CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-227

MICROBIOLOGY REVIEW

REVIEW TO HFD-590
OFFICE OF NEW DRUG CHEMISTRY
MICROBIOLOGY STAFF/HFD-805
MICROBIOLOGY REVIEW #2 OF NDA

23 January 2001

A. 1. NDA: 21-227

2. TYPE OF SUPPLEMENT: na

3. SUPPLEMENT PROVIDES FOR: na

4. APPLICANT/SPONSOR: Merck & Co., Inc
PO Box 4, BLA-20
West Point, PA 19486-0004

5. MANUFACTURING SITE: Merck & Co., Inc.
Sumneytown Pike
West Point, PA 19486

6. DRUG PRODUCT NAME:
Proprietary: CANCIDAS
Nonproprietary: Caspofungin Acetate
Drug Priority Classification: P

7. DOSAGE FORM, ROUTE OF ADMINISTRATION AND STRENGTH/POTENCY: Lyophilized Powder in a Glass Vial, Intravenous Administration, 50 mg/vial and 70 mg/vial (42 mg/mL)

8. METHOD(S) OF STERILIZATION: [redacted]

9. PHARMACOLOGICAL CATEGORY: Antibiotic

B. 1. DOCUMENT/LETTER DATE: March 15, 2000
2. RECEIPT DATE: March 16, 2000
3. CONSULT DATE: June 13, 2000
4. DATE OF AMENDMENT: January 19, 2001
5. ASSIGNED FOR REVIEW: January 19, 2001
6. SUPPORTING/RELATED DOCUMENTS:

C. REMARKS: This amendment addresses microbiology deficiencies in the NDA.

D. CONCLUSIONS: This submission is recommended for approval on the basis of product quality microbiology.

Bryan S. Riley, Ph.D.
Microbiology Reviewer

cc.: Original NDA 21-227
HFD 590/Division File
HFD 590/L. Chan
HFD 590/D. Matecka
HFD 805/Consult File
HFD 805/ B. Riley

Drafted by: Bryan Riley, Ph.D.
R/D initialed by: Peter Cooney, Ph.D.

3 page(s) have been removed because it contains trade secret and/or confidential information that is not disclosable.

MICROBIOLOGY REVIEW
DIVISION OF SPECIAL PATHOGEN AND IMMUNOLOGIC DRUG PRODUCTS (HFD-590)

NDA #: 21-227

REVIEWER : Shukal Bala
CORRESPONDENCE DATE : 07-28-00; 10-19-00
10-27-00; 11-08-00
11-16-00
CDER RECEIPT DATE : 07-31-00; 10-20-00
10-30-00; 11-09-00
11-16-00
REVIEW ASSIGN DATE : 08-02-00; 10-27-00
11-16-00; 11-17-00
11-21-00
REVIEW COMPLETE DATE: 01-12-01

SPONSOR: Merck Research Laboratories
P.O. Box 4, BLA-20
West Point, PA 19486-0004

SUBMISSIONS REVIEWED: Original, BI, BI, BI, and C

DRUG CATEGORY: Anti-fungal

INDICATION: Treatment of invasive aspergillosis in patients refractory to or intolerant of other therapies

DOSAGE FORM: Solution for intravenous administration

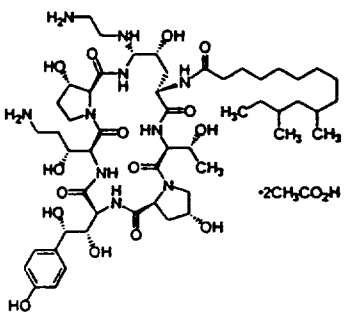
PRODUCT NAMES:

a. **PROPRIETARY:** Cancidas™

b. **NONPROPRIETARY:** Caspofungin acetate, MK-0991, L-743,872

c. **CHEMICAL:** N-(9B-(1A,2B-dihydroxy-2-(4-hydroxyphenyl)ethyl)tetracosahydro-1A,13A,22A,23A-tetrahydroxy-6B,17B-bis(1B-hydroxyethyl)2A-methyl-5,8,11,16,19,25-hexaoxo-5H-dipyrrol(2,1-C:2'1'-L)(1,4,7,10,13,16)hexa azacyclo-heneicosin-20B-yl)-4-(octyl)benzamide

STRUCTURAL FORMULA:



Molecular weight: 1213.45
Empirical formula: $C_{52}H_{88}N_{10}O_{15}.2C_2H_4O_2$

SUPPORTING DOCUMENTS: [redacted]

BACKGROUND:

Subject of this NDA is an echinocandin, Cancidas™, for the treatment of invasive aspergillosis in-patients who are refractory to or intolerant of other therapies. The sponsor has proposed to administer intravenously a single loading dose of 70 mg followed by a dose of 50 mg/day. Patients not responding to the 50 mg/day dose would be administered a higher dose (70 mg/day).

Caspofungin (MK-0991) the active drug ingredient of Cancidas™ is a water-soluble lipopeptide with activity against *Candida* and *Aspergillus* but not against *Cryptococcus*. It inhibits the synthesis of β (1,3)-D-glucan, which is important for maintaining the integrity of fungal cell walls but not mammalian cells.

The half-life (β) of the drug in humans is 10 to 12 hours. However, it is a protein-binding compound and can persist in the body. The γ half-life is 40 to 50 hours. After a 70 mg single dose, the area under the curve concentration (AUC) of caspofungin was 118.45 ug.hr/ml, and trough concentrations at 1 and 24 hours were 12.04 ug/ml and 1.42 ug/ml, respectively. After daily 70 mg multiple doses for 14 days, the AUC of caspofungin was 145.90 ug.hr/ml and trough concentrations at 1 and 24 hours were 15.58 ug/ml and 2.66 ug/ml, respectively.

The half-life of MK-0991 in mice, rats, rhesus monkeys, and chimpanzees was 5.2 to 7.6 hours (Hajdu *et al.*, 1997, Antimicrob Agents Chemother 41: 2339, reference # 51). The phamacokinetics of MK-0991 was studied in mice following intraperitoneal administration. The drug was cleared more slowly from all tissues than from plasma thereby predicting accumulation of the drug in tissues. The AUC segregated the organs into 3 exposure categories relative to plasma:

(1) The tissues with greater exposure than plasma: liver (16X), kidney (3X), and large intestine (2X).
(2) The organs with exposure equivalent to plasma: small intestine, lung and spleen.
(3) Organs with lower level of exposure than plasma: heart (0.3X), thigh (0.2X) and brain (0.06X).

SUMMARY:

The methods for testing of antifungal drugs are evolving. Attempts are being made to standardize the *in vitro* susceptibility methods and these efforts are being coordinated by the National Committee for Clinical Laboratory Standards (NCCLS). At the present time the NCCLS published guideline (M27A) describes methodology for the *in vitro* susceptibility testing of yeasts against azoles and amphotericin B (ampB) only. The NCCLS proposed guideline (M38P) describes the methodology for testing conidia forming filamentous fungi. The usefulness of these methods in predicting the *in vivo* activity for new classes of antifungal agents is not known.

It should be noted that for the purpose of this review, the term minimum inhibitory concentration (MIC) refers to no visible growth *in vitro*, and MIC-80/MIC-2 (≥80%/≥50% inhibition, respectively) represent a substantial inhibition of growth. The terms MIC_{90} and MIC_{50} indicate the concentration of the drug required for inhibiting 90% or 50% of the isolates tested respectively. The concentration required to alter the morphology of hyphae is referred to as minimum effective concentration (MEC).

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Mechanism of action

MK-0991 inhibits the activity of the enzyme glucan synthase derived from *Candida albicans* and *Aspergillus fumigatus* with 50% inhibitory concentration (IC_{50}) values of 0.6 nM and 9.6 nM respectively (Merck reports, 1995, reference # 4 and 5). The IC_{50} value against *Cryptococcus neoformans* enzyme was high (2.5 uM). This enzyme is important in the synthesis of β-(1,3)-D-glucan from glucose. Glucan is an important component of the cell wall of many fungi and the cyst stage of *Pneumocystis* but not of mammalian cells. *C. neoformans* may possess 1,6-β-glucan or other glucans (i.e., 1,3-α- or 1,6-α-glucans) in its cell wall, thus explaining decreased susceptibility to echinocandins. Studies have also shown that MK-0991's activity on the cell membrane enzyme is modulated by FKS1 gene.

It is possible that echinocandins may effect other components of the fungal cell besides the synthesis of glucan. For example, cilofungin (an analogue of echinocandin) was shown to alter sterol content. Incubation of cilofungin (0.3 and 0.6 ug/ml) with *C. albicans* for 18 hours was shown to decrease ergosterol content by 55% - 60% and glucan content by > 70% (Tables 1 and 2; Pfaller *et al.*, 1989, Eur J Clin Microbiol Infect Dis 8: 1067). There was minimal (4-13% reduction) effect on lanosterol. Chitin and mannan contents were, however, increased. It is unclear whether the changes in chitin, mannan and ergosterol are a direct effect of cilofungin or a consequence of an alteration in glucan content leading to dysregulation of carbohydrate synthesis with changes in the integrity of the cell membrane and nonspecific effect on sterol composition. A decrease in sterol component may increase the chitin content. The possibility of the effect of cilofungin on a combination of carbohydrate and lipid/sterol synthesis on cell membrane and cell wall cannot be ruled out. The effect of MK-0991 on sterol component of the cell membrane is not known.

Table 1

Effects of cilofungin on composition and synthesis of cell wall carbohydrates in *Candida albicans*. Results are mean ± standard error of a least three separate determinations. Calculations are based on μg carbohydrate per mg dry weight determined by biochemical methods, or counts per min per mg dry weight determined radiometrically by incorporation of isotope into the relevant cell wall fraction.

Concentration of cilofungin (μg/ml)	Percent of control values (mean ± SEM)						
	Growth (Dry weight)	Chitin		Glucan		Mannan	
		Bio-chemical	Radio-metric	Bio-chemical	Radio-metric	Bio-chemical	Radio-metric
0.3	18 ± 2	470 ± 92	ND	21 ± 1	ND	151 ± 4	ND
0.6	7 ± 4	625 ± 46	119 ± 4	28 ± 3	4 ± 1	166 ± 9	119 ± 1

ND = not determined.

Table 2

Effect of cilofungin on sterol composition in *Candida albicans*. Results are mean ± standard error of a least three separate determinations. Calculations are based on μg sterol per 50 μg nonsaponifiable lipid.

Concentration of cilofungin (μg/ml)	Percent of control values (mean ± SEM)	
	Ergosterol	Lanosterol
0.3	45 ± 7	96 ± 10
0.6	40 ± 1	87 ± 4

The effect of MK-0991 on human and mouse red blood cells was measured by hemolysis assay (Bartizal *et al.*, 1997, Antimicrob Agents Chemother 41: 2326, reference # 1). The minimum lytic concentrations (MLC) were measured after 2 hours of incubation at room temperature. The results in Table 3 show MK-0991 to be lytic at much higher (≥ 100 ug/ml) concentrations compared to ampB. MK-0991 was shown to persist *in vivo* for extended period. Therefore, the effect on lysis after exposure to the drug for extended period cannot be ruled out.

Table 3

	MLC (ug/ml)		
Red blood cells	**MK-0991**	**AmpB**	**Water**
Mouse	100 - 200	3	>400

Activity of MK-0991

The sponsor has tested the activity of MK-0991 against various fungal species *in vitro* and/or *in vivo*. In an initial *in vitro* experiment, 26 isolates of various fungal species (see Table 4) from the Merck culture collection were tested by broth micro-dilution method using [redacted] medium according to NCCLS reference method M27A (Merck report, 1995, reference # 35). The results indicate that the MK-0991 MICs for various *Candida* species varied from <0.008 to 0.5 ug/ml when measured at 24 hours and 0.06 to >128 ug/ml at 48 hours. MK-0991, like other echinocandins, does not exhibit complete inhibition of growth of *Aspergillus* species and the MICs were very high (8 to > 128 ug/ml). AmpB, on the other hand, inhibits the growth of *Aspergillus* with MICs ranging from 1 to 8 ug/ml. Against *Cryptococcus*, MK-0991 MICs varied from 4 to 16 ug/ml at both 24 and 48 hours. A comparison of the *in vitro* activity of MK-0991 with ampB showed that MK-0991 was more active than ampB against *Candida* but less active against *Aspergillus* and *Cryptococcus* (Table 4).

Table 4

Relative Activity of L-743,872 to Amphotericin B Against Fungal Test Strains in Susceptibility Evaluation

Strain	Ratio MIC24	Ratio MIC48	Ratio MFC
MY1028: *C. albicans*	5.90	0.01[a]	6.13
MY1055: *C. albicans*	6.10	id[b]	6.04
MY1750: *C. albicans*	3.86	id	11.62
MY1019: *C. guillermondii*	4.03	0.06	0.52
MY1010: *C. parapsilosis*	3.71	3.52	3.56
MY2099: *C. pseudotropicalis*	28.18	14.11	25.88
MY1012: *C. tropicalis*	id	id	535.61
MY1381: *C. glabrata*	5.35	5.17	2.72
MY1124: *C. tropicalis*	11.44	5.71	11.39
MY2168: *C. krusei*	id	id	id
CLY539: *C. albicans*	5.65	id	6.25
CLY494: *C. glabrata*	id	id	id
CLY495: *C. glabrata*	0.36	id	0.27
CA2: *C. albicans*	0.52	id	0.61
CA2 Parent: *C. albicans*	id	id	id
MY1051: *C. neoformans*	0.25	0.33	0.47
MY1146: *C. neoformans*	0.26	0.23	0.28
MY2061: *C. neoformans*	0.06	0.07	0.06
MY2062: *C. neoformans*	0.10	0.10	0.09
MY1976: *S. cerevisiae*	3.90	4.80	4.67
MY2140: *S. cerevisiae*	0.51	0.58	1.07
MY2141: *S. cerevisiae*	4.09	4.72	6.96
MF0383: *A. flavus*	id	id	id
MF4839: *A. fumigatus*	id	id	id
MF5668: *A. fumigatus*	id	id	id
MF5669: *A. fumigatus*	id	id	id

[a] Stippled boxes identify tests where L-743,872 is more than 2X less potent than AMB
[b] id = indeterminant

Relative activity is expressed as a ratio, calculated by dividing the mean MIC or MFC AMB values by mean MIC or MFC L-743,872 values such that numbers > 1 indicate greater potency of L-743,872.

Since then efforts have been made to determine the activity of MK-0991 against filamentous fungi by alternate methods such as measurement of alteration in morphology, viability, and substantial growth inhibition using NCCLS proposed methodology (M-38P). It is of note that these are experimental methods and their usefulness in predicting clinical outcome has not been established.

In the following sections of this review the *in vitro* activity of MK-0991 against *Aspergillus* (*in vitro* and *in vivo*) species is first discussed followed by its activity against *Candida* and other fungal species (*in vitro* and *in vivo*). The purpose of including the testing of *Candida* and other fungal species in this review is for understanding the spectrum of antifungal activity of the drug. Since these methods are not validated and the clinical efficacy against Candidiasis is still under investigation, any reference to nonclinical *Candida* studies in the label should not be considered.

In vitro* activity of MK-0991 against *Aspergillus

The *in vitro* activity of MK-0991, against various *Aspergillus* species that include laboratory and clinical isolates, was tested in 5 different laboratories. Studies by Arikan *et al.*, 1999 (ICAAC poster, 1999, reference # 43) measured the activity of MK-0991 against 82 *Aspergillus* isolates using the NCCLS proposed (M38-P) microdilution method. The activity was measured using different media [redacted] supplemented with 2% glucose [redacted] and antibiotic medium # 3 supplemented with 2% glucose [redacted] after 24, 48, and 72 hours of incubation. The MIC-80s at 48 and 72 hours were 1 to 5 folds higher than at 24 hours (Tables 5 and 6). Also, the MIC and MEC values were slightly higher in [redacted] than [redacted] and slightly lower in [redacted] than [redacted] No approved antifungal drug was used as a comparator.

Table 5: MIC and MEC values obtained in [redacted] after 24, 48, and 72 hours of incubation

Species (n)	Incubation Period	MIC (µg/mL) GM	MIC (µg/mL) Range	MEC (µg/mL) GM	MEC (µg/mL) Range
A. flavus (27)	24 h	2.72	0.25->16	0.27	0.25-0.5
	48 h	4	0.25->16	0.31	0.25-0.5
	72 h	>16	>16	0.26	0.25-0.5
A. fumigatus (26)	24 h	0.73	0.25->16	0.29	0.25-0.5
	48 h	27.7	0.25->16	0.5	0.25-0.5
	72 h	17.45	0.25->16	0.28	0.25-0.5
A. niger (17)	24 h	0.41	0.25-1	0.41	0.25-1
	48 h	0.44	0.25-2	0.42	0.25-1
	72 h	1.28	0.25->16	0.42	0.25-0.5
A. terreus (9)	24 h	0.5	0.5	0.5	0.5
	48 h	0.5	0.5	0.5	0.5
	72 h	8.64	0.5->16	0.5	0.5
A. nidulans (3)	24 h*				
	48 h	0.63	0.5-1	0.5	0.5
	72 h	0.63	0.5-1	0.5	0.5
F. solani (18)	24 h	22.6	16->16	17.3	8->16
	48 h	30.79	16->16	27.43	16->16
	72 h	>16	>16	30.8	16->16
F. oxysporum (4)	24 h	16	16	16	16->16
	48 h	>16	>16	>16	>16
	72 h	>16	>16	>16	>16

Table 6

Result (µg/mL) Species (n)	RPMI MIC/MEC	RPMI-2 MIC/MEC	AM3 MIC/MEC
A. flavus (27)	2.72/0.27	3.17/0.33	1.27/0.26
A. fumigatus (26)	0.73/0.29	1.89/0.31	0.43/0.31
A. niger (17)	0.41/0.41	0.54/0.52	0.23/0.2
A. terreus (9)	0.5/0.5	0.5/0.5	0.25/0.25
A. nidulans (3)*	0.63/0.5	0.5/0.5	0.25/0.25
F. solani (18)	22.6/17.3	24.4/22.63	19.4/16.63
F. oxysporum (4)	16/19	16/19.00	16/16

MIC=MIC-80
MIC/MEC at 24 hours
* 48 hour MIC-80

In a study by Espinel-Ingroff, 1998 (J Clin Microbiol 36: 2950; reference # 11) the activity of MK-0991 was measured against 26 *Aspergillus* isolates [*A. flavus* (11), *A. fumigatus* (13), and *A. terreus* (2)] by the NCCLS proposed (M38-P) microdilution method. These isolates were obtained from patients with severe fungal infections. The cultures were incubated from 24 to 72 hours to ensure that the growth was heavy/sufficient to determine MIC-2 values. The effect on morphology was not determined. The geometric mean MIC-2 values against *A. fumigatus* and *A. flavus*, were 2.15 (range 0.5 - >16) and 0.5 (all 11 isolates with MIC-2 of 0.5) ug/ml, respectively (Table 7). Only 2 *A. terreus* isolates were tested in this study with MIC-2 of 0.5 ug/ml. Two of the *A. fumigatus* isolates were stated to be itraconazole (ITZ) resistant (MIC of > 8 ug/ml; see Table 7, footnote). Whether these isolates were from patients refractory to treatment with ITZ was not specified. The minimum fungicidal concentrations (MFC) were determined by subculturing a small volume from the initial cultures. The MK-0991 MICs for these 2 isolates were not provided. MFCs were much higher than MIC-2 values. It is also of note that no approved antifungal agent such as ampB or ITZ was used as a comparator.

Table 7: Susceptibilities of 83 opportunistic filamentous and dimorphic fungi to SCH56592, MK-0991 and LY303366[a]

Fungus (no. tested)	Antifungal agent	MIC range (μg/ml)	Geometric mean MIC (μg/ml)	MFC range
Opportunistic filamentous fungi				
Acremonium strictum (1)	SCH56592		ND[b]	
	MK-0991		ND	
	LY303366		ND	
Aspergillus flavus (11)	SCH56592		0.10	
	MK-0991		0.5	
	LY303366		0.08	
Aspergillus fumigatus (13)[d]	SCH56592		0.13	
	MK-0991		2.15	
	LY303366		0.06	
Aspergillus terreus (2)	SCH59592		ND	
	MK-0991		ND	
	LY303366		ND	
Bipolaris spp.[e] (6)	SCH56592		0.14	
	MK-0991		1.7	
	LY303366		2.7	
Cladophialophora bantiana (5)	SCH56592		0.05	
	MK-0991		3.6	
	LY303366		2	
Fusarium oxysporum (6)	SCH56592		4.16	
	MK-0991		>16	
	LY303366		>16	
Fusarium solani (6)	SCH56592		>16	
	MK-0991		>16	
	LY303366		>16	
Phialophora spp.[f] (5)	SCH56592		0.4	
	MK-0991		2.8	
	LY303366		9	
Pseudallescheria boydii (6)	SCH56592		1.0	
	MK-0991		1.3	
	LY303366		2.5	
Rhizopus arrhizus (5)	SCH56592		2	
	MK-0991		>16	
	LY303366		>16	
Scedosporium prolificans (2)	SCH56592		ND	
	MK-0991		ND	
	LY303366		ND	
Dimorphic fungi				
Blastomyces dermatitidis (5)	SCH56592		0.05	
	MK-0991		2	
	LY303366		4	
Histoplasma capsulatum (5)	SCH56592		0.04	
	MK-0991		1.3	
	LY303366		3.6	
Sporothrix schenckii (5)	SCH56592		0.7	
	MK-0991		5.4	
	LY303366		3.9	
Total (83)				

[a] Visual MICs-2 correspond to prominent growth inhibition (approximately ≤50% of that of the growth control).
[b] For the geometric mean MIC column, ND indicates not obtained.
[c] For the MFC range column, ND indicates not done.
[d] The set included two isolates resistant to itraconazole (MICs, >8 μg/ml).
[e] *Bipolaris hawaiiensis* and *Bipolaris spicifera*.
[f] *Phialophora parasitica*, *Phialophora repens*, and *Phialophora verrucosa*.

In another study by Espinel-Ingroff (Merck report, 2000, study 2, Appendix A: reference # 62), the activity of MK-0991 was measured against 56 isolates of *A. fumigatus*, and ≥10 isolates each of other *Aspergillus* species (*A. niger*, *A. flavus*, *A. nidulans*, and *A. terreus*). The *in vitro* susceptibility was measured by the NCCLS proposed method (M38-P) using [redacted] medium. In addition, [redacted] medium and 2 inoculum concentrations of the conidial

suspension (10^3 and 10^4/ml) were used to measure the activity at 24 and 48 hours of incubation. *C. krusei* (ATCC 6258) NCCLS QC isolate was used for comparison. Also, 1 isolate each of *A. flavus* (ATCC 204304), and 2 isolates of *A. fumigatus* (ATCC 204305 and 13070) were included for testing as reference isolates (data not shown), although the M38-P document states that the ATCC numerical designations are pending. AmpB and ITZ were used as comparators. The results in Tables 8 and 9 show the MK-0991, ampB, and ITZ MIC-2 (range, geometric mean, and median) values for various isolates. The *in vitro* activity of MK-0991 varied with the medium, the concentration of the conidial suspension and the incubation time.

Table 8: Caspofungin MEC and MIC-2 data with 2 medium formulations

A. Low inoculum

A. flavus

R MEDIUM

	24 hour MEC	24 hour MIC-0	24 hour MIC-2	48 hour MIC-0	48 hour MIC-2
geomean	0.15	5.66	0.15	6.46	0.23
median	0.16	>8.0	0.16	>8.0	0.20
90th percentile	0.20	>8.0	0.2	>8.0	0.50

M-3 MEDIUM

	24 hour MEC	24 hour MIC-0	24 hour MIC-2	48 hour MIC-0	48 hour MIC-2
geomean	0.05	>8.0	0.06	7.19	0.12
median	0.05	>8.0	0.06	>8.0	0.12
90th percentile	0.12	>8.0	0.11	>8.0	0.12

A. fumigatus

R MEDIUM

	24 hour MEC	24 hour MIC-0	24 hour MIC-2	48 hour MIC-0	48 hour MIC-2
geomean	0.22	7.47	0.20	>8.0	0.35
median	0.20	>8.0	0.20	>8.0	0.50
90th percentile	0.50	>8.0	0.50	>8.0	0.50

M-3 MEDIUM

	24 hour MEC	24 hour MIC-0	24 hour MIC-2	48 hour MIC-0	48 hour MIC-2
geomean	0.08	7.32	0.07	7.35	0.14
median	0.08	>8.0	0.06	>8.0	0.12
90th percentile	0.20	>8.0	0.17	>8.0	0.20

A. nidulans

R MEDIUM

	24 hour MEC	24 hour MIC-0	24 hour MIC-2	48 hour MIC-0	48 hour MIC-2
geomean	0.31	>8.0	0.27	5.22	0.39
median	0.50	>8.0	0.50	>8.0	0.50
90th percentile	0.50	>8.0	0.39	>8.0	0.50

M-3 MEDIUM

	24 hour MEC	24 hour MIC-0	24 hour MIC-2	48 hour MIC-0	48 hour MIC-2
geomean	0.13	1.87	0.10	>8.0	0.20
median	0.12	>8.0	0.12	>8.0	0.20
90th percentile	0.20	>8.0	0.18	>8.0	0.20

A. niger

R MEDIUM

	24 hour MEC	24 hour MIC-0	24 hour MIC-2	48 hour MIC-0	48 hour MIC-2
geomean	0.14	0.19	0.17	8.06	0.18
median	0.12	0.16	0.20	>8.0	0.20
90th percentile	0.23	0.50	0.23	>8.0	0.40

M-3 MEDIUM

	24 hour MEC	24 hour MIC-0	24 hour MIC-2	48 hour MIC-0	48 hour MIC-2
geomean	0.03	0.04	0.03	0.33	0.04
median	0.06	0.06	0.03	0.16	0.06
90th percentile	0.06	0.07	0.06	>8.0	0.07

A. terreus

R MEDIUM

	24 hour MEC	24 hour MIC-0	24 hour MIC-2	48 hour MIC-0	48 hour MIC-2
geomean	0.20	>8.0	0.16	>8.0	0.27
median	0.20	>8.0	0.20	>8.0	0.20
90th percentile	0.20	>8.0	0.20	>8.0	0.50

M-3 MEDIUM

	24 hour MEC	24 hour MIC-0	24 hour MIC-2	48 hour MIC-0	48 hour MIC-2
geomean	0.03	>8.0	0.02	>8.0	0.10
median	0.03	>8.0	0.03	>8.0	0.09
90th percentile	0.09	>8.0	0.03	>8.0	0.20

B. High inoculum

A. flavus

R MEDIUM

	24 hour MEC	24 hour MIC-0	24 hour MIC-2	48 hour MIC-0	48 hour MIC-2
geomean	0.24	>8.0	0.20	>8.0	0.27
median	0.20	>8.0	0.2	>8.0	0.20
90th percentile	0.50	>8.0	0.2	>8.0	0.50

M-3 MEDIUM

	24 hour MEC	24 hour MIC-0	24 hour MIC-2	48 hour MIC-0	48 hour MIC-2
geomean	0.11	>8.0	0.12	>8.0	0.21
median	0.08	>8.0	0.06	>8.0	0.20
90th percentile	0.20	>8.0	0.20	>8.0	3.44

A. fumigatus

R MEDIUM

	24 hour MEC	24 hour MIC-0	24 hour MIC-2	48 hour MIC-0	48 hour MIC-2
geomean	0.29	>8.0	0.25	>8.0	0.38
median	0.20	>8.0	0.20	>8.0	0.50
90th percentile	0.50	>8.0	0.50	>8.0	0.69

M-3 MEDIUM

	24 hour MEC	24 hour MIC-0	24 hour MIC-2	48 hour MIC-0	48 hour MIC-2
geomean	0.13	7.41	0.11	7.91	0.16
median	0.20	>8.0	0.12	>8.0	0.20
90th percentile	0.20	>8.0	0.20	>8.0	0.20

A. nidulans

R MEDIUM

	24 hour MEC	24 hour MIC-0	24 hour MIC-2	48 hour MIC-0	48 hour MIC-2
geomean	0.42	>8.0	0.44	>8.0	0.51
median	0.50	>8.0	0.50	>8.0	0.50
90th percentile	0.90	>8.0	0.50	>8.0	0.90

M-3 MEDIUM

	24 hour MEC	24 hour MIC-0	24 hour MIC-2	48 hour MIC-0	48 hour MIC-2
geomean	0.21	6.02	0.21	>8.0	0.18
median	0.20	>8.0	0.20	>8.0	0.12
90th percentile	0.20	>8.0	0.20	>8.0	0.20

A. niger

R MEDIUM

	24 hour MEC	24 hour MIC-0	24 hour MIC-2	48 hour MIC-0	48 hour MIC-2
geomean	0.16	4.98	0.14	>8.0	0.20
median	0.18	>8.0	0.16	>8.0	0.20
90th percentile	0.23	>8.0	0.23	>8.0	0.35

M-3 MEDIUM

	24 hour MEC	24 hour MIC-0	24 hour MIC-2	48 hour MIC-0	48 hour MIC-2
geomean	0.04	0.128	0.03	2.95	0.06
median	0.06	0.06	0.03	>8.0	0.06
90th percentile	0.07	>8.0	0.07	>8.0	0.07

A. terreus

R MEDIUM

	24 hour MEC	24 hour MIC-0	24 hour MIC-2	48 hour MIC-0	48 hour MIC-2
geomean	0.12	>8.0	0.12	>8.0	0.27
median	0.12	>8.0	0.12	>8.0	0.20
90th percentile	0.20	>8.0	0.20	>8.0	0.50

M-3 MEDIUM

	24 hour MEC	24 hour MIC-0	24 hour MIC-2	48 hour MIC-0	48 hour MIC-2
geomean	0.06	8	0.05	8	0.14
median	0.06	8	0.05	8	0.20
90th percentile	0.12	8	0.20	8	0.20

Table 9: In vitro susceptibility of Aspergillus isolates to ITZ and ampB

A: ITZ (MIC-2 data) B: ampB (MIC data)

	geomean	median	90th percentile	range	# isolates used	geomean	median	90th percentile	range	# isolates used
A. flavus	0.13	0.12	0.20		13	1.38	1.00	2.00		13
A. fumigatus	0.14	0.12	0.20		58	0.94	1.00	2.00		58
A. nidulans	0.10	0.12	0.20		13	0.62	0.50	2.00		13
A. niger	0.28	0.35	0.50		10	0.76	1.00	1.00		10
A. terreus	0.04	0.06	0.11		12	1.78	2.00	4.00		12

In a study by Del Poeta *et al.*, 1997 (Antimicrobial Agents Chemother 41: 1835, reference # 36), the activity of MK-0991 was measured against 16 isolates of *Aspergillus* species (*A. fumigatus* and *A. flavus*). The cultures were prepared according to the NCCLS proposed broth macrodilution method (M38-P) using [redacted] medium with incubations for 72 hours. The MIC-80 values in Table 10 indicate MK-0991 to be effective against *Aspergillus* isolates (MIC-80 ≤ 0.09 – 3.12 ug/ml).

Table 10: *In vitro* activity of MK-0991 against 55 clinically important moulds

Species (no. of isolates tested)	MIC (μg/ml) Geometric mean[a]	MIC (μg/ml) Range	Inoculum (CFU/ml)
Alternaria sp. (1)	≤0.09		1.2×10^3
Aspergillus flavus (8)	0.20		0.6×10^3–2.0×10^3
Aspergillus fumigatus (8)	≤0.09		1.1×10^3–2.8×10^3
Curvularia lunata (4)	0.38		0.8×10^3–1.3×10^3
Exophiala jeanselmei (2)	1.10[b]		2.2×10^3
Fonsecaea pedrosoi (4)	0.13[c]		0.3×10^3–2.1×10^3
Fusarium oxysporum (5)	75.78		0.5×10^3–2.2×10^3
Fusarium solani (5)	59.46		0.6×10^3–1.1×10^3
Paecilomyces lilacinus (5)	49.98		1.1×10^3–2.6×10^3
Paecilomyces variotii (2)	≤0.09		1.5×10^3–2.3×10^3
Rhizopus arrhizus (5)	>100[d]		0.6×10^3–1.3×10^3
Scedosporium apiospermum (4)	0.38		0.2×10^3–0.8×10^3
Scedosporium prolificans (2)	8.83		0.8×10^3–1.1×10^3

[a] Unless otherwise noted, incubation was for 72 h at 30°C.
[b] Incubation was for 144 h at 30°C.
[c] Incubation was for 120 h at 30°C.
[d] Incubation was for 24 h at 30°C.

In a study by Rinaldi (Merck report, April, 2000, reference # 72) a series of *Aspergillus* isolates were tested for susceptibility to MK-0991, ampB, and ITZ by NCCLS proposed method (M38-P). The results show that the MIC_{80} values were not significantly altered by the time of incubation for most of the isolates tested (Table 11). The MK-0991 MIC-80 values for all the 25 isolates of *A. flavus* were ≤ 0.125 ug/ml at 24 and 48 hours. For *A. fumigatus*, 41 isolates were tested and the MK-0991 MIC-80 ranged from ≤[redacted]ug/ml at 24 and 48 hours. For *A. terreus*, MK-0991 MIC-80 values were ≤0.125 ug/ml against a majority of the 25 isolates tested at 24 and 48 hours. At 48 hours, only one isolate showed a MIC-80 value of >64 ug/ml.

For *A. glaucus* (n=2), *A. nidulans* (n=5), and *A. niger* (n=10) a small number of isolates were tested with 24 hour MK-0991 MIC-80 of ≤0.125 ug/ml. At 48 hours, MK-0991 MIC-80 values for a majority of the isolates were ≤ 0.125 ug/ml except for 1 isolate of *A. nidulans* (0.5 ug/ml) and 2 isolates of *A. niger* (0.25 and >64 ug/ml). The minimum lethal concentrations were much higher than the MIC-80 values for all the isolates.

Table 11

		MERCK				AMB				ITRA	
FTL #	Isolate	MIC 24	MIC 48	MLC 24	MLC 48	MIC 24	MIC 48	MLC 24	MLC 48	MIC 24	MIC 48
Control	Candida krusei	≤0.125	≤0.125	2	4	0.5	0.5	0.5	2	≤=0.015	0.03
00-153	A. flavus	≤0.125	≤0.125	>64	>64	1	2	2	2	0.03	0.06
00-87	A. flavus	≤0.125	≤0.125	>64	>64	1	1	1	2	0.03	0.06
99-1273	A. flavus	≤0.125	≤0.125	>64	>64	1	2	2	2	0.03	0.125
99-1429	A. flavus	≤0.125	≤0.125	>64	>64	0.5	0.5	0.5	1	0.06	0.125
99-1520	A. flavus	≤0.125	≤0.125	>64	>64	0.5	1	2	2	0.06	0.125
99-1706	A. flavus	≤0.125	≤0.125	1	>64	1	1	1	2	0.06	0.125
99-1738	A. flavus	≤0.125	≤0.125	>64	>64	1	1	1	1	0.06	0.06
99-1912	A. flavus	≤0.125	≤0.125	>64	>64	1	1	1	2	0.03	0.125
99-1962	A. flavus	≤0.125	≤0.125	>64	>64	1	1	2	2	0.06	0.125
99-1983	A. flavus	≤0.125	≤0.125	>64	>64	1	1	1	2	0.06	0.125
99-1992	A. flavus	≤0.125	≤0.125	>64	>64	1	1	2	2	0.06	0.125
99-2034	A. flavus	≤0.125	≤0.125	>64	>64	1	1	1	1	0.03	0.06
99-231	A. flavus	≤0.125	≤0.125	>64	>64	1	2	2	2	0.06	0.125
99-2072	A. flavus	≤0.125	≤0.125	>64	>64	1	1	1	2	0.06	0.125
99-2141	A. flavus	≤0.125	≤0.125	>64	>64	1	1	2	2	0.06	0.125
99-2137	A. flavus	≤0.125	≤0.125	>64	>64	2	2	2	2	0.06	0.06
99-2159	A. flavus	≤0.125	≤0.125	>64	>64	1	2	2	2	0.06	0.125
99-2229	A. flavus	≤0.125	≤0.125	>64	>64	2	2	2	2	0.06	0.06
99-2290	A. flavus	≤0.125	≤0.125	>64	>64	1	1	1	1	0.06	0.06
99-2309	A. flavus	≤0.125	≤0.125	>64	>64	1	1	1	1	0.06	0.06
99-2310	A. flavus	≤0.125	≤0.125	>64	>64	1	1	1	2	0.06	0.125
99-2311	A. flavus	≤0.125	≤0.125	>64	>64	1	2	2	2	0.06	0.06
99-2312	A. flavus	≤0.125	≤0.125	>64	>64	1	1	1	2	0.06	0.06
99-705	A. flavus	≤0.125	≤0.125	>64	>64	1	2	2	2	0.03	0.06
99-819	A. flavus	≤0.125	≤0.125	>64	>64	1	2	2	2	0.06	0.125
00-134	A. fumigatus	≤0.125	≤0.125	>64	>64	0.5	0.5	1	2	0.06	0.125
00-138	A. fumigatus	≤0.125	≤0.125	>64	>64	0.25	0.5	0.5	0.5	0.06	0.25
00-116	A. fumigatus	≤0.125	≤0.125	>64	>64	0.5	0.5	0.5	0.5	0.125	0.25
00-121	A. fumigatus	≤0.125	≤0.125	>64	>64	0.5	0.5	0.5	0.5	0.125	0.25
00-159	A. fumigatus	≤0.125	≤0.125	>64	>64	0.5	0.5	1	1	0.06	0.25
00-160	A. fumigatus	≤0.125	≤0.125	>64	>64	0.5	0.5	1	1	0.06	0.25
00-55	A. fumigatus	≤0.125	≤0.125	>64	>64	0.5	0.5	0.5	1	0.06	0.25

n=25

n=41

FTL #	Isolate	MIC 24	MIC 48	MLC 24	MLC 48	MIC 24	MIC 48	MLC 24	MLC 48	MIC 24	MIC 48
99-1337	A. fumigatus	0.25	0.25	1	>64	0.25	0.5	1	2	0.03	0.25
99-1538	A. fumigatus	≤0.125	≤0.125	1	>64	0.25	0.5	0.5	1	1	>8
99-1592	A. fumigatus	≤0.125	≤0.125	>64	>64	0.25	0.5	1	1	0.06	0.25
99-1632	A. fumigatus	≤0.125	≤0.125	1	>64	0.5	0.5	1	1	<=0.015	0.06
99-1643	A. fumigatus	≤0.125	≤0.125	>64	>64	0.25	0.5	0.5	1	0.06	0.125
99-1707	A. fumigatus	≤0.125	≤0.125	2	2	0.25	0.5	0.5	0.5	0.06	0.125
99-1720	A. fumigatus	≤0.125	≤0.125	2	>64	0.25	0.5	0.5	2	0.125	0.25
99-1752	A. fumigatus	≤0.125	≤0.125	>64	>64	0.25	0.5	0.5	1	0.125	0.25
99-1773	A. fumigatus	≤0.125	≤0.125	>64	>64	0.25	0.5	0.5	1	0.125	0.25
99-1774	A. fumigatus	0.25	0.25	>64	>64	0.25	0.5	1	1	0.125	0.25
99-1803	A. fumigatus	≤0.125	≤0.125	1	>64	0.25	0.5	0.5	0.5	0.25	0.5
99-1808	A. fumigatus	≤0.125	≤0.125	>64	>64	0.25	0.5	0.5	0.5	0.125	0.25
99-1820	A. fumigatus	≤0.125	≤0.125	>64	>64	0.5	1	2	2	0.125	0.25
99-1825	A. fumigatus	≤0.125	≤0.125	1	>64	0.25	0.5	2	2	0.125	0.25
99-1832	A. fumigatus	≤0.125	≤0.125	1	>64	0.5	0.5	1	2	0.125	0.25
99-1865	A. fumigatus	≤0.125	≤0.125	>64	>64	0.5	0.5	0.5	1	0.06	0.125
99-1868	A. fumigatus	≤0.125	≤0.125	2	>64	0.5	0.5	1	1	0.06	0.125
99-1965	A. fumigatus	≤0.125	≤0.125	>64	>64	0.5	0.5	1	1	0.125	0.25
99-1968	A. fumigatus	≤0.125	≤0.125	>64	>64	0.25	0.5	1	2	0.06	0.125
99-1983	A. fumigatus	≤0.125	≤0.125	>64	>64	0.5	0.5	1	2	0.06	0.25
99-2011	A. fumigatus	≤0.125	≤0.125	1	4	0.5	0.5	1	1	0.06	0.25
99-2012	A. fumigatus	≤0.125	≤0.125	>64	>64	0.5	0.5	1	1	0.125	0.25
99-2013	A. fumigatus	≤0.125	≤0.125	1	4	0.25	0.5	1	1	0.06	0.25
99-2075	A. fumigatus	≤0.125	≤0.125	2	2	0.5	1	1	2	0.5	>8
99-2119	A. fumigatus	≤0.125	≤0.125	>64	>64	0.5	0.5	1	1	0.06	0.125
99-2137	A. fumigatus	≤0.125	≤0.125	2	2	0.5	0.5	1	1	0.125	0.25
99-2230	A. fumigatus	≤0.125	≤0.125	2	2	0.5	0.5	2	2	0.125	0.25
99-2313	A. fumigatus	≤0.125	≤0.125	>64	>64	0.5	0.5	1	1	0.06	0.125
99-2314	A. fumigatus	≤0.125	≤0.125	>64	>64	0.5	0.5	1	2	0.125	0.25
99-2315	A. fumigatus	≤0.125	≤0.125	>64	>64	0.5	0.5	1	1	0.06	0.125
99-2344	A. fumigatus	≤0.125	≤0.125	>64	>64	0.5	1	1	1	0.06	0.06
99-2383	A. fumigatus	≤0.125	≤0.125	1	>64	0.5	0.5	1	1	0.06	0.06
99-2384	A. fumigatus	≤0.125	≤0.125	1	1	0.5	1	1	1	0.06	0.125
H11-20	A. fumigatus	≤0.125	≤0.125	≤0.125	0.25	0.5	1	1	2	0.125	0.25
97-300	A. glaucus	≤0.125	≤0.125	≤0.125	≤0.125	1	1	2	2	0.125	0.25
98-2574	A. glaucus	≤0.125	≤0.125	≤0.125	0.25	1	2	2	2	0.06	0.25

n=2

Table 11 continued

FTL #	Isolate	MIC 24	MIC 48	MLC 24	MLC 48	MIC 24	MIC 48	MLC 24	MLC 48	MIC 24	MIC 48	
99-1373	A. nidulans	≤0.125	≤0.125	64	>64	1	2	2	2	0.125	0.25	n=5
99-1525	A. nidulans	≤0.125	0.5	>64	>64	1	2	2	4	<=0.015	>8	
99-2087	A. nidulans	≤0.125	≤0.125	64	>64	1	2	4	8	0.06	0.125	
99-2411	A. nidulans	≤0.125	≤0.125	64	>64	1	1	16	>16	0.06	0.125	
99-247	A. nidulans	≤0.125	≤0.125	64	>64	1	2	4	4	0.03	0.06	
99-1603	A. niger	≤0.125	≤0.125	≤0.125	>64	0.5	0.5	1	1	0.5	1	
99-1937	A. niger	≤0.125	≤0.125	1	>64	0.5	0.5	0.5	1	0.25	0.5	
99-1989	A. niger	≤0.125	≤0.125	≤0.125	8	0.5	0.5	0.5	1	0.25	0.5	n=10
99-2051	A. niger	≤0.125	≤0.125	≤0.125	>64	0.5	0.5	0.5	0.5	0.25	0.5	
99-2097	A. niger	≤0.125	≤0.125	≤0.125	8	0.5	1	1	1	0.5	0.5	
99-2081	A. niger	≤0.125	>64	64	64	0.5	0.5	0.5	0.5	0.25	0.5	
99-2131	A. niger	≤0.125	≤0.125	8	8	0.5	1	1	1	0.25	0.5	
99-2142	A. niger	≤0.125	≤0.125	8	>64	0.5	0.5	0.5	1	0.25	0.5	
99-2173	A. niger	≤0.125	≤0.125	≤0.125	8	0.5	0.5	0.5	1	0.25	0.5	
99-2183	A. niger	≤0.125	0.25	1	>64	0.5	1	1	1	0.25	0.5	
00-125	A. terreus	≤0.125	≤0.125	>64	>64	2	2	2	16	0.03	0.125	
00-127	A. terreus	≤0.125	≤0.125	>64	>64	1	2	4	8	<=0.015	<=0.015	
00-184	A. terreus	≤0.125	≤0.125	>64	>64	1	2	8	>16	<=0.015	0.06	
00-99	A. terreus	≤0.125	≤0.125	>64	>64	1	2	2	4	0.03	0.06	n=25
99-1019	A. terreus	≤0.125	≤0.125	>64	>64	1	2	2	8	0.03	0.06	
99-1034	A. terreus	≤0.125	≤0.125	1	>64	1	2	8	16	0.06	0.125	
99-1121	A. terreus	≤0.125	≤0.125	>64	>64	1	2	2	4	0.125	0.125	
99-1158	A. terreus	≤0.125	≤0.125	1	>64	1	2	4	8	0.03	0.06	
99-1272	A. terreus	≤0.125	≤0.125	>64	>64	1	2	2	8	0.03	0.06	
99-1331	A. terreus	≤0.125	≤0.125	>64	>64	0.5	1	2	4	0.03	0.06	
99-1437	A. terreus	≤0.125	≤0.125	>64	>64	0.5	2	2	4	<=0.015	0.125	
99-1868	A. terreus	≤0.125	≤0.125	>64	>64	1	2	2	2	0.03	0.125	
99-2078	A. terreus	≤0.125	≤0.125	>64	>64	1	2	4	16	0.03	0.06	
99-2188	A. terreus	≤0.125	≤0.125	>64	>64	1	2	2	4	0.03	0.06	
99-2214	A. terreus	≤0.125	≤0.125	>64	>64	1	1	2	8	0.03	0.06	
99-2221	A. terreus	≤0.125	≤0.125	64	64	0.5	1	2	8	<=0.015	0.03	
99-2365	A. terreus	≤0.125	≤0.125	1	>64	1	2	4	4	0.03	0.03	
99-404	A. terreus	≤0.125	≤0.125	>64	>64	1	2	2	4	0.03	0.06	
99-405	A. terreus	≤0.125	≤0.125	>64	>64	1	1	2	4	0.03	0.06	
99-461	A. terreus	≤0.125	≤0.125	>64	>64	0.5	1	1	1	<=0.015	0.125	
99-488	A. terreus	≤0.125	≤0.125	>64	>64	1	2	4	8	0.03	0.125	

FTL #	Isolate	MIC 24	MIC 48	MLC 24	MLC 48	MIC 24	MIC 48	MLC 24	MLC 48	MIC 24	MIC 48	
99-587	A. terreus	≤0.125	>64	>64	>64	1	2	2	8	0.03	0.125	
99-598	A. terreus	≤0.125	≤0.125	>64	>64	1	2	4	16	0.03	0.06	
99-729	A. terreus	≤0.125	≤0.125	>64	>64	1	2	2	4	0.03	0.06	
R-3101	A. terreus	≤0.125	≤0.125	>64	>64	1	2	2	8	<=0.015	0.03	
99-2244	A. versicolor	≤0.125	≤0.125	≤0.125	>64	0.25	0.5	1	1	0.125	0.25	n=3
99-356	A. versicolor	16	32	64	>64	0.25	0.25	0.5	0.5	0.06	0.125	
99-740	A. versicolor	≤0.125	≤0.125	≤0.125	>64	1	2	2	2	0.06	0.125	

Over 90 clinical isolates of *Aspergillus* species obtained from 36 patients enrolled in the phase III study (protocol 019) were tested by the NCCLS proposed reference method (M38-P) for *in vitro* susceptibility to MK-0991 and other drugs (Merck report, 2000, reference # 76). The activity was measured using 2 different media [redacted] MIC values were determined after 24 (MK-0991) and 48 (ITZ) hours of incubation and expressed as no visible growth (for ampB) and the first well showing reduction in growth (approximately 80% for MK-0991 and ITZ). The results in Tables 12 and 13 show the MK-0991 values against 4 different species of *Aspergillus*. Based on MIC-80 as an end-point, the MK-0991 MIC_{90} values against *A. fumigatus* in [redacted] medium were 0.5 ug/ml and ≤ 0.03 ug/ml, respectively. The MIC_{90} values for the 11 *A. flavus* isolates from 8 patients were high (>64 ug/ml). The number of isolates for *A. niger* and *A. terreus* was small (n ≤4) with MIC_{50} of ≤ 0.03 ug/ml and 0.25 ug/ml, respectively in [redacted] medium (MIC_{50} in [redacted] medium were ≤ 0.03 ug/ml). There was no correlation between *in vitro* susceptibility and clinical outcome.

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Table 12

Minimum Inhibitory Concentrations (μg/mL) for Caspofungin, Amphotericin B and Itraconazole versus *Aspergillus* Clinical Isolates[1]

RPMI 1640 Medium

Organism (Number of Isolates)	Antifungal	Range	MIC_{90}	MIC_{90}[2]	Geometric Mean
Aspergillus fumigatus (80)	Caspofungin		0.25	0.5	0.195
	AmB		0.5	1	0.509
	ITZ		0.06	0.25	0.088
Aspergillus flavus (11)	Caspofungin		0.5	>64	1.297
	AmB		1	2	1.066
	ITZ		0.06	0.125	0.050
Aspergillus niger (4)	Caspofungin		≤0.03		0.031
	AmB		0.25		0.297
	ITZ		0.125		0.149
Aspergillus terreus (3)	Caspofungin		0.25		0.196
	AmB		4		4
	ITZ		0.06		0.044

† Broth macrodilution method (NCCLS Document: M38-P[illegible] RPMI 1640 (Biowhittaker) medium, inoculum 1 to 5 x 10^4 CFU/mL; incubation at 35°C for 24 hours (Caspofungin) or 48 hours (AmB and ITZ). MIC for caspofungin and ITZ was defined as the lowest concentration of antifungal showing significant reduction in visible growth (MIC_{90}); the MIC for AmB was defined as lowest concentration of antifungal inhibiting visible growth.

‡ At least 10 isolates are required to calculate MIC_{90}.
AmB = Amphotericin B
ITZ = Itraconazole

Data Source:
Notebooks-Flowery: AF Clinical 1 and AF Clinical 2
Study Dates: Sep-1998 to May-2000
Notebooks-Hicks: MK-0991 Clinical trials Aspergillus Book 1
Study Dates: Sep-1998 to May-2000

Table 13

Minimum Inhibitory Concentrations (μg/mL) for Caspofungin, Amphotericin B and Itraconazole versus *Aspergillus* Clinical Isolates[1]

Antibiotic Medium #3 – 2% Glucose

Organism (Number of Isolates)	Antifungal	Range	MIC_{90}	MIC_{90}[2]	Geometric Mean
Aspergillus fumigatus (80)	Caspofungin		≤0.03	≤0.03	0.033
	AmB		0.5	0.5	0.389
	ITZ		≤0.03	0.06	0.044
Aspergillus flavus (11)	Caspofungin		0.125	>64	0.866
	AmB		1	2	1.065
	ITZ		≤0.03	0.06	0.041
Aspergillus niger (4)	Caspofungin		≤0.03		≤0.03
	AmB		0.25		0.210
	ITZ		0.125		0.104
Aspergillus terreus (3)	Caspofungin		≤0.03		0.243
	AmB		2		2
	ITZ		≤0.03		≤0.03

† Microdilution broth method (NCCLS Document: M38-P[illegible]); Antibiotic Medium #3 (Difco) + 2% glucose medium; inoculum 1 to 5 x 10^4 CFU/mL; incubation at 35°C for 24 hours (Caspofungin) or 48 hours (AmB and ITZ). MIC for caspofungin and ITZ was defined as the lowest concentration of antifungal showing significant reduction in visible growth (MIC_{90}); the MIC for AmB was defined as lowest concentration of antifungal inhibiting visible growth.

‡ At least 10 isolates are required to calculate MIC_{90}.
AmB = Amphotericin B
ITZ = Itraconazole

Data Source:
Notebooks-Flowery: AF Clinical 1 and AF Clinical 2
Study Dates: Sep-1998 to May-2000
Notebooks-Hicks: MK-0991 Clinical trials Aspergillus Book 1
Study Dates: Sep-1998 to May-2000

The effect of MK-0991 on the viability of hyphae was measured by staining with fluorescein dyes which enter the cell based on its physiological state (Merck report, 2000, reference # 62; also presented by Douglas *et al.*, 2000, ICAAC, 2000, poster no. 1683). CFDA (5,6 carboxy fluorescein) is a vital stain, whereas $DiBAC_4(3)$ i.e., bis-(1,3-dibutylbarbturic acid) trimethane oxonol is a non-vital stain. *A. fumigatus* conidia were incubated in [redacted] containing 0.15% [redacted] a polyacrylate, which reduces clumping and enables *A. fumigatus* mycelia to grow as dispersed germlings) at 37°C for 14 hours. MK-0991 or other drugs were then added and cultures incubated for an additional 6 hours. The results in Figures 1 and 2 show that MK-0991 (0.3 μg/ml) was lethal at the selective areas with active cell growth i.e., the apical tips of hyphae and areas of hyphal branching. In regions of less active growth the hyphae were viable. In contrast, ampB at a concentration of 0.15 μg/ml resulted in an almost complete loss of viability of the organism. This difference is attributed to ampB's disruption of membrane activity.

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Figure 1

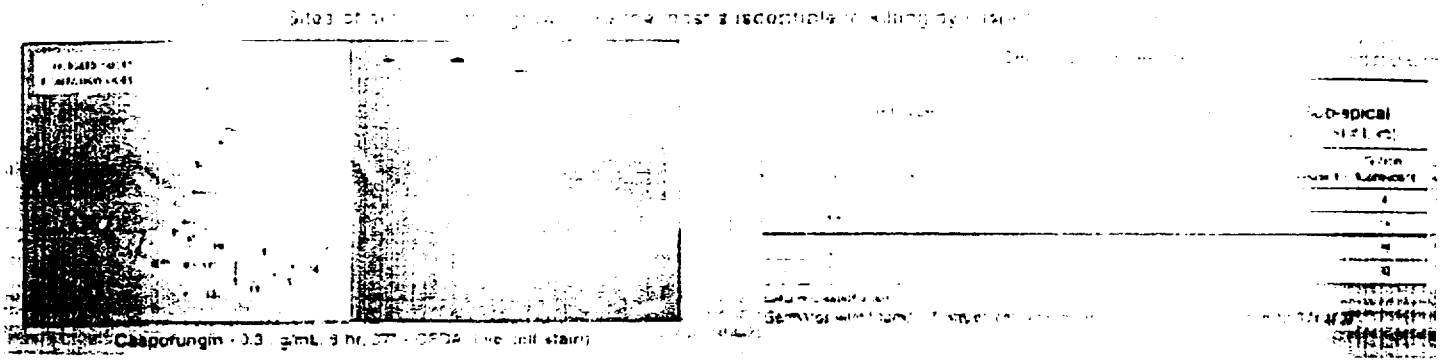
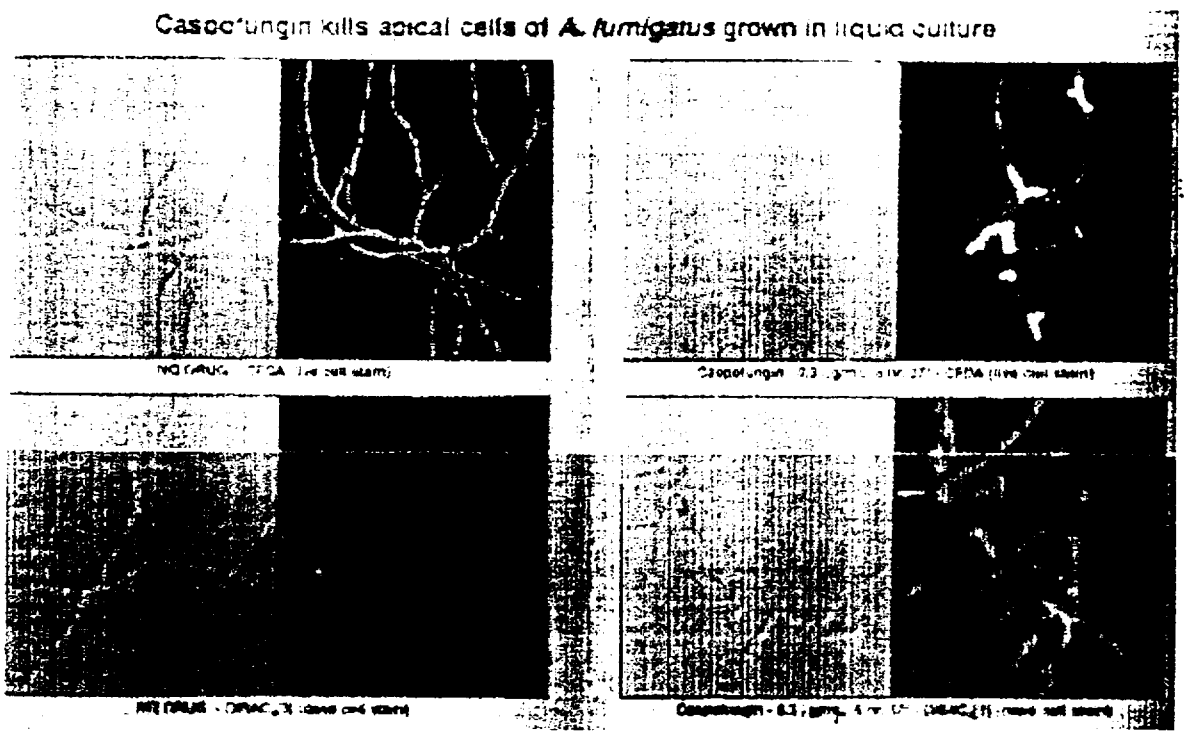


Figure 2



In vivo* activity of MK-0991 against *Aspergillus

The *in vivo* activity of MK-0991 against *Aspergillus* species was measured in immunocompromised animals. A majority of the studies measured the activity of MK-0991 against *A. fumigatus*. Only one experiment was conducted in mice to measure the activity of MK-0991 against *A. flavus*.

Activity against *A. fumigatus* in C'5 deficient mice

C'5 deficient DBA/2N mice were infected with 4.8 x 10^5 to 1.8 x 10^6 conidia of *A. fumigatus* (strain MF 5668 - ATCC 13073) by the intravenous route (Merck report, 1995, reference # 50; Abruzzo *et al.*, 1997, Antimicrob Agents Chemother 41: 2333, reference # 2). Treatment, initiated within 15 - 30 minutes or after 24 hours of infection, was b.i.d., for 5 days by intraperitoneal, intravenous, or oral routes. Animals were followed for survival over a period of 28 days after initiation of infection. AmpB was used as a comparator. The results in Figure 3 and Table 14 indicate that MK-0991, like ampB, improved the survival of mice infected with *A. fumigatus*. The ED_{50}/ED_{90} values were much higher when MK-0991 was administered by the oral route compared to intravenous or intraperitoneal routes (Table 15). Microbial burden was not measured in this study.

Table 14

Statistical Summary: Percent and Median Survival of DBA/2N Mice Challenged I.V. with *A. fumigatus* MF 5668 and Treated with L-743,872

n=10 mice/dose Unless Indicated Otherwise in [ ]

ED50 & LP., b.i.d unless noted	28 Day Percent Survival/(28 Day Median Survival) at Each Dose (mg/kg/dose)						
	Sham	0.00125	0.005	0.02	0.08	0.31	1.25
92-9	0 (7)	NT	NT	20.0 (15)	90.0* (>28)	80.0* (>28)	100.0* (>28)
92-10	0 (8)	NT	NT	40.0* (18)	100.0* (>28)	90.0* (>28)	100.0* (>28)
93-7	0 [9] (9)	0 (4)	10.0 (4)	10.0 (5)	40.0* (21)	70.0* (28)	NT*
93-12	0 (5)	0 (4)	0 (6)	30.0 (9)	90.0* (>28)	100.0* (>28)	90.0* (>28)
94-3	10.0 (5)	NT	10.0 (6)	50.0 (>28)	80.0* (>28)	90.0* (>28)	90.0* (>28)
94-4	0 (8)	NT	40.0 (16)	80.0* (>28)	90.0* (>28)	90.0* (>28)	80.0* (>28)
93-12 LP., q.4.	0 (5)	0 [9] (5)	0 (6)	10.0 (7)	90.0* (>28)	90.0* (>28)	70.0* (>28)
93-12 Delayed	0 (5)	0 (5)	10.0 (6)	0 (5)	40.0 (13)	100.0* (>28)	100.0* (>28)
93-1 I.V.	10.0 (11)	0.0 (9)	0 (10)	50.0* (23)	100.0* (>28)	90.0* (>28)	100.0* (>28)
AMB 93-12	0 (5)	NT	10.0 (6)	10.0 (5)	90.0* (>28)	90.0* (>28)	90.0* (>28)

NT = not tested.

* Denotes that survival at this dose level was statistically significant from the sham control mean at $\alpha \leq 0.05$ according to the Kaplan Meier Technique and Log Rank test.

ED50 & LP., b.i.d unless noted	28 Day Percent Survival/ (28 Day Median Survival) at Each Dose (mg/kg/dose)			
	Sham	3.13	12.5	50.0
93-5 P.O.	20.0 (21)	70.0 (28)	55.6 [9] (28)	55.6 [9] (28)
93-12 P.O.	0 (5.0)	10.0 (6)	60.0* (>28)	60.0* (>28)

* Denotes that survival was statistically significant from the sham control mean at $\alpha \leq 0.05$ according to the Kaplan Meier Technique and Log Rank test.

Table 15

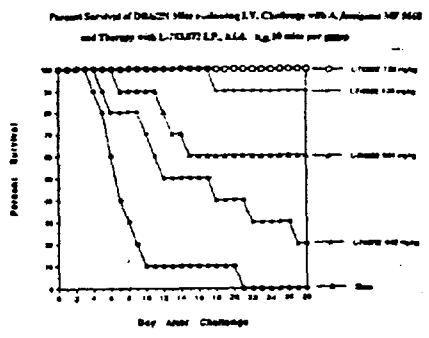
Statistical Summary: 28 Day Effective Dose 50% (ED_{50}), 90% (ED_{90}) Values, and 95% Confidence Interval of L-743,872 Against a Disseminated *A. fumigatus* MF 5668 Infection in DBA/2N Mice

ED50 & LP., b.i.d unless noted	ED50[a] (95% C.I.) (mg/kg/dose)	ED90[a] (95% C.I.) (mg/kg/dose)
92-9	0.05 (0.02, 0.011)	NE[b]
92-10	0.03 (0.01, 0.05)	NE[b]
93-7	0.13 (0.05, 0.23)	>0.31 NE[b]
93-12	0.034 (0.018, 0.063)	0.124 (0.066, 0.524)
94-3	0.042 (0.021, 0.090)	0.33 NE[b]
94-4	0.013 NE[b]	0.275 NE[b]
93-12 LP., q.4.	0.083 (0.031, 0.136)	0.444 NE[b]
93-12 Delayed	0.082 (0.046, 0.146)	0.245 (0.139, 0.968)
93-1 I.V.	0.02 (0.012, 0.033)	NE[b]
93-5 P.O.	7.98 NE[b]	>50.0 NE[b]
93-12 P.O.	20.33 NE[b]	>50.0 NE[b]
AMB ED50 93-12	0.046 (0.025, 0.090)	0.214 (0.106, 0.892)

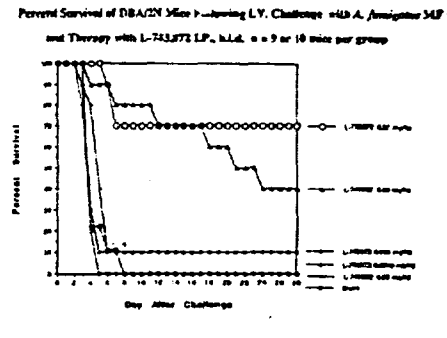
[a] ED_{50} and ED_{90} values and 95% CI determined using Robust Probit Estimates.
[b] NE = value could not be estimated.

Figure 3

(a)



(b)



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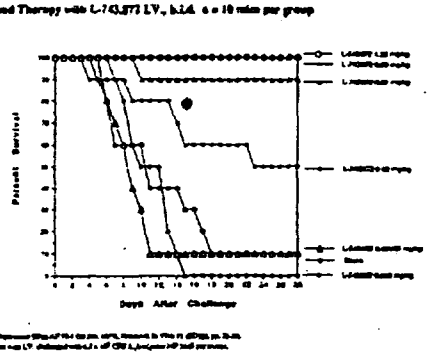
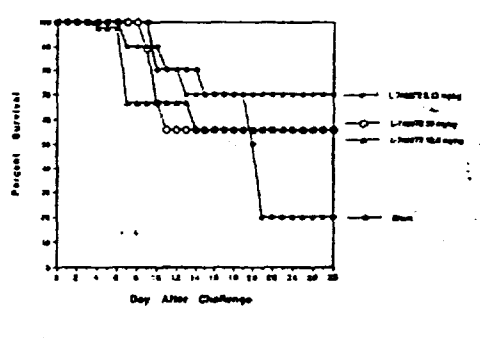
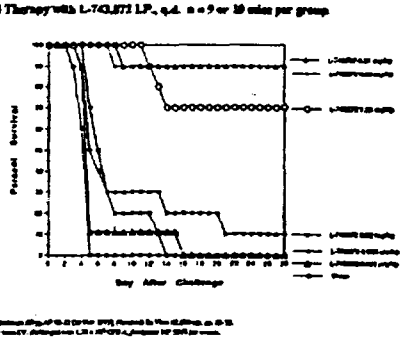


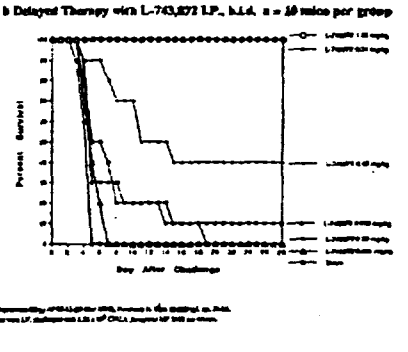
(d)



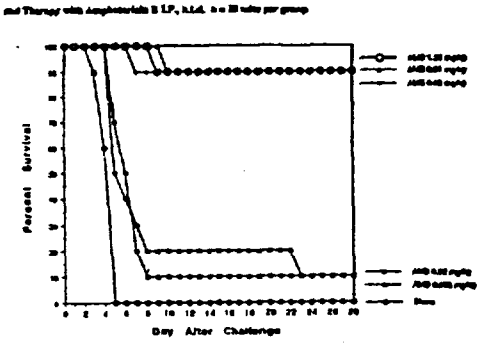
(e)



(f)



(g)



In a preliminary study using DBA/2J (C'5 deficient) mice, the effect of MK-0991 on mycological burden was measured in the kidneys (Merck report, e-mail received on December 18, 2000). Mice were infected with *A. fumigatus* (strain and the concentration of the conidial suspension used for infection was not specified). Administration of MK-0991 (1 mg/kg) or ampB (0.5 mg/kg) was initiated at the time of infection by the intraperitoneal route, for 4 days. Mice were followed for 35 days post-infection for survival and mycological burden using TaqMan® quantitative PCR and standard culture methods.

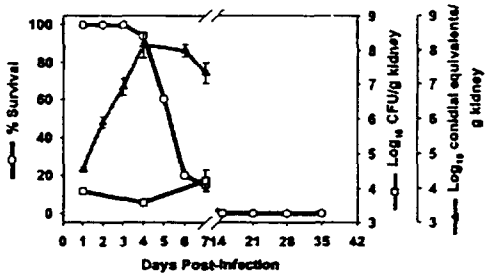
TaqMan® assay is based on the detection of 18S ribosomal RNA (rRNA) of *A. fumigatus*. It was stated that there are about 100 copies of the 18S rRNA gene in the *Aspergillus* genome and that the lower limit of detection by TaqMan® assay was <10 conidial genome equivalents. Titration of mass equivalents of *A. fumigatus* that were spiked into a naïve mouse kidney showed 1 ug of mycelial weight to be equivalent to 10^3 conidial genome equivalents. These data were not submitted for an independent review. The limit of detection for the TaqMan® and cfu methods per gm of kidney was calculated to be to be ~2000 conidial equivalents and ~300 cfu, respectively.

The results in Figure 4a show that all the infected untreated mice died by day 7 of infection. An increase in cfu was marginal from day 1 to 7. However, a significant increase in rRNA was observed from day 1 to 4 of infection. In mice treated with MK-0991 or ampB, the cfu and rRNA contents were reduced (Figures 4b and 4c). It is of note that the reduction in cfu is marginal (≤ 1 log) whereas rRNA was significantly reduced. Whether similar effects will be observed in immunocompromised and/or severely infected animals is not known.

Figure 4

(a)

Disease progression in *A. fumigatus* infected DBA/2J untreated mice as monitored by percent survival, colony forming units (CFU) and TaqMan® results.

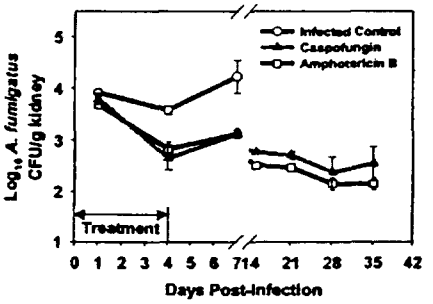


(MRL Nonclinical Report: Use of a TaqMan® assay to measure *Aspergillus fumigatus* burden and caspofungin efficacy in a murine model of disseminated aspergillosis 14-December-2000).

Each point represents the mean and standard error from 3 mice, with the exception of TaqMan® data on days 4 and 6, where 5 mice were used.

(b)

Efficacy of caspofungin and amphotericin B compared to non-medicated control DBA mice against disseminated aspergillosis as determined by colony forming units (CFU) kidney

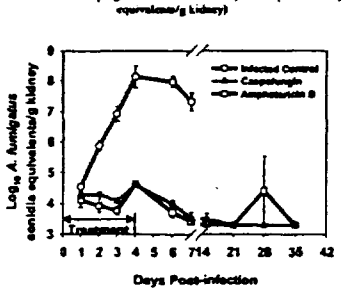


(MRL Nonclinical Report: Use of a TaqMan® assay to measure *Aspergillus fumigatus* burden and caspofungin efficacy in a murine model of disseminated aspergillosis 14-December-2000).

Each point represents the mean and standard error from 3 mice, with the exception of day 35 with AmB where only 2 mice were available.

(c)

Efficacy of caspofungin and amphotericin B compared to non-medicated control DBA/2J mice against disseminated aspergillosis as determined by the TaqMan® assay (conidial equivalents/g kidney)



Data Source: [MRL Nonclinical Report: Use of a TaqMan® assay to measure *Aspergillus fumigatus* burden and caspofungin efficacy in a murine model of disseminated aspergillosis 14-December-2000].

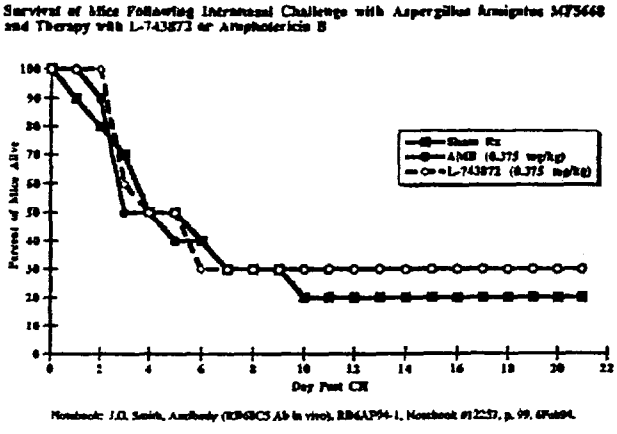
Each point represents the mean and standard error from 3 mice with the exception of days 4 and 6 in the non-medicated group, where 5 mice were used, and at day 35 in the AmB-treated animals, where only 2 mice were available.

Activity against *A. fumigatus* in agranulocytopenic mice and rabbits

(a) Pulmonary Aspergillosis:

Agranulocytopenic CD-1 mice were infected with *A. fumigatus* (MF 5668 - 2.5 x 10^7 spores) by the intranasal route (Merck report, 1995, reference # 46). Neutropenia was induced 24 hours prior to infection, by injection of a monoclonal antibody to granulocyte receptor every other day. Such treatment was shown to sustain neutropenia. The neutrophil count in the peripheral blood of neutropenic mice was shown to be < 0.2% (as determined by FACS analysis) compared to controls. Treatment, initiated intraperitoneally immediately after infection, was b.i.d., for 5 days and animals were followed for survival for 21 days post-infection. The results shown in Figure 5 indicate that both MK-0991 and ampB were not effective in protecting the infected mice. The difference between the results of this study compared to those described above could be attributed to the higher concentration of inoculum used for infection, the route of infection, the dose administered, and/or the immune status of the host.

Figure 5



From a study submitted to the IND annual report (N-303, dated October 2, 2000) it appeared that the activity of MK-0991 was tested in neutropenic rabbits and the sponsor was requested to submit the results of the study for review. These studies were conducted in Dr Walsh's laboratory. Some of the results were submitted on December 12, 2000 (e-mail) and January 3, 2001 (fax) and a teleconference was held with the sponsor and Dr. Walsh on January 3, 2001. The information received so far is as follows:

The activity of MK-0991 against *A. fumigatus* (NIH isolate # 4215, with *in vitro* MK-0091 and ampB MICs of 0.06 and 1 ug/ml, respectively) was measured in neutropenic rabbits. Persistent neutropenia was induced by the administration of 9 doses of [redacted] (days –1, 1 to 4, 8, 9, 13, and 14). In addition, macrophage and other immune cell functions were inhibited by administration of 2 doses of methyl prednisolone to enable establishment of infection. Rabbits were infected with 10^8 conidia, intratracheally on day 1 (24 hours after induction of neutropenia), and intravenous treatment with MK-0991 or ampB was initiated 24 hours post-challenge, for 12 days. The animals were followed for survival and residual mycological burden in the lungs. Only 1 of 12 of the untreated rabbits survived to day 12. The authors have stated that 8 of the 36 (22%) rabbits treated with MK-0991 survived to day 12. It is unclear which dosage group included the 8 surviving rabbits since 12 animals were used per dosage group and 3 doses (1, 3, or 6

mg/kg/day) tested. A slight improvement in mean survival days was observed in rabbits treated with MK-0991 compared to untreated control (Table 16). It is of note that all of the rabbits treated with ampB (1 mg/kg/day) died by day 12 with mean survival of 8.8 days (Table 16). The sponsor was requested to submit the survival curves in rabbits treated with ampB or various doses of MK-0991.

The reports in the literature by the same group of investigators (Francis *et al.*, 1994, J Inf Dis 169: 356) have shown about 25% to 30% survival in ampB treated rabbits and 100% survival in AmBisome treated rabbits. It is of note that the rabbits in the MK-0991 study were administered extra doses of [redacted] compared to those used in the AmBisome study. During a teleconference with the sponsor, held on Jan 3, 2001, Dr. Walsh stated that variability in survival of ampB treated rabbits was observed among various studies and is probably due to nephrotoxicity (which was influenced by water intake) and thrombocytopenia.

The effects of MK-0991 on mycological burden in the lung, pulmonary infarct score (measured by histopathological examination of the number of lobes per lung showing tissue injury), and mean lung weight were measured on the day rabbits succumbed to infection or on day 12. The results in Table 17 show that MK-0991 at a low dose (1 mg/kg/day) was effective in reducing the lung weight and infarct score but not in reducing the mycological burden, although the appearance of the colonies was different (microcolonies). Higher doses (3 and 6 mg/kg/day) were not effective. AmpB was the most effective in significantly reducing mycological burden, infarct score, and the lung weight. Dr Walsh also stated that CT scans were done on days 1, 2, 3, 5, 7, 9, 10, and 12 of treatment with MK-0991, however, the data has not been submitted to FDA for review.

Table 16: Mean survival in neutropenic rabbits infected with *A fumigatus*

Rabbit Group	Mean Survival (days)	p Value
Untreated Control(n=12)	6.92 ± 0.89	
MK 1 mg/kg (n=12)	10.42 ± 0.54	< 0.01
3 mg/kg (n=12)	10.17 ± 0.61	< 0.01
6 mg/kg (n=12)	9.50 ± 0.70	< 0.01
AmB (n=6)	8.83 ± 0.48	< 0.05

Table 17: Efficacy in Invasive Pulmonary Aspergillosis Rabbit Model

Compound (N) Dose	Lung Log [CFU/gm]	Pulmonary Infarct Score	Mean Lung Weight
Untreated Controls (12)	1.35±0.25	5.17±0.53	49.48±4.27
Caspofungin† (12) 1 mg/kg/day	1.90±0.23[d]	2.50±0.44[b]	35.21±3.30[c]
Caspofungin† (12) 3 mg/kg/day	1.42±0.17[d]	4.00±0.69[d]	37.67±3.01[d]
Caspofungin† (12) 6 mg/kg/day	1.99±0.25[d]	3.58±0.77[d]	32.45±4.26[d]
Amphotericin B (6) 1 mg/kg/day	0.13±0.08[b]	1.17±0.48[a]	17.40±2.61[a]

*control versus treated (a) p≤ 0.001, (b) p≤0.01, (c) p≤ .05, and (d) not significant
† toxicity related to increasing dosages of caspofungin was not observed.

In another experiment by Dr. Walsh's group, in neutropenic rabbits, the activity of MK-0991 as a prophylactic agent was determined. The experimental design was similar to that described above except that the treatment with MK-0991 (1 mg/kg/day) or ampB was initiated 4 days prior to challenge and continued for 12 days post-infection. Also, a lower concentration (5×10^7 conidia/rabbit) of the inoculum was used for infection. However, the data were not submitted for review.

(b) Disseminated Aspergillosis:

The activity of MK-0991 was measured in neutropenic C3H/HeN mice with disseminated *A. fumigatus* (strain MF5668, ATCC 13073 with MK-0991 and ampB MICs of 0.123 and 0.5 ug/ml, respectively) infection (Merck report, 1999, study 6, reference # 16). Three doses of monoclonal antibody (RB6-8C5), administered intraperitoneally at 2-day intervals were used to induce neutropenia. The results in Table 18 show that neutrophil counts were increased on and after day 10 (a day after the last injection of monoclonal antibody). Mice were challenged with 1 to 8×10^4 cfu of spore suspension intravenously 24 hours after initiation of neutropenia. The treatment with MK-0991 or ampB was initiated intraperitoneally, immediately after challenge, for 5 days. All the infected, untreated neutropenic mice died within 17 days of challenge whereas the uninfected neutropenic mice survived the duration of experiment. The results in Figure 6 show that MK-0991 at a dose of 1.25 mg/kg was effective in improving survival in 50% of the animals whereas ampB at 1.25 mg/kg dose improved survival by about 60%. The ED_{50} values for MK-0991 and ampB on days 7, 14 and 21 were comparable (Table 19). Mycological burdens were not measured.

Table 18

Fluorescent Activated Cell Sorting (FACS) and Differential Cell Count of RB6-8C5 (anti-GR-1) Antibody and PBS Treated C3H/HeN Mice[a]

	Number of neutrophils detected by differential (x 1000/ul) ± SD		Percent neutrophils detected by differential) ± SD		Percent neutrophils detected by FACS) ± SD	
Time Point (day)	RB6-8C5 antibody treated	PBS treated	RB6-8C5 antibody treated	PBS treated	RB6-8C5 antibody treated	PBS treated
0	0.02 ± 0.01[b]	1.18 ± 0.45[b]	1.15 ±0.37	17.63 ± 2.32	0.06 ± 0.05	11.55 ± 3.25
3	0.13 ± 0.03	1.61 ± 0.53	6.36 ± 2.10	16.48 ± 3.23	0.16 ± 0.14	10.95 ± 2.64
6	0.54 ± 0.20[b]	2.96 ± 3.54	11.68 ± 4.23	20.94 ± 12.34	0.36 ± 0.27	10.22 ± 4.67
10	9.38 ± 11.66	2.09 ± 0.63	51.48 ± 12.15	20.02 ± 2.91	62.17 ± 19.56	11.29 ± 3.02
20	3.35 ± 2.22	2.20 ± 0.32	35.98 ± 14.08	20.12 ±1.62	31.81 ± 11.84	17.90 ± 3.01

a C3H/HeN mice were made granulocytopenic by the I.P. administration of 500 µg RB6-8C5 mAb (specific for Gr-1 epitope on mouse granulocytes) on day -1 and then 250 µg RB6-8C5 on days 2 and 4. Control mice received sterile PBS I.P. on the same days. Five mice per sample point unless indicated by superscript [b] which have 4 mice per sample point.

[CL in Vitro 99 (06-Apr-1999) NB 052469 pp. 37-38]

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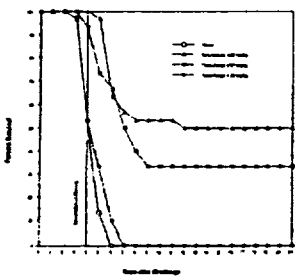
Figure 6

(a)



(b)

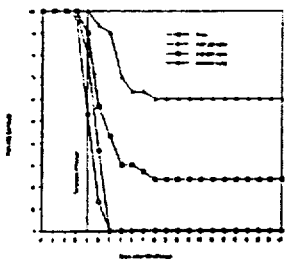


Table 19

Efficacy of Caspofungin and AmB Against a Disseminated *A. fumigatus* MF5668 Infection[a] in Monoclonal Antibody Induced-Granulocytopenic[b] C3H/HeN Mice[c]

	ED_{50} Value (mg/kg/dose)[d] [95% Confidence Interval]		
	Days Post Challenge		
Compound[e]	7	14	21
Caspofungin	0.63 [0.43, 0.97]	1.05 [0.73, 1.51]	1.05 [0.73, 1.51]
AmB	0.65 [0.46, 0.90]	0.85 [0.61, 1.19]	0.85 [0.61, 1.19]

[a] Granulocytopenic (mAb-induced) C3H/HeN mice challenged I.V. with 1.0 to 4.0 x 10^6 conidia/mouse *A. fumigatus* MF5668.

[b] C3H/HeN mice were made neutropenic by the I.P. administration of 500 µg RB6-8C5 mAb one day prior to infection and then 250 µg RB6-8C5 on days 2 and 4 post challenge.

[c] Mice treated I.P., q.d. x 5 days. Mice received first treatment immediately following infection. Thirty mice per treatment group.

[d] ED_{50} values calculated using ED50.BAS Api Program based on the Kaufman and Cutts method.

Pooled data from: JG Smith Notebook JS In Vivo II: ED_{50}AF 96-8 (16-Jan-1996) NB# 40470 pp. 93-99, ED_{50}AF 96-11 (21-Jan-1996) NB 40772 pp. 14-20, ED_{50}AF 97-2 (21-Jan-1997) NB 40772 pp. 47-52.

Activity in immunosuppressed rats and mice against *A. fumigatus*

(a) Pulmonary Aspergillosis:

The activity of MK-0991 against *A. fumigatus* (strain H11-20) was measured in immunosuppressed (cortisone treated) Sprague-Dawley rats (Bernard *et al.*, 1996, ICAAC, Abstract F39, Merck reference # 49). The dosing regimen and the route of administration for cortisone were not described. It was stated that "the untreated rats developed a progressive, rapidly-fatal, bronchopneumonia." Rats were infected with 10^6 conidia of *A. fumigatus*. In one experiment, a single dose of MK-0991 was administered 2 hours prior to infection and the survival measured on day 7. In another experiment, MK-0991 was administered at the time of infection and continued for 7 days. The results in Table 20 show that MK-0991 and ampB administered intraperitoneally as a single dose (≥ 2mg/kg) or in multiple doses (≥ 0.5 mg/kg/day for 7 days) improved survival.

Table 20

Drug	**Time treatment Initiated (hours)**	**N**	**Dose (mg/kg)**	**Survival (%)**
Experiment 1:				
Vehicle	-2	10	0	40
MK-0991	-2	10	0.5	60
MK-0991	-2	10	2	90
MK-0991	-2	10	8	100
AmpB	-2	10	4	100
Experiment 2:				
Vehicle	0	10	0 x 7 days	30
MK-0991	0	10	0.5 x 7 days	80
MK-0991	0	10	2 x 7 days	100
MK-0991	0	10	8 x 7 days	100
AmpB	0	10	4 x 7 days	100

(b) Disseminated Aspergillosis:

ICR mice were immunosuppressed by administering cyclophosphamide intraperitoneally every 3 days up to 10 days post-infection (Merck report, 1997, reference # 47). Immunosuppression was initiated 3 days prior to infection with *A. fumigatus*, strain MF5668 (1.6×10^4 conidia) by the intravenous route. The sponsor has stated that pancytopenia was confirmed but the data were not provided. Treatment with MK-0991 by the intraperitoneal route was initiated 24 hours post-infection for 14 days. AmpB and ABELCET, administered intraperitoneally, were used as comparators. Survival was measured for up to 28 days post-infection. The results in Figure 7 show that MK-0991 at a dose of ≥ 0.5 mg/kg was effective in improving survival. The ED_{50} values for MK-0991 were comparable to ampB and lower than ABELCET (Table 21). The effect on mycological burden was not measured in this study.

Figure 7

Efficacy of Delayed Treatment with MK-0991 Against Disseminated Aspergillosis in Cytoxan-Treated Mice[1,2]

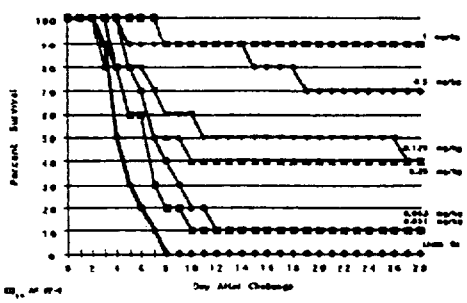


Table 21

Effective Dose 50% (ED_{50}) in mg/kg dose and 95% Confidence Intervals* for [illegible] Disseminated Aspergillosis in Cytoxan-Treated Mice[1,2]

	MK-0991	Amphotericin B	Abelcet®
Day 14	0.192 (0.126, 0.307)	0.257 (0.161, ∞)	1.225 (0.748, 2.121)
Day 21	0.226 (0.144, 0.387)	0.260 (0.155, ∞)	1.438 (0.873, 2.569)
Day 28	0.245 (0.157, 0.412)	0.264 (0.167, ∞)	1.438 (0.873, 2.569)

References: A. M. Fluttery, GA IN VIVO 17: ED50 AP97-1, Notebook # 049037, pp. 117-121, 27Mar97.
*Calculated by M. Hamer, Biometrics Research, MRL. Stored on Dusk Fluttery Project 97-25.

The effect on mycological burden was measured in another study using the pancytopenic murine model (Merck report, 1999: study 1, reference # 18). The experimental design was similar to that described above except that immunosuppression with cyclophosphamide was maintained until the end of the study (day 28). Mice were infected with the conidia suspension (1 to 2.4×10^4 cfu/mouse) intravenously and treatment with MK-0991 or ampB initiated intraperitoneally, 24 hours post-infection. The mice were followed for mycological burden. For measurement of mycological burden, ≤5 mice were sacrificed and kidneys processed for measurement of cfu and histological demonstration of infection (presence of hyphae typical of *Aspergillus* using [redacted] on days 4, 8, 15 and 28. The results in Tables 22 and 23 show that the treatment with MK-0991 reduced the cfu as observed on days 4 or 8 compared to untreated mice. It is of note that the mean log cfu in untreated mice on days 4 or 8 was about 3 to 3.5 log. Although the tissues from a majority of the treated mice were shown to be free of infection, the sensitivity of sterilization is limited by the lower limit of detection. Based on the lower limit of detection the reduction in cfu was very marginal (< 1 log). Histological findings showed abundant hyphae in the untreated mice (score 3+ to 4+) on day 4, whereas mice treated with MK-0991 or ampB showed ≤ 1+ score. The results of mycological burden on day 8 are less clear since one of the untreated mice was negative for cfu but had histological evidence of infection in 2 of the 4 sections screened. For days 15 and 28 there was no data available from the untreated mice (since all the mice were dead). All the MK-0991 and ampB treated mice showed histological scores of ≤ 1. There was no correlation between histological scores and the number of cfu. For example, on days 15 and 28 the histological score in all animals was ≤ 1+ whereas cfu/ml varied from 20 to 90 on day 15 and were completely absent on day 28. Overall, the activity of MK-0991 was comparable to ampB.

Table 22

A.

Colony Forming Units (CFU) of *Aspergillus* in the Kidneys at Time Points After Challenge and Delayed Therapy With Caspofungin or AmB in Cyclophosphamide-Induced, Chronically Pancytopenic ICR Mice[a]

Compound	Dose (mg/kg)	Mean log10 CFU/g kidneys (% Sterilization)[b] at time points after challenge			
		Day 4	Day 8	Day 15	Day 28
Caspofungin	1.00	2.74* (80)	2.77 (80)	3.02 (0)	2.54 (160)
	0.50	2.56* (100)	2.45 (100)	2.81 (20)	2.60 (80)
	0.25	2.88* (60)	2.63 (60)	2.84 (20)	2.85 (50)[d]
AmB	1.00	2.49* (80)	3.11 (20)	3.01 (0)	2.51 (100)[d]
	0.50	2.74* (60)	2.45 (40)	2.95 (50)[d]	All Dead
	0.25	2.75* (40)	3.05 (0)	3.12 (0)[d]	All Dead
Sham-Treated Water		3.51 (0)	3.04 (20)[d]	All Dead	All Dead
Sham-Treated DMSO		3.40 (0)	3.60 (0)[d]	All Dead	All Dead

Data from ED_{50} AF 98-6 (08-May-1998) Notebook *In Vivo* 18, pp. 89-97.

a. Mice were challenged I.V. with *A. fumigatus* MF5668 at 1.0 x 10^4 CFU/mouse. Kidneys aseptically collected at days 4, 8, 15 and 28 after challenge. Mice received first treatment 24 hours after challenge (delayed therapy) and were treated I.P., q.d. for 7 days. Mice were immune suppressed throughout the experimental period (28 days).

b. Mean log_{10} CFU/g at time points after challenge for paired kidneys. Five mice per group unless indicated by superscript d. Percent sterilization indicates the number of mice with no detectable *Aspergillus* where the limit of detection was 50 CFUs per pair of kidneys.

* Significant from sham (water) treated control ($p < 0.05$; Excel t-test).

B.

Colony Forming Units (CFU) of *Aspergillus* in the Kidneys at Time Points After Challenge and Delayed Therapy With Caspofungin or AmB in Cyclophosphamide-Induced, Chronically Pancytopenic ICR Mice[a]

Compound	Dose (mg/kg)	Mean log10 CFU/g kidneys (% Sterilization)[b] at time points after challenge			
		Day 4	Day 8	Day 15	Day 30
Caspofungin	1.00	1.69* (40)	1.75* (40)	1.35 (100)	1.85 (<0)
	0.50	2.12 (40)	1.86* (60)	1.69 (60)	2.30 (40)
	0.25	2.57 (0)	1.74* (40)	1.50 (60)	1.70 (80)
AmB	1.00	2.09 (0)	1.72* (60)	1.99 (40)	2.05 (40)
	0.50	2.03 (40)	2.20 (0)	2.36 (20)	2.69 (50)[d]
	0.25	2.46 (20)	2.59 (0)	1.28 (100)[d]	All Dead
Sham-Treated Water		2.87 (0)	3.25 (0)	All Dead	All Dead
Sham-Treated DMSO		3.15 (0)	2.29 (60)[d]	All Dead	All Dead

Data from ED_{50} AF 98-10 (07-Aug-1998) Notebook *In Vivo* 18, pp. 117-125.

a. Mice were challenged I.V. with *A. fumigatus* MF5668 at 2.4 x 10^4 CFU/mouse. Kidneys aseptically collected at days 4, 8, 15 and 30 after challenge. Mice received first treatment 24 hours after challenge (delayed therapy) and were treated I.P., q.d. for 7 days. Mice were immune suppressed for 28 days after challenge.

b. Mean log_{10} CFU/g at time points after challenge for paired kidneys. Five mice per group unless indicated by superscript d. Percent sterilization indicates the number of mice with no detectable *Aspergillus* where the limit of detection was 5 CFUs per pair of kidneys.

* Significant from sham (water) treated control ($p < 0.05$; Excel t-test).

The activity of MK-0991 was also measured in immunosuppressed ICR mice (pancytopenic model) infected with *A. fumigatus*, strain MF5668 (Merck report, 1999, study 8, reference # 16). Immunosuppression was induced by intraperitoneal administration of cyclophosphamide 3 days prior to challenge with 1.0 to 2.4 x 10^4 of a conidial spore suspension by the intravenous route. Immunosuppression was maintained with 9 additional doses administered at 3-day intervals. Treatment with MK-0991 or ampB was initiated 24 hours post challenge, and continued for 7 days (10 mice per group) and animals were followed for survival for up to 28 days post-challenge. The leukocyte count was shown to be low during the course of the experiment (Figure 8) and it was stated that the mortality rate was 2.5% during the 28-day period in noninfected immunosuppressed mice. The results in Table 24 show that both MK-0991 and ampB at a 1 mg/kg dose demonstrated comparable activity. Mycological burdens were not measured.

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Table 23

Colony Forming Units (CFU) and Histological Scores of *Aspergillus* in the Kidneys at Time Points After Challenge and Delayed Therapy With Caspofungin or AmB in Cyclophosphamide-Induced, Chronically Pancytopenic ICR Mice

Day 4

Treatment (I.P., q.d. x 7 days)	Mouse	Wt-g	Raw Count/ml	Log CFU/g Kidney	Total # Sections	Histological Score 0	1+	2+	3+	4+
dH_2O Sham	1	0.15	[redacted]	3.52	8					8
	2	0.19		3.72	8				8	
	3	0.16		3.27	6					6
Caspofungin 1 mg/kg	1	0.07		2.85	8	6	2			
	2	0.13		2.59	6	4	2			
	3	0.07		2.85	4	4				
	4	0.11		2.65	4	4				
	5	0.08		2.79	4	4				
Amphotericin B 1 mg/kg	1	0.13		2.58	4	4				
	2	0.18		2.44	6	6				
	3	0.2		2.39	4	4				
	4	0.15		2.51	4	4				
	5	0.15		2.51	4	4				

Day 8

Treatment (I.P., q.d. x 7 days)	Mouse	Wt-g	Raw Count/ml	Log CFU/g Kidney	Total # Sections	Histological Score 0	1+	2+	3+	4+
dH_2O Sham	1	0.26	[redacted]	3.66	8	2	6			
	2	0.19		3.41	4	2	2			
DMSO Sham	1	0.2		3.60	6	2			4	
caspofungin	1	0.12		2.61	8	8				
1 mg/kg	2	0.12		2.63	8	8				
	3	0.16		2.49	8	6	2			
	4	0.14		3.63	8	2	6			
	5	0.15		2.51	8	2	6			
Amphotericin B 1 mg/kg	1	0.18		3.74	12			12		
	2	0.19		2.41	8	8				
	3	0.2		3.40	8	8				
	4	0.09		3.22	8	6	2			
	5	0.17		2.77	8	4	4			

Day 13

Treatment (I.P., q.d. x 7 days)	Mouse	Wt-g	Raw Count/ml	Log CFU/g Kidney	Total # Sections	Histological Score 0	1+	2+	3+	4+
dH_2O Sham	1	dead	[redacted]							
	2	dead								
	3	dead								
Caspofungin 1 mg/kg	1	0.21		3.33	8	8				
	2	0.18		2.74	8	6	2			
	3	0.18		2.74	8	8				
	4	0.17		3.25	8	8				
	5	0.18		3.05	12	10	2			
Amphotericin B 1 mg/kg	1	0.18		3.22	8	8				
	2	0.19		2.90	8	8				
	3	0.13		2.89	8	8				
	4	0.13		3.06	12	12				
	5	0.16		2.97	8	8				

Day 28

Treatment (I.P., q.d. x 7 days)	Mouse	Wt-g	Raw Count/ml	Log CFU/g Kidney	Total # Sections	Histological Score 0	1+	2+	3+	4+
dH_2O Sham	1	dead	[redacted]							
	2	dead								
	3	dead								
Caspofungin 1 mg/kg	1	0.14		3.54	8	8				
	2	0.15		2.51	8	6	2			
	3	0.12		2.61	8	8				
	4	0.12		2.61	8	8				
	5	0.18		2.43	8	8				
Amphotericin B 1 mg/kg	1	0.15		2.51	8	8				
	2	0.15		2.51	8	8				
	3	0.15		2.51	8	8				
	4	dead								
	5	dead								

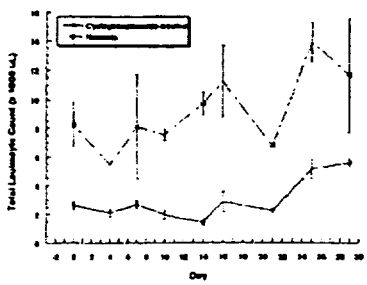
Data from Experiment ED_{50} AF 98-6 (08-May-1998), Notebook In Vivo 18, pp. 89-97.

a. Histological Scores - Sections were [redacted] and from 4 to 12 sections were examined microscopically for the presence of hyphae typical of *Aspergillus*. A subjective score of from 0 (negative- no hyphae observed) to 4+ (numerous hyphae throughout the scanned section) were assigned to each sample.

b. 0 = no detectable *Aspergillus* where the limit of detection was 50 CFUs per pair of kidneys.

Figure 8

Differential White Cell Counts of Normal and Cyclophosphamide-Treated ICR Mice[a]



Normal Data from ED_{50} AF 99-3 (30-Apr-1999) Notebook In Vivo 18, pp. 126-133.

Cyclosporin-Treated Data Pooled from ED_{50} AF 98-6 (08-May-1998) Notebook In Vivo 18 and ED_{50} AF 99-3 (30-Apr-1999) Notebook In Vivo 18, pp. 89-97 and 126-133.

Table 24

Percent Survival (Day 28) for Caspofungin and AmB Treated Mice Against a Disseminated *A. fumigatus* MF5668 (ATCC 13073) Infection[a] in Cyclophosphamide-Induced Chronically Pancytopenic ICR Mice

Treatment (24 hr Delayed) (I.P., q.d. x 7 days)	Percent Survival ED_{50} 98-6	ED_{50} 98-10	ED_{50} 99-3
Infected / Sham-treated	10.0	10.0	22.0
Caspofungin			
1.0 mg/kg	80.0	50.0	92.0
0.5 mg/kg	80.0	100.0	90.0
0.25 mg/kg	40.0	30.0	86.0
AmB			
1.0 mg/kg	80.0	50.0	90.0
0.5 mg/kg	40.0	70.0	80.0
0.25 mg/kg	30.0	30.0	96.0
Cyclophosphamide Controls			
Sham-Infected / Sham Treated	100.0	100.0	95.0
Non-Infected / Non-Treated	90.0	100.0	100.0

Data from ED_{50} AF 98-6 (08-May-1998), ED_{50} AF 98-10 (07-Aug-1998) and ED_{50} AF 99-3 (30-Apr-1999) Notebook In Vivo 18, pp. 89-97, 115-125 and 126-133, respectively

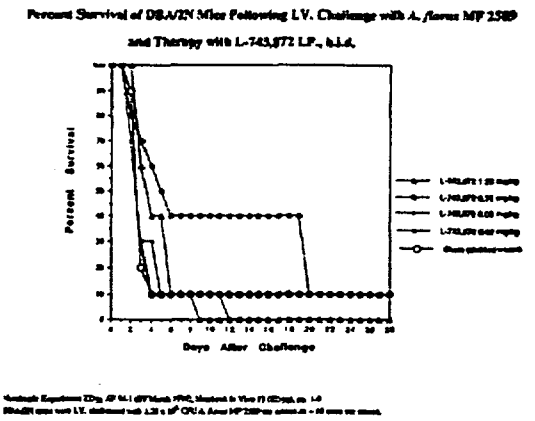
a. Mice were challenged I.V. with *A. fumigatus* MF5668 at 1.0×10^6 CFU/mouse (ED_{50} AF 98-6), at 2.4×10^6 CFU/mouse (ED_{50} AF 98-10) and 1.8×10^6 CFU/mouse (ED_{50} AF 99-3). Mice received first treatment 24 hr after challenge (delayed therapy) and were treated I.P., q.d. for 7 days. Mice were immune suppressed throughout the experimental period (28 days). Ten mice per group in studies ED_{50} AF 98-6 and ED_{50} AF 98-10. Fifty mice per group in ED_{50} AF 99-3 except for the cyclophosphamide control groups which had 20 mice per group.

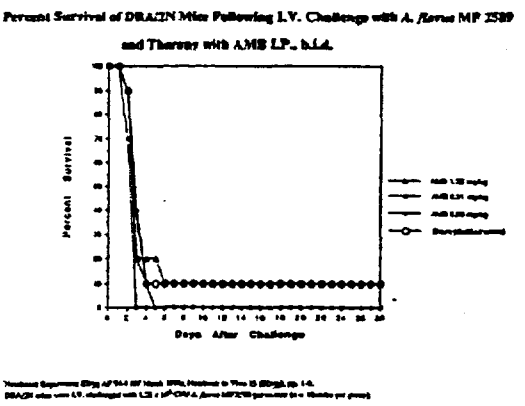
Activity in C'5 deficient mice against *A. flavus*

Complement (C'5) deficient DBA/2N mice were infected with *A. flavus* (strain MF2589 - 1.28 x 10^6 conidia) by the intravenous route (Merck report, reference # 48). Treatment with MK-0991 was initiated immediately after infection by the intraperitoneal route, b.i.d. for 5 days. The results in Figure 9 indicate that MK-0991 at a dose of 0.31 mg/kg was marginally more effective compared to higher and lower doses of the drug. AmpB was, however, not protective (Figure 9). It is unclear whether the strain of *A. flavus* used for infection was more susceptible to MK-0991 as compared to ampB. The activity of MK-0991 in immunosuppressed animals was not measured.

Figure 9

(a) (b)





No studies were conducted to measure the activity of MK-0991 *in vivo* against other species of *Aspergillus* (*A. niger*, *A.nidulans*, and *A. terreus*).

***In vitro* activity of MK-0991 against *Candida* species**

As stated above (page 2), the methods for *in vitro* testing of antifungal agents are not standardized. In an effort to standardize the *in vitro* methods for testing of MK-0991 and other antifungal agents, 3 reference isolates of *Candida* species recommended by NCCLS (*C. parapsilosis*, ATCC 22019; *C. krusei*, ATCC 6258; and *C. albicans*, ATCC 90028) were assayed in 8 different laboratories (Merck report, 1999; reference # 74). It is of note that 5 of these 8 laboratories have participated in the writing of NCCLS documents M27-A and M38-P. The assays were conducted using 3 different sources of [redacted] medium. The remaining constituents of the [redacted] broth were identical. The assays were conducted according to the NCCLS guidelines (M27-A) and incubated at 35°C for up to 48 hours. The MIC values were determined at 24 and 48 hours. The MIC for ampB was defined as the lowest concentration with no visible growth and that for 5-fluorocytosine (5-FC) or azoles as ≥ 80% inhibition, respectively. The criteria used for defining MK-0991 MIC was not described. The assays were repeated on 10 different test days. The authors described the criteria used for the definition of MIC of ampB, 5-FC, and azoles but the results for these drugs were not included in the report. These results were, however, available from a literature report (Barry *et al.*, J Clin Microbiol, 2000, 38: 3457). The control limits recommended by NCCLS are shown in Tables 25 and 26. It is of note that there is a discrepancy between the 2 reports in the range of MK-0991 MIC values at 48 hours against *C. parapsilosis*. The Merck report describes it to be 0.5 to 2.0 ug/ml (Table 26) whereas the publication by Barry *et al.*, 2000 states it to be 0.5 to 4 ug/ml (Table 25). It was stated that the MIC values for most drugs against *C. albicans* strain (ATCC 90028) were too variable to be of value for quality control purposes and other strains are currently being tested.

Table 25
(Barry *et al.*, J Clin Microbiol, 2000, 38: 3457)

Proposed MIC ranges of various antifungal agents for two quality control strains of *Candida* spp. when tested by the NCCLS microdilution procedure

Antifungal agent	MIC (μg/ml) ranges for microdilution tests with:			
	C. parapsilosis ATCC 22019 at:		*C. krusei* ATCC 6258 at:	
	24 h	48 h	24 h	48 h
Amphotericin B	0.25–2.0	0.5–4.0	0.5–2.0	1.0–4.0
Flucytosine	0.06–0.25	0.12–0.5	4.0–16	8.0–32
Fluconazole	0.5–4.0	1.0–4.0	8.0–64	16–128
Voriconazole	0.016–0.12	0.03–0.25	0.06–0.5	0.12–1.0
Ketoconazole	0.03–0.25	0.06–0.5	0.12–1.0	0.25–1.0
Itraconazole	0.12–0.5	0.12–0.5	0.12–1.0	0.25–1.0
Caspofungin (MK0991)	0.25–1.0	0.5–4.0	0.12–1.0	0.25–1.0
Ravuconazole (BMS 207147)	0.016–0.12	0.03–0.25	0.06–0.5	0.25–1.0
Posaconazole (SCH 56592)	0.06–0.25	0.06–0.25	0.06–0.5	0.12–1.0
LY303366	1.0–8.0	1.0–8.0	0.03–0.25	0.06–0.5

Table 26
(Merck report, 1999; reference # 74)

Antifungal Agent and Control Strain	24 Hour MIC (μg/ml) Range	24 Hour MIC (μg/ml) % Included	48 Hour MIC (μg/ml) Range	48 Hour MIC (μg/ml) % Included
Caspofungin (MK-0991)				
C. parapsilosis ATCC 22019	[redacted]	96.7%	[redacted]	92.1%
C. krusei ATCC 6258		98.8%		97.5%
C. albicans ATCC 90028	NOT RECOMMENDED			

Studies by Bartizal *et al.*, 1997 (Antimicrob Agents Chemother, 41: 2326, reference # 1; Merck report, 1995, reference # 6) measured the *in vitro* susceptibility of about 200 isolates of various fungal species (from the Merck culture collection) by the NCCLS recommended broth microdilution method. The MIC against the laboratory isolates showed MK-0991 $MIC_{90} \leq 1$ ug/ml against various *Candida* species except *C. guilliermondii* with MIC_{90} of 2 ug/ml (Table 27). MK-0991 was not effective against *C. neoformans* (MIC_{90} for 19 isolates tested was 32 ug/ml). MFCs were determined by subculturing 1.5 ul volume from the initial cultures. It is also of note that the MICs were comparable to the MFCs for all the *Candida* species.

A study by Espinel-Ingroff, 1998 (J Clin Microbiol 36: 2950; reference # 11) measured the activity of MK-0991 against various pathogenic yeasts by the NCCLS (M27-A) microdilution method. These were clinical isolates obtained from patients with severe fungal infections. The cultures were incubated from 24 to 72 hours to ensure that the growth was heavy/sufficient to determine the MIC values. It was stated that the MICs in general were higher at the 2nd time point tested. MK-0991 MIC_{90} values against *C. guilliermondii* and *C. krusei* were 2.0 ug/ml whereas those against other *Candida* species were ≤ 1.0 ug/ml (Table 28). MFC were comparable to MIC values against all species.

Table 27: MIC ranges, geometric MICs, MIC_{50}s, MIC_{90}s of MK-0991 and AmB for clinically relevant fungi

Organism (*n*)[a]	Antifungal agent	MIC (μg/ml)[b]				MFC (μg/ml)[c]			
		Range	50%	90%	Geometric mean	Range	50%	90%	Geometric mean
Candida albicans (40)	MK-0991		0.50	0.50	0.37		0.25	0.50	0.29
	AmB		0.25	0.25	0.25		0.125	0.25	0.18
Candida tropicalis (20)	MK-0991		0.50	1.0	0.54		0.50	1.0	0.57
	AmB		0.25	0.50	0.29		0.25	0.50	0.34
Candida parapsilosis (20)	MK-0991		0.50	0.50	0.52		0.50	0.50	0.44
	AmB		1.0	1.0	0.76		1.0	1.0	0.78
Candida lusitaniae (20)	MK-0991		0.25	0.50	0.30		0.25	0.50	0.28
	AmB		1.0	2.0	1.11		1.0	2.0	1.23
Candida guilliermondii (20)	MK-0991		1.0	2.0	1.19		2.0	2.0	1.41
	AmB		0.125	0.25	0.16		0.25	0.50	0.22
Candida krusei (20)	MK-0991		1.0	2.0	1.04		1.0	1.0	0.97
	AmB		0.25	0.50	0.30		0.25	0.50	0.28
Candida pseudotropicalis (20)	MK-0991		0.25	0.50	0.27		0.25	0.50	0.26
	AmB		0.25	0.50	0.28		0.25	0.50	0.29
Candida glabrata (20)	MK-0991		0.50	1.0	0.66		1.0	1.0	0.84
	AmB		0.25	0.50	0.25		0.25	0.50	0.26
Cryptococcus neoformans (19)	MK-0991		2.0	32.0	23.9		16.0	32.0	17.9
	AmB		0.25	0.50	0.27		0.25	0.50	0.26

[a] *n*, number of isolates tested.
[b] Broth microdilution method, RPMI 1640 medium, inocula of 0.5 × 10^3 to 2.5 × 10^3 CFU/ml, and incubation for 24 h at 35 to 37°C.
[c] Microtiter plates were shaken and 1.5-ml samples were transferred to 10-ml Sabouraud dextrose agar plates, and the plates were incubated for 24 to 48 h at 35 to 37°C.

Table 28: Susceptibilities of 104 selected pathogenic yeasts to SCH56592, MK-0991, and LY303366 as determined by a spectrophotometric procedure

Fungus (no. tested)	Antifungal agent	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MFC range (μg/ml)	MFC_{90} (μg/ml)
C. albicans (10)[a]	SCH56592		0.5	1.0		ND[c]
	MK-0991		0.5	1.0		2
	LY303366		0.06	0.25		1.0
C. albicans (10)[d]	SCH56592		<0.03	<0.03		ND
	MK-0991		0.5	1.0		1.0
	LY303366		0.06	0.06		0.5
C. glabrata (12)	SCH56592		1.0	4		≥16
	MK-0991		0.5	1.0		2
	LY303366		0.12	0.25		0.5
C. guilliermondii (8)	SCH56592		0.25	ND[e]		ND
	MK-0991		2	ND		ND
	LY303366		2	ND		ND
C. krusei (13)	SCH56592		1.0	1.0		1.0
	MK-0991		1.0	2		2
	LY303366		0.5	1.0		1.0
C. lusitaniae (12)	SCH56592		0.06	0.06		0.25
	MK-0991		1.0	2		1.0
	LY303366		1.0	2		2
C. parapsilosis (12)	SCH59592		0.25	0.5		8
	MK-0991		1.0	2		2
	LY303366		2	2		4
C. tropicalis (12)	SCH56592		0.25	0.25		ND
	MK-0991		1.0	1.0		1.0
	LY303366		0.25	0.5		1.0
C. neoformans (10)	SCH56592		0.25	0.25		0.5
	MK-0991		>16	>16		ND
	LY303366		>16	>6		ND
T. beigelii (5)	SCH56592		1.0	ND		ND
	MK-0991		>16	ND		ND
	LY303366		>16	ND		ND
Total (104)						

[a] Fluconazole MIC, >16 μg/ml; itraconazole MIC, 0.06 to >8 μg/ml.
[b] Values in parentheses are MIC-0 (SCH56592) and MIC-2 (other agents) endpoints.
[c] For MFC columns, ND indicates not done.
[d] Fluconazole MIC, ≤1.0 μg/ml; itraconazole MIC, <0.03 to 0.06 μg/ml.
[e] For the MIC_{90} column, ND indicates not obtained.

In studies by Vazquez *et al.*, 1997 (Antimicrob Agents Chemother 41: 1612, reference # 8), the activity of MK-0991 was measured against *Candida* species obtained from clinical specimens from patients with candidemia, localized mucosal disease, or asymptomatic colonization, by a NCCLS microdilution method (M27-P). For determination of MFC, a 10 ul volume from the wells showing no growth was subcultured on Sabouraud dextrose agar and incubated for 72 hours at 30°C. The results in Table 29 list the MIC values to be ≤ 0.8 ug/ml for *C. albicans*, *C. glabrata*, and *C. tropicalis* (Table 29a). Although the MK-0991 MICs against *C. krusei* and *C. lusitaniae* are in the range stated above (≤ 0.8 ug/ml), the MFCs (1.6 ug/ml) were high (Tables 29a and 29b). Against all the 5 isolates of *C. guilliermondii*, the MK-0991 MICs (1.6 ug/ml) were higher (Table 29b). The MFC against *C. guilliermondii* was very high (6.25 ug/ml). The activity of MK-0991 against fluconazole (FCZ) resistant and sensitive isolates was similar (Table 29c).

In another study by Vazquez *et al.*, 1996 (ICAAC, Abstract F-35, p106; reference # 41), it was stated that against *C. albicans*, *C. lusitaniae*, *C. krusei*, *C. tropicalis* and *C (T). glabrata*, the MK-0991 MICs were <0.25 ug/ml while those against *C. guilliermondii* and *C. rugosa* were > 10 ug/ml, thereby indicating variability in susceptibility of different *Candida* species to MK-0991. However, the complete details of the experimental design and results were not provided for an independent review.

Table 29: *In vitro* susceptibility to L-743,872 and other antifungal agents

a. *C. albicans, C. glabrata, and C. tropicalis*

Organism (no. of isolates)	Antifungal agent	MIC (µg/ml) Range	50%	90%	MFC_{90} (µg/ml)
C. albicans (50)	L-743,872		0.20	0.40	0.40
	Amphotericin		0.1	0.2	
	Flucytosine		0.16	0.64	
	Fluconazole		0.16	40	
	Itraconazole		0.01	0.40	
	Ketoconazole		0.01	0.01	
C. glabrata (21)	L-743,872		0.20	0.40	0.40
	Amphotericin		0.20	0.40	
	Flucytosine		0.08	0.08	
	Fluconazole		2.5	40	
	Itraconazole		0.20	1.6	
	Ketoconazole		0.20	0.40	
C. tropicalis (10)	L-743,872		0.20	0.40	0.40
	Amphotericin		0.20	0.20	
	Flucytosine		0.16	0.32	
	Fluconazole		1.25	40	
	Itraconazole		0.05	0.80	
	Ketoconazole		0.01	0.40	

L-743,872 demonstrates the lowest MICs and is the most active against *C. albicans, C. glabrata, C. tropicalis, C. kefyr,* and *C. parapsilosis*, with similar MIC_{50}s and narrow ranges. On the other hand, L-743,872 demonstrates less activity against *C.*

b. *C. kefyr, C. krusei, C. lustinae, C. parapsilosis,* and *C. guilliermondii*

Organism (no. of isolates)	Antifungal agent	MIC (µg/ml) Range	50%	MFC_{90} (µg/ml)
C. kefyr (5)	L-743,872		0.20	0.40
	Amphotericin		0.20	
	Flucytosine		0.08	
	Fluconazole		1.25	
	Itraconazole		0.20	
	Ketoconazole		0.01	
C. krusei (5)	L-743,872		0.80	1.6
	Amphotericin		0.80	
	Flucytosine		10	
	Fluconazole		40	
	Itraconazole		0.40	
	Ketoconazole		0.01	
C. lusitaniae (5)	L-743,872		0.80	1.6
	Amphotericin		0.10	
	Flucytosine		0.08	
	Fluconazole		0.32–0.64	
	Itraconazole		0.02	
	Ketoconazole		0.01	
C. parapsilosis (7)	L-743,872		0.20	0.40
	Amphotericin		0.20	
	Flucytosine		0.08	
	Fluconazole		0.32–0.64	
	Itraconazole		0.02	
	Ketoconazole		0.01	
C. guilliermondii (5)	L-743,872		1.6	6.25
	Amphotericin		0.40	
	Flucytosine		0.08	
	Fluconazole		5.0	
	Itraconazole		0.20	
	Ketoconazole		0.03	

c. Comparison of the in vitro susceptibility of azole susceptible and resistant strains of Candida

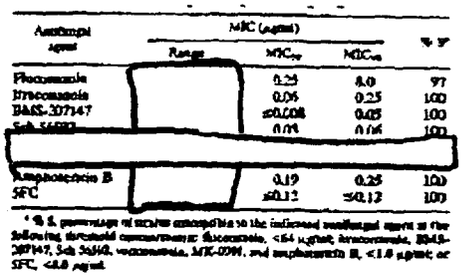
Organism (no. of isolates)*	Antifungal agent	MIC (µg/ml) Range	50%	90%	MFC_{90} (µg/ml)
C. albicans S (44)	L-743,872		0.20	0.40	0.40
	Amphotericin		0.10	0.20	
	Flucytosine		0.16	0.64	
	Fluconazole		0.16	2.5	
	Itraconazole		0.01	0.10	
	Ketoconazole		0.01	0.01	
C. albicans R (10)	L-743,872		0.20	0.40	0.40
	Amphotericin		0.05	0.20	
	Flucytosine		0.16	0.64	
	Fluconazole		40	80	
	Itraconazole		0.40	0.80	
	Ketoconazole		0.01	0.04	
C. glabrata S (10)	L-743,872		0.20	0.20	0.40
	Amphotericin		0.20	0.40	
	Flucytosine		0.04	0.20	
	Fluconazole		1.25	5	
	Itraconazole		0.20	0.40	
	Ketoconazole		0.08	0.02	
C. glabrata R (7)	L-743,872		0.40	0.80	
	Amphotericin		0.40		
	Flucytosine		8.00		
	Fluconazole		40		
	Itraconazole		1.6		
	Ketoconazole		0.40		

* S, fluconazole susceptible; R, fluconazole resistant.

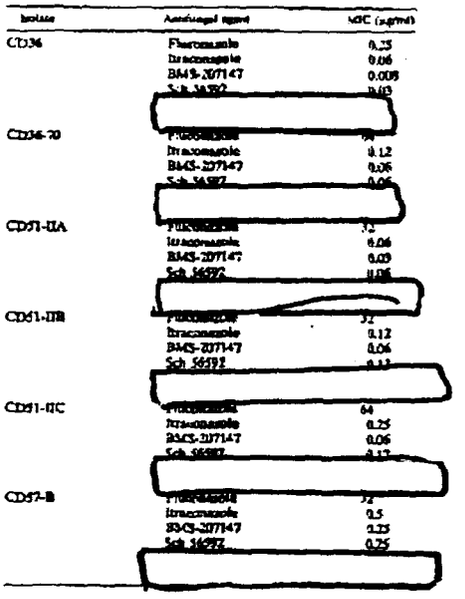
Studies by Pfaller *et al.*, 1999 (J Clin Microbiol 37: 870; reference # 9) measured the activity of MK-0991 against *Candida dubliniensis*, an opportunistic yeast pathogen recognized to be a minor component of normal human oral microbial flora. In this study, 71 isolates from 68 patients in Ireland were tested for *in vitro* susceptibility to several antifungal agents by a microdilution method recommended by NCCLS, using [redacted] medium, and incubated for 48 hours. The MK-0991 MIC values were ≤ 1 ug/ml against all isolates including 5 FCZ resistant (FCZ MIC≥32 ug/ml) isolates (Table 30).

Table 30: *In vitro* susceptibilities of *C. dubliniensis*

a: *In vitro* susceptibilities of 71 *C. dubliniensis* isolates tested against 8 antifungal agents



b: Antifungal susceptibilities of *C. dubliniensis* type strain (CD36) and of strain with decreased susceptibilities to fluconazole tested against triazole and echinocandon antifungal agents



The *in vitro* susceptibility of MK-0991 against clinical yeast isolates from patients with oropharyngeal/ esophageal candidiasis (phase IIa and phase IIb studies, protocol 003, 004, and 007 respectively, Merck reports, 2000: reference # 69, 70 and 78) was tested by NCCLS recommended method (M27-A). The clinical isolates were obtained from esophageal brushings, routine oropharyngeal swabs, and/or biopsy specimens from infected sites. All the isolates from different centers were shipped to the Central Merck Research Laboratory where the identifications of the isolates were confirmed (using [redacted] Candida, morphological testing and/ or conventional biochemical methods) before processing for *in vitro* susceptibility testing. Besides RPMI, [redacted] medium was used and the turbidity of the cultures adjusted spectrophotometrically at 550 nm. AmpB and FCZ were used as comparators. The MICs were read after 24 or 48 hours using [redacted] and [redacted] medium, respectively. Also, the MICs for MK-0991 and ampB were based on no visible growth whereas that of FCZ on inhibition of growth by 80%. The results in Tables 31 to 33 show lower MK-0991 and ampB MICs in [redacted] ompared to the [redacted] medium whereas FCZ MICs were lower in [redacted] medium. The sponsor has also stated that most isolates exhibited a sharp end point as specified above. Trailing was observed for the following species when tested in RPMI medium: *C. guilliermondii* (phase IIa and IIb studies: 12/12 and 14/14 isolates, respectively), *C. parapsilosis* (phase IIb study: 3/3 isolates), *C. tropicalis* (phase IIa and IIb: studies: 1/6 and 1/3 isolates, respectively), *C. glabrata* (phase IIa study: 1/1 isolate) and *C. albicans* (phase IIb study: 1/173 isolate). The MK-0991 MIC_{90} values against *C. albicans* and *C. glabrata* isolates were 1 and 2 ug /ml, respectively whereas that against *C. guilliermondii* was >64 ug/ml. The MIC_{90} values against other *Candida* species (*C. kefyr, C. krusei, C. lipolytica, C. parapsilosis*, and *C. tropicalis*) could not be determined since the number of isolates was low (< 10).

Table 31: phase IIa study (protocol 003)

Susceptibility (MIC in µg/mL) of Clinical Yeast Isolates From Esophageal Candidiasis to Caspofungin, Amphotericin B, and Fluconazole for Clinical Isolates in RPMI[1] and AM3[2]

Organism (No. Isolates)	Antibiotic	Range	MIC_{50}	MIC_{90}	Geometric Mean
Candida albicans (283)	Caspofungin		0.5 (0.06)	1 (0.125)	0.39 (0.05)
	Amphotericin B		0.25 (0.03)	0.5 (0.03)	0.34 (0.03)
	Fluconazole		0.25 (2)	1 (>64)	>0.39 (>2.39)
Candida guilliermondii (12)	Caspofungin		>8 (0.125)	>64 (>2)	>11.31 (>0.26)
	Amphotericin B		0.5 (0.015)	0.5 (0.015)	0.37 (0.01)
	Fluconazole		8 (8)	8 (16)	8.48 (4.69)
Candida kefyr (1)	Caspofungin				
	Amphotericin B				
	Fluconazole				
Candida krusei (7)	Caspofungin		1 (0.25)		1.30 (0.35)
	Amphotericin B		0.5 (0.06)		0.32 (0.06)
	Fluconazole		64 (32)		>58.69 (>19.03)
Candida lipolytica (2)	Caspofungin				2 (0.71)
	Amphotericin B				0.25 (0.06)
	Fluconazole				2.83 (2.83)

Susceptibility (MIC in µg/mL) of Clinical Yeast Isolates From Esophageal Candidiasis to Caspofungin, Amphotericin B, and Fluconazole for Clinical Isolates in RPMI[1] and AM3[2]

Organism (No. Isolates)	Antibiotic	Range	MIC_{50}	MIC_{90}	Geometric Mean
Candida parapsilosis (5)	Caspofungin		2 (0.5)		1.74 (0.66)
	Amphotericin B		0.25 (0.015)		0.25 (0.01)
	Fluconazole		2 (4)		2.30 (3.03)
Candida tropicalis (6)	Caspofungin		0.5 (0.06)		>0.63 (0.08)
	Amphotericin B		0.25 (0.008)		0.25 (0.01)
	Fluconazole		0.5 (1)		1.12 (0.79)
Saccharomyces cerevisiae (4)	Caspofungin		1 (0.25)		1 (0.21)
	Amphotericin B		0.125 (0.03)		0.18 (0.03)
	Fluconazole		2 (4)		2.38 (4.76)
Candida glabrata (7)	Caspofungin		1 (0.125)		1.00 (0.09)
	Amphotericin B		0.5 (0.06)		0.41 (0.06)
	Fluconazole		4 (4)		4.88 (2.69)

[1] Broth microdilution method (NCCLS Document: M27-A); RPMI 1640 media; Minimum inhibitory concentration caspofungin and amphotericin B was defined as lowest concentration of antifungal inhibiting visible growth fluconazole was defined as lowest concentration inhibiting 50% of the visible growth.

[2] Broth microdilution method (NCCLS Document: M27-A); Antibiotic Medium #3(AM3); MIC in parenthesis caspofungin and Amphotericin B was defined as lowest concentration of antifungal inhibiting visible growth fluconazole was defined as lowest concentration inhibiting 80% of the visible growth.

[3] A total of 13 isolates were originally tested. Two isolates for AN 0750 were tested in RPMI 1640 media. The profiles were similar, and therefore only one of the results was recorded in the database and table shown. The is recorded here are: caspofungin acetate MIC = >8, Amphotericin B MIC = 0.125, and Fluconazole MIC = 4.

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Table 32: phase IIa study (protocol 007)

Susceptibility (MIC in µg/mL) of Clinical Yeast Isolates From Esophageal Candidiasis to Caspofungin, Amphotericin B, and Fluconazole for Clinical Isolates in RPMI[†] and AM3[‡]

Organism (No. Isolates)	Antibiotic	Range	MIC_{50}	MIC_{90}	Geometric Mean
Candida albicans (20)	Caspofungin		0.5 (0.06)	1 (0.06)	0.68 (0.05)
	Amphotericin B		0.5 (0.03)	0.5 (0.06)	0.41 (0.03)
	Fluconazole		16 (32)	32 (>64)	>6.23 (>17.75)
Candida glabrata (1)	Caspofungin				
	Amphotericin B				
	Fluconazole				

[†] Broth microdilution method (NCCLS Document M27-A); RPMI 1640 media; Minimum inhibitory concentration (MIC) for caspofungin and amphotericin B was defined as lowest concentration of antifungal inhibiting visible growth; the MIC for fluconazole was defined as lowest concentration inhibiting 50% of the visible growth.

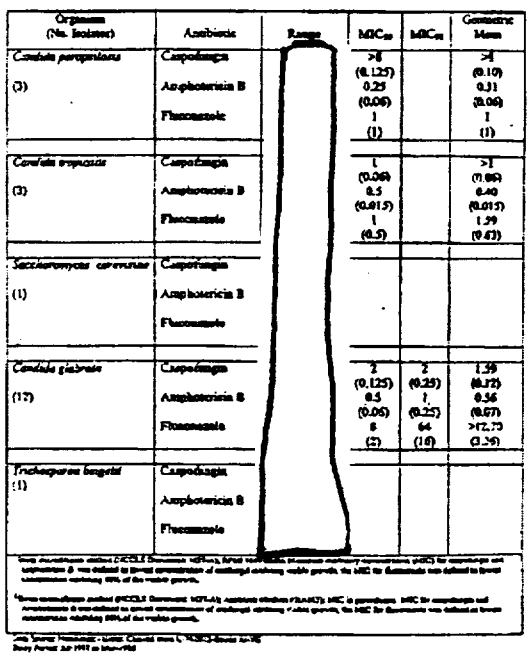
[‡] Broth microdilution method (NCCLS Document M27-A); Antibiotic Medium 3(AM3); MIC in parenthesis. MIC for caspofungin and Amphotericin B was defined as lowest concentration of antifungal inhibiting visible growth; the MIC for fluconazole was defined as lowest concentration inhibiting 50% of the visible growth.

Data Source: Notebooks - Hicks: Clinical Trials L-743872 – Books III-IX
Study Period: Jul-1997 to Jul-1999

Table 33: Phase IIb study (protocol 004)

Susceptibility (MIC in µg/mL) of Clinical Yeast Isolates From Oropharyngeal/Esophageal Candidiasis to Caspofungin, Amphotericin B, and Fluconazole for Clinical Isolates in RPMI[†] and AM3[‡]

Organism (No. Isolates)	Antibiotic	Range	MIC_{50}	MIC_{90}	Geometric Mean
Candida albicans (173)	Caspofungin		0.5 (0.06)	1 (0.125)	>0.58 (0.07)
	Amphotericin B		0.5 (0.03)	1 (0.03)	0.39 (0.03)
	Fluconazole		1 (2)	8 (>64)	>1.32 (>4.19)
Candida guilliermondii (14)	Caspofungin		>8 (0.5)	>8 (>2)	>8 (>0.71)
	Amphotericin B		0.25 (0.008)	0.25 (0.015)	0.24 (0.01)
	Fluconazole		4 (16)	16 (16)	6.56 (9.75)
Candida lusitaniae (1)	Caspofungin				
	Amphotericin B				
	Fluconazole				
Candida krusei (4)	Caspofungin		2 (0.125)		2 (0.25)
	Amphotericin B		1 (0.06)		0.84 (0.07)
	Fluconazole		64 (32)		53.82 (26.90)
Candida lipolytica (2)	Caspofungin		2 (0.125)		2 (0.18)
	Amphotericin B		0.5 (0.06)		0.5 (0.09)
	Fluconazole		2 (8)		2.83 (8)



The sponsor has also consolidated the results of the *in vitro* susceptibility testing of isolates from all the patients enrolled in all clinical trials (protocol 003, 004, 007, 014, and 020: Merck report, 2000; reference # 62). It appears from Table 34 that overall 900 isolates were tested. All the isolates were processed as described above. Table 34 shows the MICs in [redacted] medium after 48 and 24 hour of incubation respectively. The MK-0991 MIC_{90} in [redacted] medium against *C. albicans* was 1 ug/ml compared to that against *C. glabrata, C. krusei,* and *C. tropicalis* were 2 ug/ml. The MIC_{90} against *C. guilliermondii* and *C. parapsilosis* were high (>8 ug/ml). No correlation was observed between 24 hour MICs and clinical outcome (Merck report, 2000, reference # 71)

Although it was stated that the isolates were collected from patients enrolled in protocols 003, 004, 007, 014, and 020, the microbiological raw data from studies 014 and 020 were not available for review. The combined number of patients enrolled in studies 003, 004, and 007 was about 282. Study 020 enrolled about 177 patients but the number and species of isolates collected and tested in this study were not provided. There was no information available from study 014 for review. From the results of studies 003, 004 and 007 it is clear that multiple isolates of the same species from the same patient were tested. In the absence of genotyping it is difficult to conclude whether the multiple isolates of the same species collected from the same patient were indeed different.

Table 34

A.

Minimum Inhibitory Concentrations (µg/mL) for Caspofungin, Amphotericin B (AmB) and Fluconazole (FCZ) Versus Clinical Isolates[a] in RPMI 1640 Medium

Organism (Number of Isolates)	Antibiotic	Range	MIC_{50}	MIC_{90}	Geometric Mean
Candida albicans (771)	Caspofungin	[redacted]	0.5	1	0.54
	AmB		0.5	0.5	0.37
	FCZ		0.5	8	0.96
Candida glabrata (74)	Caspofungin		2	2	1.34
	AmB		0.5	1	0.55
	FCZ		8	16	>6.57
Candida guilliermondii (44)	Caspofungin		>8	>8	>8.26
	AmB		0.25	0.5	0.27
	FCZ		8	8	6.32
Candida kefyr (1)	Caspofungin				
	AmB				
	FCZ				
Candida krusei (18)	Caspofungin		2	2	1.65
	AmB		0.5	1	0.54
	FCZ		64	64	50.80
Candida lipolytica (7)	Caspofungin		2		1.64
	AmB		0.5		0.37
	FCZ		2		2.44
Candida lusitaniae (1)	Caspofungin				
	AmB				
	FCZ				
Candida parapsilosis (16)	Caspofungin		4	>8	3.67
	AmB		0.5	0.5	0.35
	FCZ		1	2	1.08
Candida tropicalis (31)	Caspofungin		1	2	>1.89
	AmB		0.5	0.5	0.43
	FCZ		2	4	1.87

[a] Microdilution broth method (NCCLS Document: M27-A); RPMI 1640 (Biowhittaker) medium; inoculum 10^4 CFU/mL; incubation at 35°C for 48 hr. MIC for caspofungin and AmB was defined as lowest concentration of antifungal inhibiting visible growth; the MIC for FCZ was defined as lowest concentration inhibiting 80% of the visible growth.

[b] Trailing seen (80% inhibition of growth was seen at 1 to 2 ug/mL).

B.

Minimum Inhibitory Concentrations (µg/mL) for Caspofungin, Amphotericin B (AmB) and Fluconazole (FCZ) Versus Clinical Isolates[a] in Antibiotic Medium #3

Organism (Number of Isolates)	Antibiotic	Range	MIC_{50}	MIC_{90}	Geometric Mean
Candida albicans (771)	Caspofungin	[redacted]	0.06	0.125	>0.06
	AmB		0.03	0.03	0.03
	FCZ		2	>64	3.43
Candida glabrata (74)	Caspofungin		0.125	0.25	0.10
	AmB		0.06	0.5	0.10
	FCZ		7	16	2.83
Candida guilliermondii (44)	Caspofungin		0.5	>2	>0.51
	AmB		0.015	0.015	0.01
	FCZ		8	16	>6.62
Candida kefyr (1)	Caspofungin				
	AmB				
	FCZ				
Candida krusei (18)	Caspofungin		0.5	1	0.46
	AmB		0.06	0.125	0.07
	FCZ		32	32	25.5
Candida lipolytica (7)	Caspofungin		0.125		0.18
	AmB		0.03		0.04
	FCZ		4		3.28
Candida lusitaniae (1)	Caspofungin				
	AmB				
	FCZ				
Candida parapsilosis (16)	Caspofungin		1	2	0.74
	AmB		0.015	0.06	0.02
	FCZ		1	4	1.41
Candida tropicalis (31)	Caspofungin		0.06	0.125	0.05
	AmB		0.015	0.03	0.02
	FCZ		1	2	1.05

[a] Microdilution broth method (NCCLS Document: M27-A); Antibiotic Medium #3; inoculum 10^3 CFU/mL; incubation at 35°C for 24 hr. MIC for caspofungin and AmB was defined as lowest concentration of antifungal inhibiting visible growth; the MIC for FCZ was defined as lowest concentration inhibition 80% of the visible growth.

In a study by Ernst *et al.*, 1999 (Diag Microbiol Infect Dis 33: 75; reference # 26) attempts were made to correlate MIC/MIC-80 values with the alteration in morphology. For this, 2 isolates each of *C. albicans*, *C. glabrata* and *C. tropicalis* were tested for *in vitro* susceptibility to MK-0991 by the NCCLS microdilution method. The inhibition of growth was determined either visually or spectrophotometrically (Table 35, see footnote), after 48 hours of incubation.

Electron microscopic observations showed round budding and branching forms in control cultures. After exposure to MIC-80 concentrations of MK-0991, structural changes consistent with inhibition of growth were observed. Only cellular debris was observed in cultures exposed to MIC equivalent concentrations. The authors concluded that MIC-80 values represent more accurately the antifungal activity of MK-0991 rather than MICs. The number of isolates tested was very small and appears that only 1 isolate of *C. albicans* was followed for morphological changes. It is unclear whether other isolates (listed in Table 35) were followed for morphological changes after exposure to the drug.

Table 35: Results of *in vitro* susceptibility

Isolate	$MIC_{80\%}$[a] (μg/mL)	$MIC_{80\%}$[b] (μg/mL)	$MIC_{100\%}$ (μg/mL)	MFC (μg/mL)	EC_{50} (μg/mL)
C. albicans 90028	0.03	0.03	0.25	0.06	0.87
C. albicans OY31.5	0.03	0.03	0.12	0.25	0.67
C. glabrata 350	0.03	0.03	0.25	0.5	2.27
C. glabrata 582	0.03	0.03	0.12	0.06	1.15
C. tropicalis 2697	0.03	0.06	0.25	0.25	0.64
C. tropicalis 3829	0.03	0.12	0.5	0.5	1.00

$MIC_{80\%}$[a], determined by visual inspection for 80% inhibition of growth compared with control.
$MIC_{80\%}$[b], determined by spectrophotometric methods for 80% inhibition of growth compared with control.
$MIC_{100\%}$, determined by visualization of complete inhibition (clear well).
MFC Minimum fungicidal concentration.
EC_{50}, 50% maximal effect concentration.

In vitro* activity against fungal species other than *Aspergillus* and *Candida

Studies by Arikan *et al.*, 1999 (ICAAC poster, 1999, reference # 43) measured the activity of MK-0991 against 22 *Fusarium* isolates using the NCCLS proposed (M38-P) microdilution method. The activity was measured using different media [redacted] supplemented with 2% glucose [redacted] and antibiotic medium # 3 supplemented with 2% glucose [redacted] after 24, 48, and 72 hours of incubation. MK-0991 exhibited minimal effect against *Fusarium* species (Tables 5 and 6). No known antifungal drug was used as a comparator in this study.

A study by Espinel-Ingroff, 1998 (J Clin Microbiol 36: 2950; reference # 11) measured the activity of MK-0991 against 5 to 6 isolates each of *Fusarium*, *Histoplasma*, and *Blastomyces* species by the NCCLS proposed (M38-P) microdilution method. In addition, 19 isolates of *C. neoformans* were tested according to the NCCLS M27-A microdilution method. These isolates were obtained from clinical specimens from patients with severe fungal infections. The cultures were incubated from 24 to 72 hours to ensure that the growth was heavy/sufficient to determine MIC-2 values. The effect on morphology was not determined. The geometric mean MK-0991 MIC-2 values against *H. capsulatum* and *B. dermatitidis* varied from 0.5 to 8 ug/ml whereas that against *Fusarium* species were >16 ug/ml. (Table 7). The MFCs were not determined. No approved antifungal agent such as ampB or ITZ was used as a comparator. MK-0991 was not effective against *C. neoformans* (≥16 ug/ml, Table 27).

In another study by Del Poeta *et al.*, 1997 (Antimicrobial Agents Chemother 41: 1835, reference # 36), the activity of MK-0991 was measured against a small number of isolates of *Fusarium* and *Rhizopus* species. The cultures were assayed according to the proposed NCCLS broth macrodilution method (M38-P) using [redacted] medium and incubated for 72 hours. The MIC-80 values in Table 10 indicate MK-0991 to be ineffective against *Fusarium* (MIC-80 ≥ 50 ug/ml) and *Rhizopus* (MIC-80 ≥ 100 ug/ml) species.

Four isolates of *C. neoformans* from the Merck culture collection were tested by broth micro-dilution method using [redacted] medium [NCCLS reference method M27A (Merck report, 1995, reference # 35)]. The results indicate that the MK-0991 MICs varied from 4 to 16 ug/ml at both 24 and 48 hours. A comparison of the *in vitro* activity of MK-0991 with ampB showed that MK-0991 is less active against *Cryptococcus* (Table 4).

In vitro Susceptibility of clinically resistant and laboratory mutated isolates to MK-0991:

MK-0991, like ampB, was shown to be active against 23 clinical isolates of *Candida* species [*C. albicans* (n=9), *C. tropicalis* (n=1), *C. glabrata* (n=3), or *C. lusitaniae* (n=2)] and *C. neoformans* (n=8) collected from patients failing therapy with ampB, 5-FC or FCZ (Merck report, 1995, reference # 33; Bartizal *et al.*, 1997, Antimicrob Agents Chemother, 41: 2326, reference # 1). It is of note that the majority of the isolates tested were resistant to FCZ. There were 2 isolates resistant to ampB or 5-FC only, 3 resistant to 5-FC+FCZ, and 1 to ampB+5-FC. The duration of treatment with the antifungal agents, history, etc. were not described. These isolates were obtained from Dr Rinaldi's Mycology reference laboratory. The MK-0991 MICs were ≤ 2 ug/ml (Table 36). The MK-0991 MICs against *C. neoformans* were high [redacted]

Table 36: Susceptibilities of antifungal resistant isolates or isolates from patients with clinical failure after treatment with AmB, FCZ, and 5FC

Organism	Resistance or failed outcome after treatment with the following drug:	Culture no.	MIC (μg/ml)			
			MK-0991[a]	AmB	FCZ	5FC
Candida albicans	Susceptible	489	0.25	0.50	0.06	0.06
	5FC	536	0.125	1.0	0.50	8.0
	5FC	544	0.125	0.50	0.50	>32.0
	AmB	537	1.0	4.0	1.0	1.0
	FCZ	538	0.125	0.50	2.0	0.06
	5FC and FCZ	543	0.25	0.50	2.0	>32.0
	FCZ	539	0.25	0.50	32.0	0.06
	FCZ	540	0.125	0.50	2.0	0.125
	FCZ	541	0.50	1.0	>32.0	0.06
	FCZ	542	0.125	0.50	32.0	1.0
Candida tropicalis	Susceptible	425	0.25	0.25	0.125	0.50
	FCZ	545	0.125	1.0	>32.0	0.06
Candida glabrata	FCZ	257	0.50	0.25	>32.0	0.03
	FCZ	494	0.50	0.50	>32.0	0.03
	5FC and FCZ	535	0.50	1.0	>32.0	>32.0
Candida lusitaniae	Susceptible	298	0.50	1.0	0.50	≤0.01
	AmB and 5FC	533	2.0	8.0	0.125	>32.0
	AmB	531	1.0	8.0	0.50	≤0.01
Cryptococcus neoformans	Susceptible	34	16.0	0.25	NT[b]	NT
	FCZ	525	32.0	0.50	8.0	1.0
	FCZ and 5FC	526	32.0	1.0	16.0	4.0
	FCZ	527	16.0	1.0	8.0	2.0
	FCZ	528	16.0	1.0	8.0	2.0
	FCZ	529	32.0	1.0	4.0	2.0
	FCZ	530	16.0	1.0	4.0	2.0
	FCZ	531	16.0	1.0	4.0	2.0
	FCZ	532	16.0	0.50	32.0	2.0

[a] Broth microdilution method, RPMI 1640 medium, inocula of 1×10^3 to 5×10^3 CFU/ml, incubation for 24 to 48 h at 35 to 37°C.
[b] NT, not tested.

In another study, the activity of MK-0991 was tested against a panel of clinical isolates of *C. albicans* and *C. neoformans* resistant to other antifungal agents (ampB, 5-FC, FCZ and ketoconazole, i.e., KTZ) and laboratory derived echinocandin resistant (ech-r) isolates of *C. albicans* and *C (T). glabrata* (Merck report, 1995, reference # 33). It is unclear whether the echinocandin used for deriving ech-r isolates was indeed MK-0991 or some other echinocandin. The results in Table 37 indicate comparable MK-0991 MICs against the ampB, FCZ, 5-FC or KTZ resistant and susceptible strains. The echinocandin resistant isolates were less susceptible (about 2 to 8 fold increase in MICs) to MK-0991 with MICs varying from 1 to 4 ug/ml.

Table 37: MFC values of L-743,872 and ampB against antifungal resistant yeast isolates

Organism	Resistance Pattern	CL#	L-743,872 MFC (µg/ml)	AMB MFC (µg/ml)
Candida albicans	None (susceptible)	489[a]	0.25	0.50
	5-Flucytosine (5-FC)	536	0.125	1
	Amphotericin B (AMB)	537	0.25	8
	5-FC & Fluconazole	538	0.125	0.50
	Fluconazole	539	0.25	0.50
	Fluconazole	540	0.125	0.50
	Fluconazole	541	0.25	1
	Ketoconazole	542	0.25	0.50
	Fluconazole	543	0.25	1
	5-FC	544	0.06	0.50
	Echinocandin	490	2	0.50
	Echinocandin	491	2	0.50
	Echinocandin	492	2	0.50
	Echinocandin	493	2	0.50
Candida tropicalis	None (susceptible)	425[a]	0.25	0.25
	Fluconazole	545	0.25	1
Torulopsis glabrata	None (susceptible)	257[a]	0.50	0.125
	5-FC	494	0.50	0.50
	5-FC	535	0.25	1
	5-FC & Echinocandin	495	1	0.50
	5-FC & Echinocandin	496	2	0.50
	5-FC & Echinocandin	497	2	1
	5-FC & Echinocandin	498	2	1
	5-FC & Echinocandin	499	4	0.50
Candida lusitaniae	None (susceptible)	298[a]	0.50	1
	AMB	533	1	8
	AMB	534	1	8
Cryptococcus neoformans	None (susceptible)	34[a]	16	0.25
	Fluconazole	525	32	1
	Fluconazole	526	32	1
	Fluconazole	527	16	1
	Fluconazole	528	16	1
	Fluconazole	529	16	0.5
	Fluconazole	530	16	1
	5-FC	531	16	1
	Fluconazole	532	16	1

Notebook: BVAL III-CP pp. 96-101, pp. 110-127, BFeb95-GMarch95.
[a] Y-489, Y-425, Y257, 298 and Y34 are susceptible isolates.

In another study, the activity of MK-0991 was tested against 30 *C. albicans* isolates by NCCLS (M27-T) method (micro dilution vs. macrodilution method) using [redacted] mediums and cultures incubated for 24 to 48 hours (Nelson *et al.*, 1997, J Med. Vet Mycology 35: 285; reference # 32). Some of these isolates were stated to be resistant to FCZ or ampB (Table 38). The results in Table 38b showed higher MK-0991 MICs at 48 hours compared to the 24-hour time point. Also, the MK-0991 MICs were higher when tested in [redacted] medium as compared to the [redacted] The results by microdilution and macrodilution method were comparable. Although some of the isolates were stated to be resistant to FCZ or ampB, the basis of resistance was not specified. The MK-0991 MICs for both resistant and susceptible isolates were similar.