feature of human and animal heart failure. Sordahl, L.A. et al (1973) Mitochondria and sarcoplasmic reticulum function in cardiac hypertrophy and failure. Am J Physiol. 224(3):497-502. It is not clear if isavuconazole contributed to this increased heart weight, through an effect on calcium channels (or its affinity for other molecules which may control calcium homeostasis) but the effect seems to be drug related.

Isavuconium-related organ weight changes in the adrenals and liver, in male and/or female monkeys after 39 weeks of dosing at 40 mg/kg, appeared to be similar to findings in other toxicology studies. At doses below the MTD, changes were generally slight and there was no mortality. The adrenal weight changes appeared to correlate with the microscopic finding of enlarged vacuolated cells in the adrenal cortex zona fasciculata in males.

No drug-related changes in organ weights and no macroscopic or microscopic findings were noted in monkeys administered isavuconazonium at 10 mg/kg dose levels. Therefore, the NOAEL was 10 mg/kg, which was associated with an AUC of 15. µg*h/mL in week 39. This NOA was equivalent to about 12 % of the clinical exposure

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Study title:	Two-week infusion toxicity study of [redacted] (b) (4) in monkeys (including recovery study)
Key study findings:	Reversible increase in liver weights, injection site irritation, hepatocellular hypertrophy
Study no.:	9766-TX-0011
Document location	EDR
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	September 1999
GLP compliance:	GLP
QA report :	Yes
Drug, lot #,	MTS2111
% purity:	98.93 %

Methods

Doses:	0, 10,30 and 60 mg/kg/day
Species/strain:	Monkey, cynomolgus
Number/sex/group (main study):	3/sex/dose (Main study animals) 1/sex/dose (Recovery 1 control and 1 high dose)
Age:	4-6 years old
Weight	Males (3.7-4.2 kg) ; females (2.6 to 3.2 kg)
Route	Intravenous
Formulation	pH 3.5 adjusted saline.
[Isavuconazonium]	1 to 4 mg/mL
Infusion rate	6.25 ml/kg/hr for 2 hours

This study was only described briefly since drug was administered under conditions that differed from the clinical dosing. Specifically, later nonclinical studies and clinical trials used drug that was filtered and infused at a slower rate. Slower infusion rates resulted in lower toxicity. In this study monkeys were about 3 kg therefore infusion rate was about 0.3 mL/min.

Clinical signs
At 60 mg/kg only, isavuconazonium was associated with vomiting, incomplete eyelid opening, loose passage or diarrhea, low spirit and decreased spontaneous motor activity. A significant decrease in food consumption was detected in the high dose group from day 2 onward, but this resolved towards the end of week 2.

Clinical Chemistry
Mean cholesterol values were about 29-47% lower at 30 and 60 mg/kg in male and female monkeys on Day 14. Mean triglyceride (TG) values were 2x control levels at 30 mg/kg and 3.5 x controls at 60 mg/kg/day in males, with smaller increases in females (+19 % and +67%, respectively). These values were no different from controls at the end of the recovery period.

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Table T22. Cholesterol levels (mg/dL) in monkey after 14 daily intravenous doses of isavuconazonium

Isavuconazonium dose (mg/kg)	0	10	30	60
	Cholesterol levels (mg/dL)			
Females	139	140	94	98
Males	151	132	107	81

Organ weights
Increased absolute liver (+15%, +47%) and adrenal (+20%, +114%) weights were observed in mid and high dose animals respectively. Low thymus was seen in all drug treated groups (-43% to 68 %) and uterus weights (-57 %) was reduced in the high dose females.

Necropsy findings
Liver and adrenal enlargements were observed in high dose animals at the end of dosing. No changes observed at the end of the recovery period.

Histopathological findings
Hepatocellular hypertrophy (slight) observed in one male and one female of the high dose group. The change in the male was accompanied by fatty change of the centrilobular hepatocytes. Furthermore, an increase of lipid droplets in the cortical cells of the zona fasciculata was observed in one male and one female of the high dose group. Atrophy of the thymus was observed in one male and two females in the high dose group as the secondary changes related to the treatment. There were no drug-related changes in the middle and low dose groups or at the end of the recovery period, the changes observed at the end of the treatment period were not observed.

Reversibility
There were no drug-related changes at the end of the recovery period. The changes observed at the end of the treatment period were not observed.

Discussion

Intravenous isavuconazonium at 60 mg/kg is associated with adverse clinical signs (vomiting, incomplete eyelid opening, loose passage or diarrhea, low spirit and decreased spontaneous motor activity), liver and adrenal enlargement, increased liver and adrenal weights, reduced uterus weights, hepatocellular hypertrophy, decreased cholesterol, increased triglycerides and thymic atrophy. Fatty change of the centrilobular hepatocytes and the increase of lipid droplets in the cortical cells of the zona fasciculata may reflect phospholipidosis.

These findings are consistent with the findings seen in other animal studies of isavuconazonium. Several of these effects were observed at the mid dose (increased triglycerides, decreased cholesterol, increased liver and adrenal weights) and thymus weights were reduced even at the 10 mg/kg dose. The NOAEL could therefore only be estimated at < 10 mg/kg/day. These changes were reversible upon the cessation of dosing.

Reference ID: 3689714

Study title:	6-Week Intravenous (Infusion) Toxicity Study in the Rat with a 4-Week Recovery
Key study findings:	Deaths, injection site irritation
Study no.:	9766-TX-0009
Document location	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	22 April 2005
GLP compliance:	(b) (4)
QA report :	Yes
Drug, batch #,	17CH03.NJ00001
% purity:	91.5%

Methods

Doses:	0, 10, 20, 40/30 mg/kg/day. The 40 mg/kg dose was reduced to 30 mg/kg due to early mortalities
Species/strain:	Rat, HanRcc:WIST(SPF)
Number/sex/group (main study):	6/sex/dose (Main study animals) 3/sex/dose (interim sacrifice,) 3/sex/dose(Toxicokinetics and recovery)
Age:	approximately 8 weeks old (males)
Weight	Males (mean 229g) ; females (mean 160g)
Route	Intravenous
Formulation	0.9 % NaCl, adjusted to pH 4 with HCl Prior to infusion the solutions were passed through a sterile filter (Sarstedt Filtropur) then transferred to sterile bottles using a sterile syringe.
[Isavuconazonium]	0.5, 1 and 2 mg/mL
Infusion rate	10 ml/kg/hour over 2 hours Group 1 and 4 animals were treated over 4 hours/day between days 24 and 28 due to local tolerance problems.

Reference ID: 3689714

5.2 ALLOCATION

Dose Levels [mg/kg BW/day]		Group 1 control* 0	Group 2 10	Group 3 20	Group 4 40/30**
Males	A	1-6	14-19	27-32	40-45
	B	7-10	20-23	33-36	46-49
	C	11-13	24-26	37-39	50-52
Females	A	53-58	66-71	79-84	92-97
	B	59-62	72-75	85-88	98-101
	C	63-65	76-78	89-91	102-104

* Control animals were treated with the vehicle (0.9% NaCl) adjusted to pH 4
** 40 mg/kg/day on treatment days 1 to 4, 30 mg/kg/day from treatment day 5
A Main Study animals
B Interim Sacrifice animals
C Animals for Toxicokinetics and Recovery

Duration of treatment
14 days (Allocation B)
28 days (Allocation A and C from Group 4)
42 days (Allocation A and C from Groups 1-3)

5.11.1 NECROPSY

Sacrifice:

After 15 days	12 May 2005 (Allocation B from Groups 1-4)
After 28 days	25 May 2005 (Allocation A from Group 4)
After 42 days	08 June 2005 (Allocation A from Groups 1-3)
After 56 days	22 June 2005 (Allocation C from Group 4)
After 70 days	06 July 2005 (Allocation C from Group 1)

Results:

Mortality and Clinical signs:

Two males and five females at 40/30 mg/kg/day died in the course of the study. Five deaths occurred within the first 4 days of treatment, and two high dose females died after 23 days of treatment. The cause of death was not established.

Necrosis and reversible swelling and of the infusion site (tail) was noted at 20 and 40/30 mg/kg/day from Week 3 onwards. Animals were checked at least twice daily.

Body weights:

There were no toxicologically significant effects of isavuconazonium on bodyweights in any animals. Body weights were recorded once during acclimatization, and thereafter twice weekly (on Days 1 and 3 per week)

Food consumption:

Food consumption was 21 % lower in high dose females during the first week which included data from the animals that died. There were no other toxicologically significant drug-related differences in food consumption between treated and control animals. The quantity of food consumed by the animals in each cage was recorded once a week during the study. Food consumption was calculated per animal and per day.

Reference ID: 3689714

Ophthalmoscopy:

There were no toxicologically significant drug-related differences in ophthalmoscopy findings between treated and control animals. Ophthalmoscopic examinations were performed in all animals at acclimatization, Weeks 2, 6 and in Groups 1 to 3 at recovery end.

Clinical laboratory investigations

Blood and urine were collected for hematology, clinical chemistry and urinalysis at Week 2, Week 4, Week 6, Week 8 and Week 10. Prior to blood sampling and during urine collection, the animals were deprived of food for at least 18 hours. Water was provided throughout the food deprivation period. Blood samples were taken from the orbital sinus of the animals (before the daily treatment) under isoflurane anesthesia and collected into tubes containing the appropriate anticoagulant. Urine was collected during the 18 hours fasting period into a specimen vial, using a metabolic cage.

Hematology:

There were no toxicologically significant effects on hematology parameters. A few parameters showed statistically significant differences between treated animals and controls but changes were either slight or not dose-related or within the normal range. For example, Week 6 reductions in Red cell volume distribution width (RDW) in females at 10 mg/kg/day (- 28%) and 20 mg/kg/day (-32) were related to the high RDW values observed in control animals in this study (very upper end of the historical control data). Values in the treated animals were within the range of historical controls

Clinical chemistry:

No toxicologically important blood chemistry findings were recorded. The lower bilirubin levels (~20 %) detected at 10 and 20 mg/kg were of no toxicological significance. Other changes were slight, not dose-related, related to a few animals only and generally within the normal physiological range.

Blood chemistry and urinalysis parameters were determined for all surviving animals at the end of the treatment period.

Urinalysis:

No toxicologically relevant differences in qualitative or quantitative urinary parameters were observed in any test article-treated groups. For urine collection, the animals were individually placed in metabolic cages. The urine was collected in the presence of thymol crystals.

Postmortem Evaluations:

All animals surviving to the end of their observation period were anaesthetized by intraperitoneal injection of pentobarbitone and sacrificed by exsanguination.

Samples of the following tissues and organs were collected from all animals at necropsy and fixed in neutral phosphate buffered 4 % formaldehyde solution except for eyes with optic nerve which were fixed in Davidson's solution or epididymides and testes which were fixed in Bouin's solution:

Reference ID: 3689714

Adrenal glands
Aorta
Auricles - ear tattoo
Bone (sternum, femur including joint and with bone marrow)
Brain – including section of medulla/pons, cerebral and cerebellar cortex
Cecum
Coagulating glands
Colon
Duodenum
Epididymides (fixed in Bouin's solution)
Esophagus
Extraorbital lacrimal glands
Eyes with optic nerve and Harderian glands (fixed in Davidson's solution)
Fat tissue (white)
Heart
Ileum, with Peyer's patches
Infusion site
Jejunum, with Peyer's patches
Kidneys
Liver
Larynx
Lungs, infused with formalin
Lymph nodes – mesenteric, mandibular
Mammary gland area
Nasal cavities
Ovaries
Pancreas
Pharynx
Pituitary gland
Prostate gland
Rectum
Salivary gland – mandibular, sublingual
Sciatic nerve
Seminal vesicles
Skeletal muscle
Skin
Spinal cord – cervical, midthoracic, lumbar
Spleen
Stomach
Testes (fixed in Bouin's solution)
Thymus
Thyroid gland / parathyroid gland
Tongue
Trachea
Urinary bladder, infused with formalin
Uterus with cervix
Vagina
Zymbal's glands
All gross lesions and tissue masses

Gross pathology
There were no other toxicologically significant differences in gross pathology findings between treated and control animals.

Organ weights
The following organ weights were recorded for all animals killed on the scheduled dates of necropsy: Adrenals, Brain, Epididymides, Heart, Kidneys, Liver, Ovaries, Spleen, Thymus, Testes.

Thymus weight was decreased by 26 % in high dose males but not females. Other changes were either marginal or not dose related or showed opposite trends based on time or sex evaluated. For example, at the end of dosing (Day 42), marginal, but statistically significant decreases in kidney weights (-10 %) were recorded in males at 10 and 20 mg/kg/day, but statistically significant increases (+20 %) were recorded the recovery period.

The body weight of each animal was recorded for all animals before sacrifice at the end of the treatment and treatment-free periods. The organs specified above were weighed wet as soon as possible after dissection.

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

At the injection site, hemorrhage, vasculitis/perivasculitis, necrosis and/or thrombosis were diagnosed. Other findings were usually in one animal/sex/group. Thyroids were not evaluated.

Besides the injection site findings, no toxicologically significant differences were observed.

Toxicokinetics:

Blood sampling was performed at 2, 4 and 12 hours after administration start on Days 1, 14and 42 according to the following time schedule. On Day 28, sampling was only performed at 4 and 12 hours after administration start:

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Isavuconazonium (BAL8557) was always detectable in the 2-hour blood samples (end of infusion) in the 10 and 20 mg/kg dose groups but 4 hours after start of the infusion BAL8557 levels were very low and the prodrug was no longer detectable by the 12 hour time point.

Male rats showed lower exposure to BAL4815 than female rats given the same dose of, BAL8557 and the highest plasma concentration at the end of the infusion was almost dose-proportional. After termination of the infusion plasma concentrations of BAL4815 declined slowly with measurable levels at 12 hours after start of the infusion in male and female rats at all doses.

Table T23 Maximum plasma concentrations (ng/mL) after a 2-h infusion in the rat 6-week intravenous toxicity study

	Male				Female		
Dose (mg/kg)	**Day 1**	**Day 14**	**Day 28/42**		**Day 1**	**Day 14**	**Day 28/42**
Isavuconazonium (BAL8557)							
10	27.4	134	22.4		34.2	95.9	33.1
20	48.8	253	67.5		73.1	190	59.6
40/30	122	345	BLQ		176	230	NS
Isavuconazole (BAL4815)							
10	380	698	644		509	888	1060
20	718	1291	1180		1075	1681	1440
40/30	2152	2031	2730		1584	1761	NS
Cleavage Product (BAL8728							
10	364	512	451		306	328	349
20	858	1042	993		655	847	695
40/30	1894	1419	1900		1168	1164	NS

Mean of 2 or 3 animals, Data are expressed in ng/mL. BLQ: all values below the limit of quantification, NS: no sample

Discussion

When administered at 10 ml/kg/hour over 2 hours, 40 mg/kg isavuconazonium was associated with mortality and severe local irritation at the infusion site which resulted in the dose being reduced to 30 mg/kg from Day 5 onwards. On Day 24, as a result of continued injection site effects, the infusion rate was reduced so drug was administered over a four hour period. Despite these changes, the 30 mg/kg dose had to be terminated on Day 28. Animals in the 20 mg/kg dose group showed reversible swelling at the infusion site. The NOAEL was 10 mg/kg which had a mean Cmax of 852 ng/ml.

The steady state isavuconazole Cmax in patients treated at the recommended maintenance dose (200 mg/day) was 7499 ng/mL. The NOAEL was equivalent to about 11 % of the human dose based on Cmax comparisons. Mortality was observed at a Cmax of 2730 ng/mL, which is about one third of the clinical dose based on Cmax comparisons.

Reference ID: 3689714

Study title:	6-Week Toxicity Study by Daily 2-Hour Intravenous Infusion in Cynomolgus Monkeys with a 4-Week Treatment-Free Period
Key study findings:	Reversible increase in liver weights, hepatocellular hypertrophy, injection site irritation,
Study no.:	9766-TX-0012
Document location	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	7 April 2005
GLP compliance:	Yes
QA report :	Yes
Drug, batch #,	17CH03.NJ00001
% purity:	91.5% [listed as 96.5 % on p14]

Methods

Doses:	0, 10, 20 or 40 mg/kg
Species/strain:	Monkey, cynomolgus
Number/sex/group (main study):	3/sex/dose (Main study animals) 1/sex/dose (Recovery 1 control and 1 high dose)
Age:	At least 24 months old
Weight	Males (mean 2.8 kg) ; females (mean 2.7 kg)
Route	Intravenous
Formulation	Sterile isotonic saline solution (0.9% NaCl), pH 4. Prepared daily and used within 8 hours. Prior to infusion the solutions were passed through a 0.22 µm sterile filter (Sarstedt Filtropur) then transferred to bottles using a syringe.
[Isavuconazonium]	1 to 4 mg/mL
Infusion rate	5 ml/kg/hour over 2 hours (initially) then over 4 hours for groups 1 and 4.See Table T17

Table T24: Group assignments

Group	Number of animals	Dose-level (mg/kg/day)	Concentration (mg/mL)	Dosage-volume (ml/kg)
1	4 males + 4 females	0	0	10 and then 20*
2	3 males + 3 females	10	1	10
3	3 males + 3 females	20	2 and then 1**	10 and then 20**
4	4 males + 4 females	40	4 and then 2*	10 and then 20*

Infusion duration extended to 4 hours *: from week 3 for males and 4 for females. **: from week 4 for males and 5 for females.

Reference ID: 3689714

Results:

Mortality and Clinical signs:

There were no unscheduled deaths during the study. Dose related induration was present in nearly all test-treated animals. It started earlier at higher drug concentrations (day 12 at 40 mg/kg, day 19 at 20 mg/kg and day 26 at 10 mg/kg). All mid-and high-dose animals had induration, and 2/3 animals/sex in the low-dose group showed this finding. No induration was present in control animals, although swelling of the infusion site was seen (on a single occasion on day 40, or from day 42) in 2/4 control males. The induration was also associated with swelling of the infusion sites in some animals. The dosing at 40 mg/kg was stopped after 4-weeks, since it was no longer possible to find a patent superficial vein for infusion.

Body weights:
There were no toxicologically significant effects of isavuconazonium on bodyweights in any animals. The body weight of each animal was recorded at least twice before group allocation, on the day before the first treatment and then once a week until the end of the study.

Electrocardiography
There were no toxicologically significant differences in EKG parameters between treated and control animals. Electrocardiographic examinations were performed on all animals before the beginning of the treatment period and at least 2 hours after dosing on one occasion at the end of the treatment period, and at the end of the treatment-free period. The electrocardiograms were recorded using a Delta 3 Plus Cardioline, standard leads I, II and III. In the first instance, the heart rate, PQ and QT intervals and the QRS-complex duration were determined on lead II. The corrected QT interval, QTc, was also calculated using the Bazett formula.

Ophthalmoscopy:
No ophthalmological lesions were recorded in any animal at any time during the study. Ophthalmological examinations were performed on all animals before the beginning of the treatment period and on one occasion at the end of the treatment periods.

Bone marrow
Slightly reduced cellularity of the sternal bone marrow was seen in two males receiving 40 mg/kg/day. This finding was not present in the females of the same group, or in animals receiving 20 mg/kg/day. Bone marrow smears were prepared from the sternum of all animals sacrificed on completion of the treatment and treatment-free periods.

Clinical laboratory investigations
Blood and urine were collected for hematology, clinical chemistry and urinalysis once before the beginning of the treatment period, at the end of treatment and at the end of treatment-free periods. Blood samples were taken from the cephalic, saphenous or femoral veins without anesthesia (before the daily treatment) and collected into tubes containing the appropriate anticoagulant. Prior to blood sampling and during urine collection, the animals were deprived of food for an overnight period of at least 14 hours. The urine was collected in the presence of thymol crystals.

Hematology
Many of the changes observed were considered most likely as secondary to the local irritation at the infusion sites and the associated inflammation, and problems to find suitable veins for infusion.

At 10 mg/kg, consistent changes included increased WBC's (up to +21 %). At 20 mg/kg, neutrophils were increased (up to + 138 %) and WBC's were increased in males (+67 %) but decreased in females (-13 %).
At 40 mg/kg changes affected WBC (increased by up to + 73 %), RBC's (decreased by as much as 34 %), hemoglobin (decreased by as much as 35 %), packed cell volume (decreased by as much as 37 %), platelets

Reference ID: 3689714

(increased by as much as 67 %) and neutrophils increased by as much as 128 %. Other changes were not toxicologically significant since changes were either slight or not dose-related or within the normal range.

Clinical chemistry:
No toxicologically important blood chemistry findings were recorded.

Urinalysis:
No toxicologically relevant differences in qualitative or quantitative urinary parameters were observed in any test article-treated groups.

Postmortem Evaluations:

Gross pathology
Enlarged livers were noted in individual animals given 20 or 40 mg/kg/day. Hematomas at the infusion sites were noted in all groups including the controls, but were most frequently seen in the groups receiving 10 or 20 mg/kg/day. In addition, induration and edema of the infusion sites and thickening of the veins were noted at a high incidence in the test-treated groups.

At the end of the treatment-free period, hematomas at the infusion sites were more frequent in the drug- treated animals. Edema at one infusion site was recorded in a previously treated male.

A complete macroscopic *post-mortem* examination was performed on all study animals. On completion of the treatment or treatment-free period, after at least 14 hours fasting, all animals were tranquilized using an intramuscular injection of ketamine hydrochloride, anesthetized by an intravenous injection of thiopental sodium and sacrificed by exsanguination. For all study animals, the tissues specified in the Tissue Procedure Table were preserved in 10% buffered formalin (except for the eyes with optic nerve which were fixed in Davidson's fixative, and the testes and epididymides which were preserved in Bouin's fluid). In addition, bone marrow smears were prepared from the sternum of all animals sacrificed on completion of the treatment and treatment-free periods for bone marrow differential cell count.

Histopathology

End of Treatment

Diffuse hepatocellular hypertrophy was noted in one and two males receiving 10 and 40 mg/kg/day, respectively, and in one, three and three females receiving 10, 20 and 40 mg/kg/day, respectively.

At the infusion sites of drug-treated animals showed moderate to marked fibrosis, inflammation, hemorrhage and edema in the perivascular/subcutaneous tissue, and intimal thickening, mural necrosis, inflammation and thrombus formation in the veins. Infusion sites changes were also noted in the controls, but were far less frequent and generally milder in nature.

Recovery animals

Infusion sites changes (subcutaneous hemorrhage, subcutaneous and perivascular fibrosis, subcutaneous inflammation and intimal thickening) persisted until the end of the reversibility period, and these findings were more common in the previously test-article treated animals than in controls. The severity of the inflammatory and fibrotic changes was reduced compared to terminal findings indicating partial recovery.

Reference ID: 3689714

TISSUE PROCEDURE TABLE

Organs	Organ weights	Preservation of tissues	Microscopic examination
Macroscopic lesions		X	X
Adrenals	X	X	X
Aorta		X	X
Brain (including medulla/pons cerebellar and cerebral cortex)	X	X	X
Cecum		X	X
Colon		X	X
Duodenum		X	X
Epididymides	X	X	X
Esophagus		X	X
Eyes		X	X
Femoral bone with articulation		X	
Gall bladder		X	X
Heart	X	X	X
Ileum		X	X
Infusion sites (3 levels)		X	X
Jejunum		X	X
Kidneys	X	X	X
Larynx		X	X
Liver	X	X	X
Lungs with bronchi		X	X
Lymph nodes (mandibular and mesenteric)		X	X
Mammary gland area		X	X
Optic nerve		X	X
Ovaries	X	X	X
Oviducts		X	X
Pancreas		X	X
Pituitary gland		X	X
Prostate		X	X
Rectum		X	X
Salivary glands (parotid and submandibular)		X	X
Sciatic nerve		X	X
Seminal vesicles		X	X
Skeletal muscle		X	X
Skin		X	X
Spinal cord (cervical, thoracic and lumbar)		X	X
Spleen	X	X	X
Sternum with bone marrow		X	X
Stomach		X	X
Testes	X	X	X
Thymus	X	X	X
Thyroids with parathyroids	X	X	X
Tongue		X	X
Trachea		X	X
Ureters		X	X
Urinary bladder		X	X
Uterus (corpus and cervix)		X	X
Vagina		X	X

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Organ weights

At the end of dosing, liver weights were increased by 17 to 35 % at 10, 20 and 40 mg/kg, but increases were resolved by the end of the recovery period.

The body weights of all animals sacrificed at the end of the treatment or treatment-free period were recorded before necropsy, and the organs specified in the Tissue Procedure Table were weighed wet as soon as possible after dissection. The ratio of organ weight to body weight (recorded immediately before sacrifice) was calculated.

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

Toxicokinetics
Isavuconazole levels (Cmax) were proportional to isavuconazonium dose and no accumulation or gender differences were seen. Exposure to isavuconazole (AUC) was suprapproportional to isavuconazonium dose during the infusion period.

Blood samples for the determination of plasma levels of the active moiety BAL4815 were taken from all groups on day 1 and in week 2, following the change of drug formulation concentration and infusion duration, for the high and mid-dose groups, and during the last week of treatment (as relevant) for all groups at 2, 4, 8 and 24 hours after the start of the infusion.

Table T25 Toxicokinetics in the cynomolgus monkey 6-week intravenous toxicity study

Dose (mg/kg)	Timing	Male			Female		
		C_{max} (ng/mL)	t_{max} (h)	AUC_{last} (ng·h/mL)	C_{max} (ng/mL)	t_{max} (h)	AUC_{last} (ng·h/mL)
Isavuconazole (BAL4815)							
10	Day 1	1850	2	13900	1800	2	13300
	Day 29/41	2610	2	13600	2150	2	13600
20	Day 1	3360	2	37600	3990	2	34300
	Day 29/41	4460	4	31100	15300	4	56100
40	Day 1	7480	2	80700	7280	2	84800
	Day 29/41	11800	4	89000	7260	4	77100

Summary and conclusions

Six weeks of daily intravenous isavuconazonium (administered by 2-h intravenous infusions) to rats at 40 mg/kg resulted in severe local irritation at the infusion site which resulted in the group being terminated at week 4. Most of the other findings in this study have been observed in previous nonclinical studies including dose-dependent increases in liver weights at all doses which correlated with diffuse hepatocellular hypertrophy. Liver findings were resolved by the end of the 4-week recovery period. Due to infusion site irritation and the increased liver weights in females (+17 %) seen at the lowest dose, the NOAEL could only be estimated to be ≤ 10 mg/kg. Mean AUC value at 10 mg/kg was13.6 µg*h/mL or 11 % of the AUC seen in patients receiving isavuconazonium at 200 mg/day.

Reference ID: 3689714

7 Genetic Toxicology

Study title:	Mutagenicity Evaluation of [redacted] (b) (4) in the Ames Test
Study no.:	9766-TX-0023
Study report location:	EDR
Conducting laboratory:	[redacted] (b) (4)
Date of study initiation:	November 23, 1999
GLP compliance:	[redacted] (b) (4)
QA statement:	Yes
Drug, batch #	JO-1 0179
Purity:	98.99 %

Key Study Findings: Isavuconazonium showed no evidence of genotoxicity at doses up to 1000 µg/plate, with or without metabolic activation.

Methods	
Strains:	*Salmonella typhimurium*, TA1535, TA97, TA98, TAI 00 and TA102.
Concentrations in definitive study:	10 to 1000 µg/plate (standard assay) 5 to 500 µg/plate (preincubation assay).
Basis of concentration selection:	In a range-finding experiment with the TA100 tester strain using standard plate incorporation version, ±S9, toxic effects were apparent at concentrations greater than 500 µg/plate (-S9) and greater than 1580 µg/plate (+S9) respectively) by reduction of background growth and by reduction or absence of revertant colonies. There was also precipitation of test compound at concentrations greater than 1580 µg/plate.
Negative control:	Deionized water
Positive controls:	2-Nitrofluorene, 0.5 µg/plate Sodium azide, 1 µg/plate ICR 191, 1 µg/plate Mitomycin C, 0.4 µg/plate 2-aminoanthracene, 4 µg/plate
Formulation/Vehicle:	Deionized water
Incubation time:	Standard plate incorporation assay: 2 days Preincubation modification assay: preincubation for 30 minutes, incubation for 2 days)

Standard Ames test procedure
Test tubes containing 2 ml of 0.7 % agar medium were autoclaved and kept in a pre-warmed water bath at 42°C to 45°C. The following solutions were added in order: 0.2 ml of the histidine/biotin mixture corresponding to 21 µg L-histidine and 24.4 µg biotin, 0.1 ml of isavuconazonium at different concentrations or of the solvent or 0.05 ml of the controls which were thawed shortly before use, 0.1 ml of the overnight cultures of the bacterial strain, 0.5 ml of the S9 mixture where metabolic activation was needed. The S9 mixture was replaced by 0.5 ml sodium phosphate buffered saline 0.2 M, pH 7.4 in the treatment without metabolic activation. The contents of the tubes were mixed and poured immediately onto Vogel-Bonner minimal agar plates. There were three replicate plates for the test compound and negative control and two replicate plates for the positive controls. When the top agar had hardened, the plates were incubated at 37ºC, upside down for 2 days.

Reference ID: 3689714

Liquid preincubation assay
In the modified procedure the following solutions are added in order: 0.1 ml of the isavuconazonium solutions or of the solvent or 0.05 ml of the controls, 0.5 ml of sodium phosphate buffered saline 0.2 M, pH 7.4 or 0.5 ml of the S9 mixture and 0.1 ml of the overnight cultures of the bacterial strain. The test tubes were incubated and shaken for 30 minutes at 37°C. 2.2 ml soft agar supplemented with 21 µg L-histidine and 24.4 µg biotin was added afterwards and the content of the tubes were mixed and poured on Vogel-Bonner minimal agar plates. Three replicate plates for the test compound and negative control or two replicate plates for the positive controls were incubated at 37°C, upside down, for 2 days. Colonies were usually counted electronically using a DOMINO automatic image analysis system (Perceptive Instruments, Haverhill, Suffolk, England) after having inspected the background lawn for signs of toxicity. Plates exhibiting precipitate or contamination were counted manually.

Study Validity
This study was valid because(1) bacterial tester strains were selected based on ICH guidelines, (2) positive controls showed expected response, (3) dose selection was adequate based on the presence of precipitate. (4) S9 (purchased from (b) (4) was verified using positive controls requiring metabolic activation.

Results
Dose selection assay: In the range finder assay with strain TAlOO, toxic effects were apparent at ≥ 500 µg/plate and ≥ 1580 µg/plate (-S9 and +S9, respectively) by reduction of background growth and by reduction or absence of revertant colonies. Precipitation was apparent at concentrations ≥ 1580 µg/plate. (See Table G1, below.

Table G1 Isavuconazonium toxicity to tester strains

Concen-tration µg/plate	Precipitation in standard assay	Precipitation in preincubation assay	Revertants per plate (two plates average) -S9	+S9	Toxicity on VB plates -S9	+S9
0	-	-	117	129	growth	growth
50	-	-	131	162	growth	growth
158	-	-	144	152	growth	growth
500	-	-	78	169	Reduced growth	growth
1580	-	-	-	57	Reduced growth	Reduced growth
5'000	-	-	-	-	No growth	Reduced growth

Main Study
Isavuconazonium did not induce any dose related increase of the number of revertant colonies/plate in any of the five tester strains See Table G2. Some reduction of background growth was visible in the plate incorporation assay at 1000 µg/plate (-S9) for all strains except TA97. When repeated using the preincubation assay, there was still no increase in revertant counts even though more pronounced toxicity was observed in the (data not shown). A repeat assay with lower dose levels performed with strains TA98, TAl00 and TA102 (-S9) at lower concentrations (data not shown) also showed no increase in mutant frequencies. The mutant frequencies of the controls were in the range of historical control values. The positive controls induced significant increases in the mutant frequencies, verifying the sensitivity of the strains used.

Reference ID: 3689714

Table G2: Effect of isavuconazonium on number of revertant colonies in plate incorporation test in the presence and absence of S9 activation

Test Article	Conc (µg/plate)	TA98	TA100	TA1535	TA97	TA102
		Tester strain				
		Number of revertant colonies				
Without metabolic activation						
ISN	0	12±3	189±11	11 ± 6	286±17	387±18
ISN	10	10±2	198±38	12 ± 1	296±52	380±11
ISN	31.6	14±1	202±29	14±2	307±23	428±9
ISN	100	12±2	232±15	11±1	302±3	377±23
ISN	316	16±3	189±15	13±3	299±22	311±17
ISN	1000	10±2§	68±13§	6±1 §	305±17	90±7§
ICR 191	1				756±45	
2-NF[1]	0.5	211±23				
Na azide[2]	1		684±17	1183±68		
2-AA[5]	4	20±3	206±37	9±2	284±30	389±13
With metabolic activation						339±19
ISN	0	16±8	207±7	7 ± 4	330±11	319±28
ISN	10	14±0	217±22	9 ± 2	325±13	339±19
ISN	31.6	16±3	208±9	8±5	320±13	349±27
ISN	100	17±8	199±19	6±1	340±12	335±9
ISN	316	23±2	213±8	10±4	318±23	279±8
ISN	1000	23±5	154±9	11±5	371±33	157±48§
[5]2-AA	4	2255±54	3905±22	207±3	1525±34	606±6

[1]2-Nitrofluorene, [2] Sodium azide, [4]4-Nitroquinoline-N-Oxide [5]2-amino-anthracine

Discussion
Isavuconazonium showed no genotoxic potential in this study. The evaluation was valid, conducted according to (b) (4). The strain selection was adequate and consistent with ICH guidelines. Concentrations were appropriate, based on toxicity and precipitates observed in the range finding study. Control revertant counts were within the range of historical controls and the positive controls resulted in increased revertants as expected, with and without S9. The solvent was appropriate. In buffer solutions below pH 7 the compound shows little decay in 24 hours.

Conclusion
Isavuconazonium did not show any evidence of genotoxic potential in the presence or absence of metabolic activation. Other studies (Study 9766-TX-0031) have also shown that the active drug isavuconazole (BAL4815) showed no mutagenic or clastogenic potential in the modified Ames assay.

Reference ID: 3689714

7.2 *In Vitro* Mouse Lymphoma Assay

Study title: (b) (4) Mouse Lymphoma Cell Mutation Test (ML/TK)
Study no.: 9766-TX-0027
Study report location: EDR
Conducting laboratory : (b) (4)
Date of study initiation: November 18, 1999
GLP compliance: (b) (4)
QA statement: Yes
Drug, batch #: JO-10179
Purity (%) 98.99.%
Key Study Findings: Isavuconazonium showed no evidence of mutagenic potential.
Method
Cell line: mouse lymphoma L5178Y *tk+'* cells
Assay version Microwell version
Solubility Isavuconazonium was soluble in water
Metabolic activation S9 from the livers of Sprague Dawley rat was obtained from (b) (4)
Concentrations in definitive study: 3-hour incubation: 36.4, 51, 71.4, 100, 140 µg/mL (±S9)
24- hour incubation: 5, 10, 20, 30, 40 µg/mL (-S9)

Basis of concentration selection:
In a non-GLP study cells were exposed to isavuconazonium at concentrations up to 5000 µg/mL. Cytotoxic effects were observed in absence as well as in presence of metabolic activation. Without S9-mix the adapted relative survival at Day 0 decreased to 54 % at 78 µg/mL (and 0 % at 156 µg/mL) for the 3 h exposure time and to 10% at 39 µg/mL (0 % at 78 µg/mL) for the 24 h exposure time. With S9-mix the (3 h treatment time) the adapted relative survival at Day 0 was reduced to 70 % at 78 µg/mL and to 0 % at 156 µg/mL. See Table G3 and G4. The concentrations selected for the main experiments were therefore up to 140 µg/mL (3h, -S9), up to 40 µg/mL (24 h, -S9) and up to 140 µg/mL (3h, +S9).

Criteria for positive
Test item was considered mutagenic if it induced a concentration-related increase in mutant frequency, the mutant frequency was above 2 times the negative (vehicle) control mutant frequency and this effect was reproducible in an independent repeat experiment.

Study outcome
In the absence of metabolic activation (study 514M99/2), the mutant frequency in the presence of 100 µg/mL isavuconazonium with a 3h incubation was 2.6 times the controls. The 24-hour incubation (study 514M99/2) in the absence of metabolic activation did not result in an increase in mutant frequency.

Reference ID: 3689714

Table G3: Relative cell counts and viability of mouse lymphoma *tk'''* cells after 3h exposure to (b) (4) in the absence of metabolic activation. (514M99/2)

Metabolic Activation	Test Article	Dose Level (mcg/mL)	Treatment period of 3 h (Assay ID: 514M99/2) ADRS (%)	Mutant Frequencies (Mutants per 10^6 viable cells) small	large	total	Proportion small/large colonies
Without Activation	Isavuconazonium	0	95	61	132	190	0.46
		36.4	104	74	190	280	0.39
Treatment time: 3 h		36.4	100	48	164	217	0.29
		51	84	88	203	295	0.43
		51	94	86	162	244	0.53
		71.4	64	94	163	263	0.58
		71.4	70	121	164	286	0.74
		100	10	204	241	457*	0.85
		100	6	321	325	649*	0.99
		140	0	†	†	†	†
		140	0	†	†	†	†
	4-nitroquinoline 1-oxide	0	105	50	174	229	0.29
		0.1	90	318	396	756	0.80

Table G4: Relative cell counts and viability of mouse lymphoma *tk'''* cells after 24h exposure to (b) (4) in the absence of metabolic activation. (514M99/4)

Metabolic Activation	Test Article	Dose Level (mcg/mL)	Treatment period of 24 h (Assay ID: 514M99/4) ADRS (%)	Mutant Frequencies (Mutants per 10^6 viable cells) small	large	total	Proportion small/large colonies
Without Activation	Isavuconazonium	0	94	48	237	287	0.20
		5	90	54	151	210	0.36
Treatment time: 24 h		5	102	48	209	250	0.23
		10	85	44	236	286	0.19
		10	76	52	225	282	0.23
		20	32	84	191	270	0.44
		20	33	48	250	292	0.19
		30	8	53	166	218	0.32
		30	5	86	231	305	0.37
		40	1	†	†	†	†
		40	1	†	†	†	†
	Methylmethan-sulfonate	0	106	45	213	251	0.21
		5	56	760	958	2196	0.79

Table G5: Relative cell counts and viability of mouse lymphoma *tk'''* cells after 3h exposure to (b) (4) in the presence of metabolic activation. (514M99/4)

Metabolic Activation	Test Article	Dose Level (mcg/mL)	Treatment period of 3 h (Assay ID: 514M99/3) ADRS (%)	Mutant Frequencies (Mutants per 10^6 viable cells) small	large	total	Proportion small/large colonies
With Activation	Isavuconazonium	0	93	41	102	138	0.40
		36.4	108	46	140	186	0.33
Treatment time: 3 h		36.4	105	65	142	208	0.46
		51	98	54	192	237	0.28
		51	116	42	169	212	0.25
		71.4	87	50	137	177	0.36
		71.4	85	27	133	158	0.20
		100	48	129	238	371*	0.54
		100	38	109	175	276*	0.62
		140	7	†	†	†	†
		140	6	†	†	†	†
	Benzo[a]pyrene	0	108	34	167	200	0.20
		1	70	330	459	854	0.72
		2	24	750	877	1718	0.86

Reference ID: 3689714

Table G6: Relative cell counts and viability of mouse lymphoma *tk+/-* cells after 3 h exposure to (b) (4) in presence of metabolic activation.

Metabolic Activation	Test Article	Dose Level (mcg/mL)	Treatment period of 3 h (Assay ID: 514M99/5)				
			ADRS (%)	Mutant Frequencies (Mutants per 10^6 viable cells)			Proportion small/large colonies
				small	large	total	
With Activation	Isavuconazonium	0	103	61	272	343	0.22
Treatment time: 3 h		36.4	91	95	334	434	0.28
		36.4	93	72	250	325	0.29
		71.4	55	99	302	425	0.33
		71.4	63	79	238	319	0.33
		100	21	186	396	613*	0.47
		100	29	148	413	602*	0.36
		120	5	†	†	†	†
		120	7	†	†	†	†
		140	2	†	†	†	†
		140	3	†	†	†	†
	Benzo[a]pyrene	0	97	56	292	338	0.19
		1	55	463	772	1346	0.60
		2	17	750	1033	2163	0.73

ADRS: Adapted relative survival
†Relative survival after treatment below 10%
*p≤0.05 Dunnett test statistically significant difference from control group

Study Validity

The study was deemed valid for the following reasons: 1) data included cytotoxicity, plating efficiency determination, colony counts and mutant frequencies for treated and control cultures and mutant colony sizing on appropriate cultures; 2) mutant frequency (MF) in the untreated/vehicle controls was within an acceptable range; untreated/vehicle plating efficiency (PE) at the time of mutant selection was within an acceptable range (65-120 %); suspension growth (SG) was between 8-32 for the 3 hour treatment, 3) positive controls exhibited an increase above background of at least 300 X 10^{-6} with at least 40% of the induced MF in small colonies and an upper limit of cytotoxicity greater than a relative total growth (RTG) of 10%; 4) maximum concentrations with relative total growth (RTG) values between 10 and 20% were achieved.

Discussion

In the absence of metabolic activation (study 514M99/2), the mutant frequency observed at the 100 µg/mL concentration of isavuconazonium with a 3h incubation was 2.6 times the controls. However, this concentration was associated with excessive toxicity as indicated by the low suspension growth (1.4-2.4). In study 514M99/3, the mutant frequency in the presence of 100 µg/mL isavuconazonium with metabolic activation was about 1.9 times control. When the same conditions were repeated, (Study 514M99/4), mutant frequency in the presence of 100 µg/mL isavuconazonium was about 1.8 times control. There was therefore no reproducible doubling of the mutant frequency. The moderate increases were also obtained at the 100 µg/mL concentration which had a suspension growth of (1.4-2.4) indicating excessive toxicity. Since the suspension growth at 100 µg/mL was outside of the range for a valid study (8-32), and since no dose with an acceptable suspension growth produces a reproducible doubling of the mutant frequency, isavuconazonium was considered negative in this assay.

Conclusion
Isavuconazonium is considered negative in the mouse lymphoma assay.

Reference ID: 3689714

Study title:	Micronucleus Assay in Bone Marrow Cells of the Rat with BAL8557 (Water Soluble Azole).
Study no.:	9766-TX-030
Study report location:	EDR
Conducting laboratory:	(b) (4) [redacted]
Date of study initiation:	December 17, 2001
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #:	MTS 2/20
% purity:	97.5 %

Key Study Findings: BAL8557 was not genotoxic in the rat micronucleus assay.

Methods	
Doses in definitive study:	6.25, 12.5, 25mg/kg
Frequency of dosing:	Single
Route of administration:	Intravenous
BAL8557 dose volume:	5 mL/kg
Positive control dose volume	5 mL/kg
Formulation/Vehicle:	0.9°/o NaCI
Species/Strain:	Rat: Wistar Hanlbm: WIST (SPF)
Number/Sex/Group:	6/sex/dose/group
Satellite groups:	Toxicokinetics (2/sex/dose time point)
Basis of dose selection:	In a previous experiment, dyspnea, decrease in spontaneous motor activity, tremor, staggering gait, and jumping were observed at 40 mg/kg, and no changes were observed at 10 and 20 mg/kg.
Negative control:	0.9°/o NaCI
Positive control:	Cyclophosphamide (30 mg/kg, i.v.)

Isavuconazonium chloride was formulated in 0.9°/o NaCI and a single dose administered intravenously at doses of 6.25, 12.5 or 25 mg/kg (5 ml/kg). Twenty-four and 48 hours after administration, the bone marrow cells were collected for micronuclei analysis. Animals were sacrificed by cervical dislocation, after which the femora were removed and the epiphyses were cut off. The bone marrow was then flushed out with fetal calf serum (FCS), using a syringe. The nucleated cells were separated from the erythrocytes using the method of Romagna. Briefly, the cell suspension was passed through a column consisting of a-Cellulose (Sigma) and Cellulose (Sigmacell type 50). The columns were then washed with Hank's buffered saline. The cell suspension was centrifuged at 1500 rpm (390 x g) for 10 minutes and the supernatant was discarded. The pellet was resuspended in a small drop of FCS and spread on slides. The smear was air-dried and then stained with May-GrOnwald (MERCK, D-64293 Darmstadt)/Giemsa (Gurr, BDH Limited Poole, Great Britain). Cover slips were mounted with EUKITT (KINDLER, D-79110 Freiburg). At least one slide was made from each bone marrow sample. Evaluation of the slides was performed using NIKON microscopes with 1OOx oil immersion objectives. At least 2000 polychromatic erythrocytes (PCE) were analyzed per animal for micronuclei. To describe a cytotoxic effect, the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and expressed in normochromatic erythrocytes per 2000 PCEs. The analysis was performed with coded slides. Ten animals (5 males, 5 females) per test group were evaluated as described.

Reference ID: 3689714

Criteria for positive response
A test item is classified as mutagenic if it induces either a dose-related increase or a clear increase in the number of micronucleated polychromatic erythrocytes in a single dose group. The Mann-Whitney test was used to evaluate the results.

Study Validity
The study was deemed valid because (1) pharmacokinetics data showed good exposure, which were comparable to those seen in the clinic. (2) The single dose study showed frank toxicity (3) Data from the negative and positive control animals demonstrate that they were producing the expected responses and that laboratory is able to reliably detect micronuclei in the test mice and (4) the formulation and administration of the drug was appropriate

Results

Toxicity

Table G7: Toxicity observed after a single intravenous 25 mg/kg dose of isavuconazonium

toxic reactions	hours post-treatment male / female					
	1 h	2-4 h	6 h	24 h	30 h	48 h
reduction of spontaneous activity	2/2	2/2	2/2	1/1	1/0	0/0
abdominal position	2/2	1/1	1/1	0/1	0/0	0/0
eyelid closure	0/0	0/0	0/0	0/0	0/0	0/0
ruffled fur	2/2	2/1	1/1	0/1	0/1	0/0

The animals treated with 25 mg/kg showed toxic effects including reduction of spontaneous activity, eye closure, ruffled fur, and prostration. Since the 40 mg/kg dose resulted in mortality, the 25 mg/kg dose was selected for the definitive study.

Toxicokinetics

Plasma isavuconazole concentrations (measured 2 hours after dosing) were slightly less than dose proportional between 6 and 25 mg/kg and included values of 1120 and 1450 ng/ml, which are within the range of Cmax values recorded on Day 12 in patients dosed at the 200 mg/day. See 'A phase 1, randomized, double-blind, placebo and active controlled, parallel study to evaluate the effect of repeat doses of isavuconazole on cardiac repolarization in healthy adult subjects'.

Table G8 Individual and mean plasma concentrations of isavuconazole (ng/ml) in male and female rats after intravenous isavuconazole

	Male		Female	
Dose (mg/kg)	**5 min post-dose**	**2 h post-dose**	**5 min post-dose**	**2 h post-dose**
Isavuconazole (BAL4815)				
6.25	118	286	353	407
12.5	923	482	1450	734
25	1150	796	202	1120

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Table G9: Summary of Micronucleus Test Results

test group	dose mg/kg b.w.	sampling time (h)	PCEs with micronuclei (%)	range	PCE/ NCE
vehicle	0	24	0.095	0 – 5	2000/ 1834
BAL8557	6.25	24	0.095	0 – 5	2000/ 1782
BAL8557	12.5	24	0.085	0 – 4	2000/ 1884
BAL8557	25	24	0.120	0 – 6	2000/ 1644
positive control	30	24	1.155	10 - 39	2000/ 3174
BAL8557	25	48	0.060	0 - 4	2000/ 1664

The data show that the number of PCE's with micronuclei in the isavuconazonium-treated animals did not significantly differ from the vehicle control values based on analysis by the Mann Whitney test. The increase of the micronucleus rates in the positive control treated animals was significantly higher than the vehicle control.

Discussion
In the present study, single intravenous isavuconazonium chloride doses between 6.25 and 25 mg/kg resulted in plasma concentrations of isavuconazole similar to those observed at clinical exposures. At these doses isavuconazonium chloride did not induce micronuclei in polychromatic erythrocytes. Since the study was valid and conducted according to established guidelines and controls showed the appropriate responses, it is concluded that isavuconazonium chloride was nongenotoxic in the mouse micronucleus assay.

8 Carcinogenicity
The applicant has not conducted a carcinogenicity study and maintains that the drug (b) (4) . This statement is at odds with the data which show that the median duration of dosing for mucormycosis patients is 102 days and individual patients have been dosed over 800 days.

Reference ID: 3689714

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title:	BAL8557: Study of Fertility and Early Embryonic Development to Implantation in the Rat
Report no.:	BAP00636
Study no:	9766-TX-0049
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 14, 2005
Date of first dosing:	Males: June 21, 2005 Females: July 5, 2005
GLP compliance:	(b) (4)
QA statement:	Yes
Drug, batch #:	17CH03.NJ00001
% purity:	91.5 %
Methods	
Doses:	0, 10, 30 or 90 mg/kg/day
Frequency of dosing:	Males: once daily during a 4-weeks pre-pairing period, during pairing and up to Day 6 post coitum. Females: once daily during a 2-week pre-pairing period, during pairing and up to Day 6 post coitum.
Dose volume:	10 mL/kg/day
Route of administration:	Oral (gavage)
Formulation/Vehicle:	Purified water (pH 3.5)
Species:	Rat (*Rattus norvegicus*)
Strain:	Wistar
Number/Sex/Group:	22 rats/sex/dose group

Control animals received purified water, pH 3.5 at 10 ml/kg/day. Males were necropsied after treatment end and females on day 14 *post coitum.* Examinations included monitoring of general condition, feed consumption and body weight development, organ weights (liver), fertility and reproductive parameters, and Toxicokinetics monitoring.

Key Study Findings: No adverse effects on fertility or early reproductive parameters were detected.

Mortality and Clinical signs
All animals survived to the scheduled date of necropsy and there were no drug-related clinical signs recorded during this study.

Body Weight
There were no biologically significant differences in body weight in males or females when the drug-treated animals were compared to controls. Body weights were recorded daily.
Feed Consumption
There were no biologically significant differences in feed consumption between drug-treated animals and controls. Food consumption of the males and females was recorded weekly during the pre-pairing period. No food

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consumption was recorded during the pairing period. During the after-pairing period the food consumption of males was recorded weekly. Food consumption of mated females was recorded for Days 0-7 and 7-14 post coitum.

Stability and Homogeneity
The purity of isavuconazonium was found to be above 95.8% and the concentration of isavuconazonium in the formulations ranged between 90.4 and 98.8 % of the nominal doses and so drug preparations were deemed acceptable.

Table R1 Summary of performance of mated females.

FEMALES SCHEDULED FOR CAESAREAN SECTION

	GROUP 1 0 MG/KG	GROUP 2 10 MG/KG	GROUP 3 30 MG/KG	GROUP 4 90 MG/KG
Percentage mating	100.0	95.5	100.0	100.0
Fertility index (%)	100.0	90.9	90.9	100.0
Conception rate (%)	100.0	95.5	90.9	100.0
Gestation index (%) (Caesarean section)	100.0	100.0	100.0	100.0

Necropsy
There were no significant differences between the groups in fertility parameters (percentage mating, fertility index, conception rate, gestation index). All mated females were pregnant. There were no biologically significant differences between the treated groups concerning the relevant reproduction data (mean number of females with live embryos at termination, mean numbers of Corpora lutea, pre- or post-implantation loss, embryonic resorptions, and mean number of embryos). Liver weights were increased by 17 % in high dose male rats only.

Table R2: Isavuconazole pharmacokinetics in rats

Dose (mg/kg)	Timing	Male			Female		
		C_{max} (ng/mL)	t_{max} (h)	AUC_{last} (ng·h/mL)	C_{max} (ng/mL)	t_{max} (h)	AUC_{last} (ng·h/mL)
Isavuconazole (BAL4815)							
10	Day 1	314	4	2410	983	1	6810
	Week 3 or GD 6	324	4	2440	645	4	5790
30	Day 1	993	4	8100	2806	1	18400
	Week 3 or GD 6	1297	4	9270	1882	4	17400
90	Day 1	6091	4	46200	5634	4	53700
	Week 3 or GD 6	2675	4	21600	4507	4	41300

Discussion and conclusion
Female rats exposed to isavuconazonium a doses up to 90 mg/kg/day achieved isavuconazole exposures of 41 µg*h/mL (see Table R2) which was about one third the exposure in the clinic (AUC 121 µg*h/mL). While rats showed increased liver weights at these exposures, isavuconazonium had no adverse effects on fertility or reproductive parameters.

Reference ID: 3689714

9.2 Embryonic Fetal Development

Study title:	BAL8557: Study for Effects on Embryo-Fetal Development in the Han Wistar Rat
Study no:	9766-TX-0052
Report no:	BAP00411
Study report location:	EDR
Conducting laboratory :	(b) (4)
Date of study initiation:	June 23, 2004
Start of mating:	June 29, 2004
GLP compliance:	Yes
QA statement:	Yes
Drug, batch #:	17CH02.NJ00002
% purity:	99.3

Key Study Findings

Treatment with 30 and 150 mg/kg isavuconazonium resulted in increased incidences of rudimentary cervical ribs, rudimentary supernumerary ribs and zygomatic arch fusions. Additional findings at 150 mg/kg included increased incidences of supernumerary ribs and lumbar vertebral arch 4 with additional ossification.

Methods

Doses:	0, 6, 30 and 150 mg/kg/day
Frequency of dosing:	Daily from Day 6 to 17 postcoitum.
Dose volume:	10 mL/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	Purified water, adjusted to pH 3.5 with 1 N HCI)
Species:	Rat (*Rattus norvegicus*)
Strain:	HanBri:WIST (SPF)
Number/Group:	22 rats/dose group
Satellite groups:	3 or 9 female rats/dose group for toxicokinetics

Pregnant females were dosed with isavuconazonium as described above. On day 21 post coitum, females were killed by $C0_2$ asphyxiation and the fetuses removed by Caesarean section. The fetuses were sexed, weighed individually, examined for gross external abnormalities and either micro dissected or stained with alizarin red S to detect abnormalities and variations. On days 6 and 17 post coitum, blood samples of approximately 0.5 ml was taken at 0 (predose), 1, 2, 4, 8 and 24 hours after dosing from satellite animals.

Observations and Results

Mortality and Clinical signs

All females survived until scheduled Caesarean section and no test item-related clinical signs were noted in any group. Animals were checked daily for mortality and clinical signs.

Body Weight

There were no biologically significant differences in bodyweights or bodyweight gain between treated and control animals. Body weights were recorded daily from day 0 until day 21 post coitum.

Reference ID: 3689714

Feed Consumption
Slight reductions in feed consumption were observed in high dose animals. While these changes were statistically significant, they were slight, (< 10 %).

Table R3: Toxicokinetics: Mean isavuconazole pharmacokinetics parameters after dosing with isavuconazonium.

Pharmacokinetic Parameters of BAL4815						
Dose Groups (mg/kg)	Gestation Days	$T_{1/2}$ (h)	T_{max} (h)	C_{max} (ng/mL)	AUC_{last} (ng.h/mL	AUC_{inf} (ng.h/mL)
0	6	NC	NC	NC	NC	NC
0	17	NC	NC	NC	NC	NC
6	6	7.90	2.00	233	3180	3700
6	17	9.40	2.00	398	3790	4560
30	6	6.40	2.00	1540	19400	21200
30	17	7.60	2.00	1730	20800	23600
150	6	7.70	2.00	6640	101000	117000
150	17	6.10	4.00	6240	86700	93900

NC: Not Calculated

T_{max} (h) values were between 2-4 hours after dosing on Day 6 or Day 17. C_{max} and AUC $_{(0\text{-}24h)}$ values increased in proportion with dose. C_{max} values on Day 17 were similar and did not indicate any drug accumulation.

Stability and Homogeneity
The concentration and stability of the test article preparations were evaluated by HPLC. Data showed that the test article formulation was stable for at least four hours. The purity of all BAL8557 samples has been found to be above 98.2 % and their content between 91.4 and 101.0 % of expected values.

Necropsy
The incidence of post-implantation loss and number of fetuses per dam were unaffected by treatment with BAL8557 at all dose levels. Other changes which were not statistically significant or dose dependent were not considered biologically significant. No drug-related gross pathology findings were observed at necropsy. Liver weights were increased by 14 % in high dose animals, but there were no differences in the histopathology findings between the treated and control animals.

Table R4: Bone abnormalities and variations (% fetuses affected) in the offspring of rats dosed with increasing doses of isavuconazonium

Dose of isavuconazonium (mg/kg)	0	6	30	150
No. Litters Evaluated	21	20	22	20
Abnormalities				
Zygomatic arch fusion (process of maxilla to jugal)	3	2	6	12
Rudimentary cervical rib (uni/bilateral)	4	7	14	62
Additional ossification of lumbar vertebral arch 4	0	0	0	3
Variations				
Supernumerary ribs	0	1	3	15
Rudimentary supernumerary ribs ‡	27	43	60	80

Reference ID: 3689714

Cesarean Section Data
There were no toxicologically relevant, drug-related effects on sex ratios, weights of live fetuses or external findings. Drug-related increases in bone abnormalities (defined as structural changes in a fetus that could impair its health or development) were seen at all doses. Findings included increased incidence of rudimentary cervical ribs (7%, 14% and 62% of fetuses affected at 6, 30 and 150 mg/kg respectively, compared with 4% in controls), increased incidence of zygomatic arch fusions, (6% and 12% of fetuses affected, at 30 and 150 mg/kg respectively, compared with 3% in the control) and increased incidence of lumbar vertebral arch 4 with additional ossification was increased (3% of fetuses affected compared with 0% in the control). Drug-related skeletal variations (fetal changes that occur within the normal population under investigation and is unlikely to adversely affect survival or health) included increased supernumerary ribs (15% of animals at 150 mg/kg, compared to 0 % in controls) and increased rudimentary supernumerary ribs in 43%, 60% and 80% of animals at 6, 30 and 150 mg/kg respectively, compared to 27 % on controls)

Discussion

Dosing in the study was determined to be adequate since AUC values at the high dose on Day 7 of gestation (117 µg*h/mL) were comparable to clinical exposures. The human AUC at the maintenance dose of 200 mg/day was 121 µg·h/mL (Study No. 9766-CL-0017). Bone abnormalities and variations such as zygomatic arch fusion, rudimentary cervical rib and rudimentary supernumerary ribs were increased at least two-fold at 30 mg/kg isavuconazonium. On Day 17 of gestation, animals dosed with isavuconazonium at 6, 30 and 150 mg/kg in this study showed isavuconazole exposures of 4.5, 23.6 and 93.9 µg*h/mL. This means that at doses as low as one fifth of the clinical exposures at the maintenance dose of 200 mg/day, bone abnormalities were observed. At doses comparable to the clinical dose, increased rudimentary supernumerary ribs were observed in 80 % of the animals and rudimentary cervical ribs were observed in 62 % of animals.

Conclusion
Isavuconazonium administration was associated with increases in bone abnormalities and variations in rats. Skeletal changes have also been observed in the offspring of dams treated with other azoles. Women should be made aware of the potential risks of skeletal toxicity in their offspring and should be advised to use birth control when taking CRESEMBA.

Reference ID: 3689714

Study title:	BAL8557: Study for Effects on Embryo-Fetal Development in the Himalayan Rabbit
Study no:	9766-TX-0055
(b) (4) Report no:	BAP00412
Study report location:	EDR
Conducting laboratory :	(b) (4)
Date of study initiation:	August 27, 2004
Start of mating:	August 31, 2004
GLP compliance:	(b) (4)
QA statement:	Yes
Drug, batch #:	17CH02.NJ00002
% purity:	99.3

Key Study Findings: 45 mg/kg isavuconazonium (less than one tenth of the clinical exposure) resulted in increased incidence of rudimentary cervical ribs and increased incidence of additional artery arising from aortic arch.

Methods

Doses:	0, 10, 20 and 45 mg/kg/day
Frequency of dosing:	Daily from Day 6 to 18 postcoitum.
Dose volume:	4 mL/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	purified water, adjusted to pH 3.5 with 1 N HCI)
Species:	Rabbit
Strain:	Himalayan
Number/Group:	20 rabbits/dose group
Satellite groups:	Blood samples from three females/group were collected on the first and last day of treatment at 0.5, 2, 4, 10 and 24 hours after dosing.
Study design:	Pregnant Himalayan rabbits received BAL8557 from gestation days 6-18. Maternal examinations included monitoring of general condition, feed consumption and body weight, reproductive data and toxicokinetics monitoring. Caesarean sections were performed on gestation day 28 and fetal examination (external, visceral and skeletal) was performed.

Observations and Results

Mortality and Clinical signs
There were no drug-related deaths. Animals were checked at least twice daily for mortality and clinical signs.

Body Weight
There were no drug-related effects on bodyweights or bodyweight gain. Body weights were recorded daily from day 0 until day 28 post coitum.

Reference ID: 3689714

Feed Consumption
Mean food consumption was not affected by treatment with isavuconazonium. Food consumption was recorded for the following periods: days 0-6, 6-11, 11-15, 15-19, 19-24 and 24-28 post coitum.

Toxicokinetics

In Himalayan rabbits, isavuconazole C_{max} and AUC showed a dose-proportional increase with isavuconazonium dose on Day 6 of gestation. T_{max} was 3 hours. At 45 mg/kg the AUC was about 6 % of the clinical exposure.

Table R5: Pharmacokinetics parameters of isavuconazole in Himalayan rabbits on gestation Day 6/18 after dosing with isavuconazonium.

Dose (mg/kg)	Gestation day	Isavuconazole (BAL4815)		
		C_{max} (ng/mL)	t_{max} (h)	AUC_{last} (ng·h/mL)
10	6	94.1	3	992
	18	55.0	3	585
20	6	134	3	1980
	18	168	3	2280
45	6	444	3	6950

Stability and Homogeneity
The concentration and stability of the test article preparations were evaluated by HPLC. Data showed that the test article formulation was stable for at least four hours. The purity of all BAL8557 samples has been found to be above 98.0 % and their content between 93.4 and 100.8 % of expected values.

Necropsy
There were no drug related effects on reproductive parameters. Incidence of post-implantation loss and number of fetuses per dam were unaffected by treatment with isavuconazonium. No drug-related gross pathology findings were observed at necropsy.

Cesarean Section Data
There were no toxicologically relevant, drug-related effects on sex ratios or weights of live fetuses. At 45 mg/kg/day an increased incidence of an additional artery arising from aortic arch was noted (16% of fetuses affected compared with 7% in the vehicle control). The incidence of uni- or bilateral rudimentary cervical ribs was increased (27%) at 45 mg/kg compared to controls (12 %). The incidence in the vehicle control was high compared to incidence in historical control data so the incidence was only considered increased in the high dose group.

Table R6: Variations and bone abnormalities and in the offspring of rats dosed with isavuconazonium

Dose of isavuconazonium (mg/kg)	0	10	20	45
No. Litters Evaluated	19	19	18	19
Variations				
Additional artery arising from aortic arch	7	2	7	16
Abnormal findings				
Rudimentary cervical rib (uni/bilateral)	12	20	15	27

Reference ID: 3689714

Discussion and conclusion

Dosing was considered to be adequate since body weight gains was slightly reduced compared to controls when higher doses (60 mg/kg) were administered in the dose range finding study. For this discussion, abnormalities are defined as structural changes in a fetus that could impair its health or development and variations are defined as fetal changes that occur within the normal population under investigation and is unlikely to adversely affect survival or health. Rabbits dosed with isavuconazonium at 45 mg/kg bore pups which showed an increased incidence of a variation (additional artery arising from aortic arch) and increased incidence of abnormalities (uni- or bilateral rudimentary cervical ribs). These findings occurred at an exposure that was about 0.06 times the clinical exposure. Women should be made aware of the potential risks of defects to the offspring before taking this drug and women of childbearing age should be advised to use birth control.

Reference ID: 3689714

9.3 Prenatal and Postnatal Development

Study title:	BAL8557: Study for Effects on Pre- and Postnatal Development Including Maternal Function in the Han Wistar Rat
Astellas study no:	9766-TX-0056
Study report location:	EDR
Conducting laboratory:	(b) (4)
Date of study initiation:	29 May, 2006
Experimental start date:	5 June, 2006
GLP compliance:	(b) (4)
QA statement:	Yes
Drug, batch #,	17CH03SD.HQ00002.02
% purity:	85.2 %

Key Study Findings

Oral administration of isavuconazonium to pregnant rats from gestation day 6 to post-partum day 20 at a dose of 90 mg/kg was associated with increased post-natal loss (post-partum day 0 to 4). The NOEL for the dams was 30 mg/kg while that for the F1 generation was 90 mg/kg.

Methods

Doses:	0, 10, 30, 90 mg/kg
Frequency of dosing:	Once daily from Day 6 after mating to Day 20 postpartum.
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Purified water, adjusted to pH 3.5
Species/Strain:	Rat (*Rattus norvegicus*) HanRcc:WIST (SPF)
Number/Sex/Group:	22 pregnant dams/group
Satellite groups:	Toxicokinetics phase: 6 pregnant F_0 dams/dose level

BAL8557 was administered to mated female rats (F_0 generation) from day 6 *post coitum* to day 20 *post-partum.* Control animals received the vehicle alone. Examinations included monitoring of mortalities, clinical symptoms, feed consumption, body weight development and peri-/postnatal observations of F0 and F1 animals, and physical development, behavioral tests and reproductive performance of F1 animals. Plasma samples for pharmacokinetic examinations were collected from satellite animals (6/dose) at 1, 4 and 12 hours after dosing on day 6 *post coitum* and day 20 *post-partum*, and plasma and milk samples were collected at 4 hours after dosing on day 16 *post-partum.*

Results

F_0 dams	
Survival:	All females survived until the scheduled necropsy.
Clinical signs:	There were no drug-related adverse clinical findings.
Body weight:	There were no biologically significant differences between drug-treated dams and controls throughout gestation or lactation.
Feed consumption:	There were no biologically significant differences in feed consumption between drug-treated dams and controls.
Gestation length/index:	No test item-related effects were noted during gestation and parturition.

Reference ID: 3689714

Toxicokinetics

Table R7: Toxicokinetics in the rat pre- and postnatal development study. Study 9766-TX-0056

Dose (mg/kg)	Timing	Isavuconazole (BAL4815) C_{max} (ng/mL)	Isavuconazole (BAL4815) AUC_{last} (ng·h/mL)	Inactive Cleavage Product (BAL8728) C_{max} (ng/mL)	Inactive Cleavage Product (BAL8728) AUC_{last} (ng·h/mL)
10	Gestation Day 6	541	4970	374	1580
	Post Partum Day 20	536	4370	338	862
30	Gestation Day 6	1760	16300	930	3870
	Post Partum Day 20	1670	13000	1110	3590
90	Gestation Day 6	6820	63000	1830	12000
	Post Partum Day 20	6810	59600	2510	9410

In this study, systemic exposure (AUC) to isavuconazole was almost proportional to isavuconazonium dose in plasma and milk. The mean concentrations of isavuconazole in the maternal milk were 1700, 9030, and 29000 ng/mL at 10, 30, and 90 mg/kg, respectively, with milk concentrations exceeding plasma concentrations by up to 17-fold in individual animals.

Table R8: Isavuconazole pharmacokinetics in plasma and milk of rats dosed with isavuconazonium

Daily Dose (BAL8557, mg/kg)			0 (Control)	10	30	90
F_0 Females	Toxicokinetics: BAL4815 (active drug)					
	Plasma:	AUC (ng.h/mL), Day 6 p.c.	-	4970	16300	63000
		Cmax (ng/mL), day 16 p.p.	-	554	1830	4060
	Milk:	Mean conc. (mg/mL), Day 16 p.p.	-	1700	9030	29000
		Ratio (milk/plasma), Day 16 p.p. [1]	-	3.1	5.3	9.1

Drug was present in milk at all doses and levels were supraproportional to dose on Day 16 pp.

Formulation homogeneity: The drug preparation was demonstrated to be stable over four hours. Drug was prepared fresh daily and used within four hours. The content of the different dosing solutions tested was between 91.2 % and 102.8 %.

F_1 Generation
Litter size, survival, sex ratio:
There was a slight increase in the number of stillborn pups/litter (0.2 and 0.6 at the 30 and 90 mg/kg compared with 0 in controls). There were no drug-related effects on sex ratio.

At 90 mg/kg, post natal loss was statistically significantly increased (16.7% of live pups at birth compared to 0.7% in the control group). One female lost 7 surviving pups, another female which had one dead pup at the first litter check, lost the rest of its litter. Another female lost 15 pups, leaving only 2 remaining live pups, and another lost 3 pups. A further 4 litters lost one pup each. These losses were considered to be drug-related.

At 10 and 30 mg/kg body weight/day, post natal loss was similar to that of the control group.

Clinical signs: There were no drug-related clinical signs in the offspring.
Body weight: There were no drug-related differences in bodyweights the offspring

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Physical development: There were no biologically significant drug-related differences in developmental indices the offspring

Behavioral assessments: There were no biologically significant drug-related differences in developmental indices the offspring

Sexual maturation: There was no drug-related effect on sexual maturation as assessed by vaginal opening for females and preputial separation in males.

Reproduction: There were no drug-related differences in reproduction performance in the F_1 animals

Table R14: Litter data for Main phase females

F_0 Females continued	**Food Consumption (g/animal/day)**				
	GD 0 to 6	20.9	20.2	19.7	20.7
	GD 6 to 11	22.4	21.9	21.1	21.3
	GD 11 to 15	24.6	23.9	23.5	24.1
	GD 15 to 21	25.5	24.6	24.8	25.2
	LD 1 to 4	34.1	33.3	36.0	33.0
	LD 4 to 7	43.5	42.3	43.6	40.9
	LD 7 to 14	57.1	54.7	56.0	53.5
	Mean Duration of Gestation (Days)	21.2	21.4	21.5	21.5
	Abnormal Parturition	0	0	0	0
	Mean No. of Implantations	13.5	13.6	13.5	14.5
	Gestation index (%) (No. females with living pups / No. female pregnant)	100	100	100	100
F_1 Litters (Preweaning)	**No. Litters Evaluated**	22	20	22	22
	Mean No. Liveborn Pups/Litter	12.4	12.4	12.5	12.8
	Mean No. Stillborn Pups/Litter	0.0	0.0	0.2	0.6
	Postnatal Loss (%, Days 0-4 pp)	0.1	0.1	0.0	2.1
	Pup Sex Ratios (Males:Females) at LD 0	50:50	43:57#	45:55	52:48
	External Examination at the first litter check after parturition				
	No milk in stomach	---	---	---	+
	Birth Index (%) (No. Liveborn pups / No. Implantations)	91.6	91.2	92.9	88.4
	Viability Index (%) (No. Alive pups on LD4 / No. Live-born pups)	99.3	99.2	99.6	83.3##
	Weaning Index (%) (No. Alive pups on LD21 / No. Alive pups on LD4)	100	96.8#	99.4	98.7
	Male Pup Body Weights (g)				
	Day 0 post partum	6.1	6.4	6.0	5.9
	Day 21 post partum	49.7	48.9	50.9	47.9
	Female Pup Body Weights (g)				
	Day 0 post partum	5.7	5.9	5.7	5.7
	Day 21 post partum	47.8	47.1	48.6	45.8
	Pup Clinical Signs During Lactation Period	---	---	---	---
	Developmental Indices†	---	---	---	---
	Behavioral Tests‡	---	---	---	---

Discussion

Administration of isavuconazonium to rats in utero and during early postnatal development resulted in increased numbers of stillborn pups and increased post-natal loss between days 0 and 4 *post-partum*. The exposure (AUC) to isavuconazole at the high dose was about 61 µg*h/mL, which corresponded to about half the exposures observed in the clinic. The human AUC recorded at the maintenance dose of 200 mg/day was 121.4 µg*h/mL in Study No. 9766-CL-0017.

There were no significant adverse effects on physical development, neurological assessments, sexual maturation or reproduction in the offspring. Exposure to isavuconazole was approximately dose-proportional in plasma and milk, with milk concentrations exceeding plasma concentrations by factors up to 17 for individual animals.

Conclusion

Increased numbers of stillborn pups, increased peri-natal loss and high drug levels in milk were observed at isavuconazonium doses half the clinical exposures. Patients should be advised of these findings and should be on birth control if they are of child-bearing age.

Reference ID: 3689714

Integrated Summary and Safety Evaluation

Astellas Pharma Global Development, Inc. is seeking to market oral and intravenous CRESEMBA (isavuconazonium sulfate) for the treatment of patients with invasive aspergillosis and invasive mucormycosis who are at least 18 years old. The nonclinical toxicology data submitted in support of the approval of CRESEMBA were adequate to support these indications. Nonclinical studies covered durations of up to 39 weeks for the oral dosing and 6 weeks for intravenous dosing. In the study of isavuconazonium in aspergillosis patients, the mean treatment duration was 47 days, while the mucormycosis patients were treated for a median of 102 days. The mean steady state exposures ($AUC_{[0-24h]}$) observed following oral dosing of 200 or 600 mg were a 121 and 353 µg*h/mL, respectively, with a mean elimination half-life of 130 hours.

Pharmacology

The active moiety, isavuconazole is a triazole antifungal agent, which inhibits the conversion of lanosterol to ergosterol via its inhibition of the cytochrome P-450 dependent enzyme, lanosterol 14-alpha-demethylase. The presence of isavuconazole in the fungal cell is believed to result in the depletion of ergosterol within the fungal cell membrane, thereby damaging the fungal cell membrane. Isavuconazole was shown to inhibit both the hERG-mediated potassium currents and the L-type calcium channels, $I_{Ca,L}$.

Pharmacokinetics

There was good absorption of CRESEMBA after oral administration, with bioavailability between 62 % (rats) and 87 % (monkeys) after a single oral dose. Isavuconazonium was rapidly converted to isavuconazole, with a $t_{1/2}$ of less than 2 min in rat, rabbit, cynomolgus monkey, and human plasma. Isavuconazole was eliminated with a $t_{½}$ of 5 h in rats and 10 h in monkeys. Except for the active moiety isavuconazole, no individual metabolite was observed with an AUC > 10 % of drug-related material. In some rat and monkey studies, induction of CYP3A and/or CYP2B was observed.

Isavuconazole is extensively bound to proteins, with plasma protein binding above 96% for all species, including humans (99%). Quantitative whole body autoradiography data showed that, after repeated oral dosing in rats, drug-related ^{14}C -radioactivity concentrations were highest in the adrenal cortex and liver, followed by the small intestinal mucosa, brown fat, Harderian gland, pancreas, intra-orbital lacrimal gland, kidney cortex, adrenal medulla, stomach mucosa and the thyroid. After intravenous administration to pregnant rats, isavuconazole-related radioactivity was detected in the fetuses, in some instances at levels comparable to that in the maternal plasma. In pregnant rats dosed with isavuconazonium, milk concentrations of drug exceeded plasma concentrations by up to 17-fold.

Toxicology

Isavuconazonium sulfate was associated with liver effects in rats, mice and monkeys after oral or intravenous administration. Adrenal findings were only observed in monkeys, while thyroid effects were only observed in rats. While adverse effects were related to dose, no new toxicities developed as drug was administered for longer durations. Besides the hemorrhage, vasculitis/perivasculitis, necrosis and/or thrombosis seen at the injection site with the intravenous dosing, there were no new toxicities compared to oral dosing. This is consistent with the 67 % bioavailability of isavuconazonium. Humans appear to better tolerate this drug than the monkeys used for these studies since the clinical exposure appears to be above the maximum tolerated dose in the 39 week monkey study. However, this may also relate to the age of the monkeys used, which appeared to be prepubescent, based in the immature epididymides observed at necropsy.

The following were adverse events of special interest:

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During the course of the nonclinical evaluations, adverse events of special interest were observed in the heart, liver, adrenals, thyroids and the fetus.

Shortened QT
Simulation experiments using a human torso bidomain model have predicted that drugs like isavuconazonium which inhibit both the hERG-mediated potassium currents and the L-type calcium channels, $I_{Ca, L}$ will have a net effect of shortening the QT interval. Zemzemi, N. et al (2014) *Effects of L-type calcium channel and human ether-a-go-go related gene blockers on the electrical activity of the human heart: a simulation study. Europace. 2014, Sep 15. pii: euu122.* Clinical trials have confirmed this prediction as CRESEMBA shortened the QTc interval in a concentration-related manner in clinical trials. CRESEMBA was therefore contraindicated in patients with Familial Short QT syndrome.

Liver toxicity
Enlarged livers with increased liver weights and hepatocellular hypertrophy and/or vacuolation were seen in male and female rats, mice and monkeys dosed with isavuconazonium. Although these findings were not associated with liver necrosis and rarely resulted in increased liver enzymes, they were detected after two weeks of dosing (in preliminary studies) and at exposures lower than those expected at the clinical dose .These findings were, however, reversible when drug was stopped and so patients would be expected to recover from these effects once the drug treatment is over.

Nonetheless, the CRESEMBA prescribing information instructs physicians to evaluate liver-related laboratory tests at the start and during the course of CRESEMBA therapy and to monitor patients who develop abnormal liver-related laboratory tests during CRESEMBA therapy for the development of more severe hepatic injury. They are also advised to discontinue CRESEMBA if clinical signs and symptoms consistent with liver disease develop that may be attributable to CRESEMBA.

Adrenal toxicity
Increases in adrenal weights and/or vacuolation/hypertrophy of adrenocortical cells were observed in monkeys, but there were no atrophic or necrotic lesions. The applicant has ascribed this finding to the effects of enzyme induction. The increase in size and weights of both liver and adrenal may in fact relate to phospholipidosis, which has been observed with previous azoles and is evidenced by the increased vacuolation of the hepatocytes and adrenocortical cells. Unfortunately, without electron microscopy data, phospholipidosis cannot be definitively confirmed. The finding was general reversible except at doses more than double the clinical exposures. The clinical significance of these adrenal findings remains unclear.

Injection site
Injection site reactions were severe in the nonclinical studies and despite dilution of drug and increasing infusion times, some dose groups had to be terminated because of injection site damage. In the clinic, injection site findings are more common with isavuconazole than with voriconazole. Physicians are instructed to stop the infusion if injection site reactions become too severe.

Thyroid toxicity
In rats, repeated administration of isavuconazonium was associated with an increase in thyroid weights and thyroid follicular cell hypertrophy/hyperplasia, but these effects were not observed in monkeys. Humans handle thyroid hormones differently from rats and so these changes to the rat thyroid gland my not indicate any risk to humans. See *Wu, K-M et al, (2006) Preclinical development of new drugs that enhance thyroid hormone metabolism and clearance: Inadequacy of using rats as an animal model for predicting human risks in an IND and NDA. American Journal of Therapeutics 13, 141-144. (2006).*

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Fetal toxicity
When administered to pregnant animals during early embryo-fetal development, isavuconazonium induced skeletal abnormalities and/or variations in the rat and rabbit at doses as low as one tenth of the systemic exposure at the maintenance dose of 200 mg/day. Also, when dams were dosed orally during pregnancy and through the weaning period, there was increased perinatal mortality among rat pups. Isavuconazole was detected in the milk of lactating dams at levels up to 17 times higher than plasma levels. Women who become pregnant while on CRESEMBA are encouraged to contact their doctor. CRESEMBA should be used during pregnancy only if the potential benefit to the patient outweighs the risk to the fetus. Skeletal abnormalities have also been associated with the administration of other azole antifungals such as ketoconazole and fluconazole.

Bone fractures
Bone fractures were increased in the juvenile monkeys dosed with isavuconazonium at doses similar to clinical exposures. This dose was above the maximum tolerated dose for these monkeys as indicated by morbidity which resulted in humane sacrifices. While a single high dose female rat showed a diaphysis fracture of the femur in the 26 week study in adult rats, it is not clear that this fracture was drug-related. This finding may relate to the effects of isavuconazonium on calcium homeostasis. Since the drug is not indicated for patients below 18 years of age, this finding should have little impact on patients.

The applicant is seeking the approval of isavuconazonium for the treatment of very sick patients with two diseases which are often fatal. With the exception of pregnant women, the doses proposed have been associated with findings which are either monitorable, reversible or of questionable toxicological significance. All the pivotal studies were conducted in the most relevant species, the monkeys, with supporting data from rats and mice.

The applicant maintains (b) (4), but individual patients have been treated for almost 3 years. On one hand, no new toxicities were observed during the longest nonclinical study (39 weeks in monkeys) which indicates a favorable safety profile. However, if patients are going to be treated beyond 3 months, carcinogenicity studies will need to be conducted.

Unlike voriconazole, isavuconazonium has the advantage that it is not formulated in hydroxypropyl-beta-cyclodextrin HPBCD. HPBCD has its own toxicities which could complicate the treatment of these very sick patients.

Summary

There are no nonclinical data that would preclude the approval of CRESEMBA. While the exposure multiples at the LOAEL are small compared to the clinical exposures, the adverse effects are reversible and manageable or of questionable significance to man. The product will not be used in patients younger than 18 years of age and the label will carry warnings for patients with liver disease and pregnant women. The extensive nonclinical program supports the use of CRESEMBA for invasive aspergillosis and invasive mucormycosis. Carcinogenicity studies should be conducted as a Phase 4 commitment.

Reference ID: 3689714

Summary Table: Safety margins for adverse events of special interest

Toxicity	Species	LOAEL* Mg/kg	Exposure multiple based on AUC**
Liver, weight increase	Rat, Monkey	30	0.1
Liver, Hepatocellular hypertrophy	Rat, Monkey	30	0.1
Thyroid weight increase	Rat	90	0.3
Thyroid follicular cell hypertrophy	Rat	90	0.3
Adrenal, increased weight	Monkey	40	0.7
Adrenal, enlarged vacuolated cortical cells	Monkey	40	0.7
Fetal skeleton	Rat, rabbit	45	0.1
Bone fractures	Monkeys	20	0.8

**Compared to the human AUC at the maintenance dose of 200 mg/day isavuconazole = 121,402 ng·h/mL

Reference ID: 3689714

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/s/

OWEN G MCMASTER
01/20/2015

WENDELYN J SCHMIDT
01/21/2015

I concur with Dr. McMaster's assessment of the data submitted. I agree that the studies submitted were sufficient to determine the safety of isuvaconazole, and I concur with Dr. McMaster's interpretation of the data.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Numbers: 207500 207501 **Applicant:** Astellas **Stamp Date:** 07/08/2014

Drug Name: CRESEMBA **NDA/BLA Type:** NEW

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	√		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	√		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	√		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	√		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	√		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	√		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	√		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	√		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	√		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	√		
11	Has the applicant addressed any abuse potential issues in the submission?		√	
12	If this NDA/BLA is to support an Rx to OTC switch, have all relevant studies been submitted?			n/a

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? ___Yes_____

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Owen McMaster August 13, 2014

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

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

/s/

OWEN G MCMASTER
08/14/2014

WENDELYN J SCHMIDT
08/14/2014