Table 38

a:

MICs for fluconazole and amphotericin B obtained by M27-T and modified M27-T conditions, respectively, against *Candida* isolates used in this study

Group*	N	Fluconazole M27-T MIC†	Modified Amphotericin B M27-T MIC‡
AMB resistant	7	0·0625–4	1– > 64
AMB and FLU resistant	1	> 64	> 64
FLU resistant	3	64– > 64	0·0625–0·25
Susceptible	15	0·25–32	0·0625–0·5

*AMB, amphotericin B; FLU, fluconazole.
†All MICs are reported in μg ml^{-1}.
‡M27-T was modified by use of Antibiotic Medium 3 broth, a microdilution format, and reading after 24 h of incubation. These modifications are known to enhance the ability of M27-T to detect resistance to amphotericin B [6,7]. The isolates included in the amphotericin B resistant group were *C. albicans* (2), *C. lusitaniae* (4) and *C. parapsilosis* (1). The one isolate which exhibited resistance to both amphotericin B and fluconazole was a *C. krusei*. The isolates in the fluconazole resistant group were *C. glabrata* (4), *C. krusei* (1), *C. lusitaniae* (1) and *C. tropicalis* (1). The susceptible group included *C. albicans* (4), *C. glabrata* (3), *C. guilliermondii* (1), *C. lusitaniae* (1), *C. parapsilosis* (3) and *C. tropicalis* (3).

b:

MICs for pneumocandins L-733,560 and L-743,872 obtained under different assay conditions

Time of reading / Group (n)	RPMI 1640		Antibiotic Medium 3	
	24 h range	48 h range	24 h range	48 h range
L-733,560				
AMB resistant (7)				
AMB and FLU resistant (1)				
FLU resistant (7)				
Susceptible (15)				
All isolates (30)				
Microdilution: all isolates (30)				
L-743,872				
AMB resistant (7)				
AMB and FLU resistant (1)				
FLU resistant (7)				
Susceptible (15)				
All isolates (30)				
Microdilution: all isolates (30)				

AMB, amphotericin B; FLU, fluconazole. All MICs are reported in μg ml^{-1} and, except for as marked, were determined in the microdilution format. The pooled results obtained in the microdilution format are shown in the final row of the isolates on L-733,560 and L-743,872.

Some other properties of MK-0991:

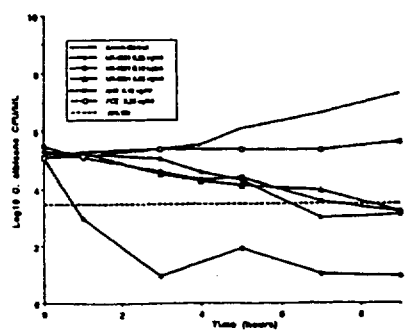
Some of the other properties (such as fungicidal activity determined by time kinetics, effect of serum proteins, and post antifungal effect) of MK-0991 as an antifungal agent are limited to studies against some of the *Candida* species only. These studies are discussed below:

The **fungicidal activity** of the drug was determined either by subculturing 1.5 ul aliquots from the initial culture or time kinetics studies. As discussed above MFCs were found comparable to MICs against some of the *Candida species* but not against *Aspergillus* (Merck reports, 1995, reference # 4, 5, and 35; Bartizal *et al.*, 1997, Antimicrob Agents Chemother, 41: 2326, reference # 1; Espinel-Ingroff, 1998, J Clin Microbiol 36: 2950, reference # 11; Vazquez *et al.*, Antimicrob Agents Chemother 41: 1612, reference # 8).

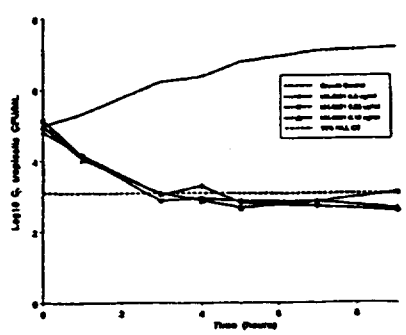
Growth inhibition time kinetic studies indicate that MK-0991 kills *C. albicans* in 5.6 to 10 hours. The results in Figure 10 show that killing of *C. tropicalis* (data based on 2 separate experiments) was faster by about an hour compared to *C. albicans* (Merck report, 1995, reference # 24; Bartizal *et al.*, 1997, Antimicrob Agents Chemother, 41: 2326, reference # 1). The killing effect of ampB was faster than MK-0991 (Figure 10(a)). FCZ was, however, fungistatic.

Figure 10: Time kill study

(a) *C. albicans*



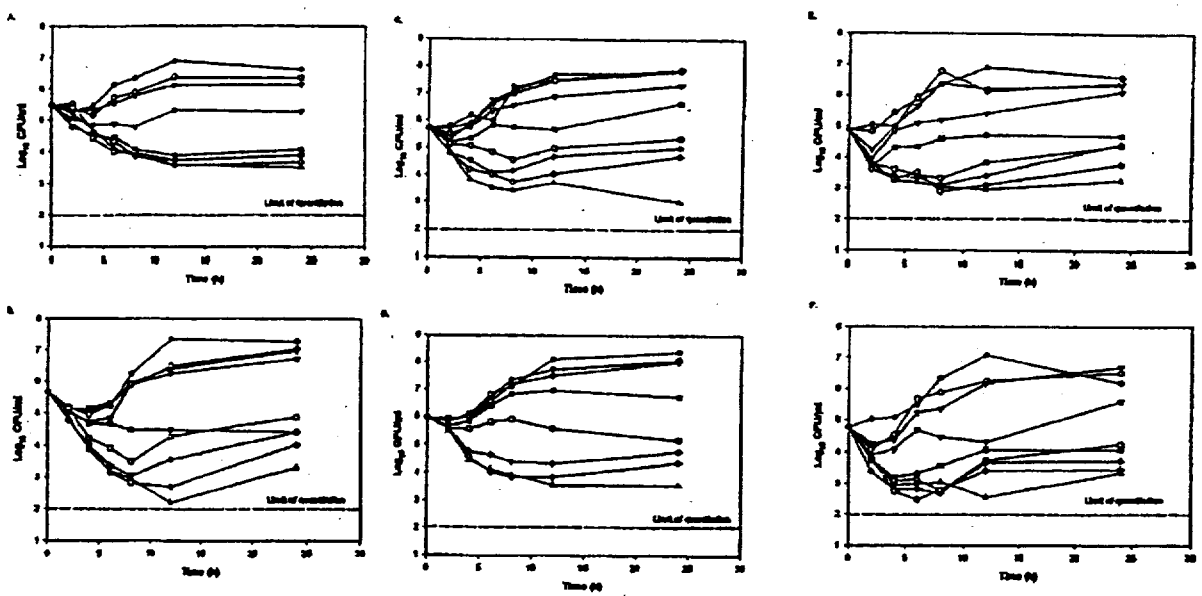
(b) *C. tropicalis*



In another study by Ernst *et al.*, 1999 (Diag Microbiol Infect Dis 33: 75; reference # 26) the time kill studies were performed against 6 yeast isolates at an inoculum of 10^5 to 5 x 10^5 CFU/ml. The results in Figure 11 show that the activity against various isolates of the 3 *Candida* species tested varied from fungicidal ($3\log_{10}$ reduction in CFU) to fungistatic at the different concentrations. For example, against *C. albicans*, the MK-0991 concentrations of ≥ 4X the MIC after 6 to 8 hours of exposure decreased the CFU by only > 1.5 log. The maximal activity was observed at concentrations equivalent to 16X the MIC. Against *C. glabrata*, the fungicidal activity was observed at 12 and 24 hours at high concentrations. These cultures were incubated for variable times (0, 2, 4, 6, 8, 12 and 24 hours) and small aliquots were subcultured for enumeration of colony counts. MK-0991 was clearly fungistatic against the 2 isolates of *C. tropicalis*.

Figure 11: Time-kill plots for *C. albicans* 90028 (A),OY315 (B); *C. glabrata* 350 (C), 582 (D); *C. tropicalis* 2697 (E) and 3829 (F) at the following concentrations

(●), 0.125 × $MIC_{80\%}$ (○), 0.25 × $MIC_{80\%}$ (▼), 0.5 × $MIC_{80\%}$ (▽), 1 × $MIC_{80\%}$ (■), 2 × $MIC_{80\%}$ (□), 4 × $MIC_{80\%}$ (◆), 8 × $MIC_{80\%}$ (◇), 16 × $MIC_{80\%}$ (▲).



The results show that the fungicidal activity of MK-0991 varies with the species and strain of *Candida*. It is also dependent on the concentration and duration of drug exposure.

The effect of serum proteins on the activity of MK-0991 was tested against *C. albicans* using human and mouse sera between the concentrations of 0 - 50 % in [redacted] broth medium (Merck report, 1995, reference # 22; Bartizal *et al.*, 1997, Antimicrob Agents Chemother, 41: 2326, reference # 1). In [redacted] medium, the MIC and MFC values were not significantly altered whereas in [redacted] medium the MFC values were increased up to 4/8-fold (Table 39). MICs were comparable to MFCs. The significance of this finding to the activity *in vivo* is unclear.

Table 39: Effects of human and mouse serum on susceptibility of *C. albicans* MY 1055 to MK-0991 and AmB*

Medium	MFC (µg/ml)	
	AmB	MK-0991
YNBD plus human serum		
Plain	0.25	≤0.06
10%	0.25	≤0.06
20%	0.5	≤0.06
30%	0.25	≤0.06
40%	0.25	≤0.06
50%	0.25	0.125
YNBD plus mouse serum		
Plain	0.25	≤0.06
10%	0.25	≤0.06
20%	0.5	≤0.06
30%	0.25	≤0.06
40%	0.25	≤0.06
50%	0.25	0.125
RPMI plus mouse serum		
Plain	0.25	≤0.06
10%	0.25	≤0.06
20%	0.25	≤0.06
30%	0.25	0.125
40%	0.25	0.25
50%	0.5	0.5

* The broth microdilution method was used. The compounds were diluted in fresh pooled human or mouse serum, and then RPMI 1640 medium was added

The drug is less effective against *Aspergillus* species as compared to *Candida*. Whether serum proteins or albumin will alter the activity of MK-0991 against *Aspergillus* species is not known. However, it is of note that RPMI medium used for *in vitro* testing contains several amino acids.

The **post antifungal effect (PAFE)** of MK-0991 was measured using 2 isolates each of *C. albicans* and *C. neoformans* (Ernst *et al.*, 2000, Antimicrob Agents Chemother 44: 1108; reference # 57). The *in vitro* susceptibility was measured by the NCCLS recommended broth microdilution method using [redacted] medium buffered with [redacted] and the MICs determined after 48 and 72 hours of incubation at 35°C. The results in Table 40 show that both of the *C. albicans* isolates were susceptible to MK-0991 (MIC = 0.03 ug/ml) whereas the *C. neoformans* isolates were not (MIC > 2 ug/ml). The PAFE was determined by incubating cultures of the 4 isolates with the drug at varying concentrations (0.125 to 4 X MIC) and incubated for varying time intervals (0.25 to 1 hour). The cultures were washed by repeated centrifugation and the fungal pellet suspended in warm medium and viable colony counts determined at varying time intervals (2, 4, 6, 8, 10, 12, and 24 hours). After1 hour of exposure to the drug MK-0991 at concentrations ≥ 1X MIC showed a PAFE of > 12 hours against 2 of the *C. albicans* isolates (Table 41 and Figure 12). AmpB was effective at 0.25 to 0.5X MIC against the 2 isolates tested with PAFE of >12 hours. FCZ exhibited no PAFE.

MK-0991 alone or in combination with FCZ did not exhibit any PAFE against *C. neoformans*.

Table 40: Results of susceptibility tests

Isolate	MIC (μg/ml)			
	FLC	MK-0991	LY303366	AMB
C. albicans 90028	0.25	0.03	0.015	1.0
C. albicans OY31.5	0.25	0.03	0.015	0.5
C. neoformans 625.012	4	>2	>2	1.0
C. neoformans 1425.019	4	>2	>2	1.0

FLC=FCZ

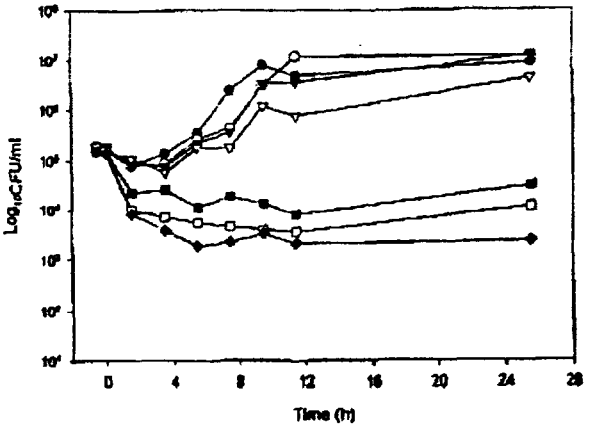
Table 41: PAFE after 1.0 h of drug exposure time for concentrations ranging from [redacted] times the MIC for *C. albicans* OY31.5 and 90028

Drug exposure (multiple of MIC)	PAFE (h)			
	FLC	MK0991	LY303366	AMB
C. albicans OY31.5				
0.125	NT*	0	>12	2
0.25	NT	0	>12	4
0.5	NT	0	>12	>12
1.0	0	>12	>12	>12
2.0	0	>12	>12	>12
4.0	0	>12	>12	>12
C. albicans 90028				
0.125	NT	0	0	2
0.25	NT	0	>12	>12
0.5	NT	0	>12	>12
1.0	0	>12	>12	>12
2.0	0	>12	>12	>12
4.0	0	>12	>12	>12

* NT, not tested.

Figure 12:

PAFE of MK-0991 in *C. albicans* 90028 following 0.5 h of exposure to the drug at various concentrations. ◆, control; ○, 0.125 times the MIC; ▽, 0.25 times the MIC; ▽, 0.5 times the MIC; ■, 1 times the MIC; □, 2 times the MIC; ◆, 4 times the MIC.



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ON ORIGINAL

In vivo* activity of MK-0991 against *Candida

The *in vivo* activity of MK-0991 against *Candida* was determined in immunocompromised and immunocompetent mice.

(a) Studies in complement (C') 5 deficient mice

(i) Effect on mycological burden:

C'5-deficient DBA/2N mice were infected with *C. albicans* (strain MY1055, 3.4 to 9.4 x 10^4 cfu) by the intravenous route (Merck report, 1995, reference # 29). Treatment was initiated 15 - 30 minutes after infection, with different doses of MK-0991 (b.i.d for 4 days) administered by different routes (intraperitoneal, intravenous and oral). The animals were necropsied 3 days after discontinuation of treatment and the fungal burden measured in the kidneys. MK-0991 administered by the intraperitoneal route, at doses ≥ 0.09 mg/kg, was effective in reducing the microbial burden by about 2 to 3 log (Table 42). Treatment by the oral route at doses ≥ 12.5 mg/kg was effective in reducing the fungal burden. Treatment by the intravenous route at a dose of 0.375 mg/kg was effective in reducing microbial burden. The reduction in microbial burden with ampB (administered by intravenous or intraperitoneal route) was not statistically different from the sham treated mice. It is also of note that delaying initiation of treatment by 24 hours post-infection reduced the antifungal activity.

Similar observations were made in another study where MK-0991 was administered by different routes and measuring efficacy in a mouse 4-day target organ assay (Merck report, 1999, study 1, reference # 16). C'5 deficient DBA/2N mice were infected intravenously with *C. albicans* MY1055 (7.2 x 10^4). Treatment with MK-0991 at different doses, b.i.d., was initiated 15 to 30 minutes after challenge for 2 days by different routes. On day 4, the mice were sacrificed and kidneys from 5 mice per group processed for measurement of mycological burden. The results in Table 43 show that parenteral (intraperitoneal, subcutaneous, intramuscular, or intravenous) administration of MK-0991 at ≥0.09 mg/kg was effective in reducing the cfu in kidneys. The oral administration of MK-0991 was less effective. The mycological burden in organs other than kidneys was not measured. Also, no known antifungal agent was used as a comparator.

The effect of MK-0991 on mycological burden in tissues other than kidney was measured in another study (Merck report, 1999, study 2, reference # 16). The experimental design was similar to that described above except that a lower concentration (6.2 x 10^4 cfu) of the inoculum of *C. albicans* strain MY1055 was used for infection. The activity of MK-0991 was compared with ampB and both drugs were administered intraperitoneally. The results in Table 44 show that MK-0991 was effective in reducing cfu in liver, kidney, spleen and brain. The reduction in cfu was comparable to those observed in ampB treated mice. However, when the % sterilization is compared then variability in activity of MK-0991 vs. ampB in different organs was observed. For example, at a 0.375 mg/kg dose, response was better in mice treated with MK-0991 in kidneys and brain whereas ampB showed a better response in liver and spleen. The reasons for such variability are unclear, but could reflect differences in pharmacokinetics/pharmacodynamics of the drugs.

Table 42:

Efficacy of L-743,872 and Amphotericin B (AMB) Following I.V. Induction of a Disseminated *Candida albicans* MY1055 Infection in DBA/2N Mice[1].

TOKA #[2] (I.P., b.i.d unless noted)	Mean log10 CFU/g Kidney, Percent Sterilization ( ) and Percent Change From Control at Each Dose (mg/kg/dose)[4]										Pooled Std Dev.
	Sham	0.00125	0.005	0.01	0.02	0.05	0.09	0.18	0.375	1.5	
93-13	5.75 (0)	NT[4]	NT	NT	5.16 (0) -74.63	NT	2.19* (100) -99.97	NT	2.13* (100) -99.97	NT	0.73
93-2	4.92 (0)	6.68 [1] (0)	5.28 [1] (0)	NT	4.26 (0)	NT	2.34 (80)	NT	2.27 (100)	NT	NE[5]
93-3	6.96 [1] (0)	NT	7.96 [1] (0) >100	NT	5.67 (0) -94.87	NT	2.45* (80) -99.99	NT	2.25* (100) -99.99	NT	0.90
93-10	6.24 (0)	5.62 (0) -75.99	4.89* (0) -95.45	NT	4.45* (0) -98.25	NT	2.21* (100) -99.99	NT	2.21* (100) -99.99	NT	0.98
93-11	5.67 (0)	NT	5.33 (0)	4.62 (0)	4.41 (0)	3.03 (0)	2.28 (80)	NT	NT	NT	
94-1	6.46 [1] (0)	NT	6.47 [1] (0) 1.35	5.82 [1] (0) -96.80	5.49 [4] (0) -99.99	3.34* (20) -99.99	2.15* (100) -99.99	NT	NT	NT	1.37
94-7	6.30 (0)	NT	6.36 [4] (0) -93.15	7.09 (0) 95.98	5.39* (0) -96.11	3.19* (0) -99.98	2.29* (80) -99.99	NT	NT	NT	0.62
94-11	6.04 (0)	NT	5.33 (0) -80.68	5.50 (0) -71.03	5.08 (0) -89.19	3.17* (0) -99.57	2.22* (60) -99.99	NT	NT	NT	
94-1 s.d.	6.86 [3] (0)	NT	All Dead[6]	6.59 (0) -46.13	6.23 (0) -76.27	5.37* (0) -96.80	2.39* (40) -99.99	2.26* (100) -99.99	2.20* (100) -99.99	NT	0.50
94-1 Delayed	6.86 [3] (0)	NT	3.72 [1] (0) -99.93	NT	5.93 [1] (0) -88.16	NT	2.55* (0) -99.99	NT	2.17* (80) -99.99	2.14* (80) -99.99	0.28
93-2 I.V.	4.69 (0)	5.99 (0)	5.30 (0)	NT	4.66 (0)	NT	4.83 (0)	NT	2.26 (100)	NT	NE
AMB 93-11	5.67 (0)	NT	5.69 (0)	NT	4.70 (0)	NT	3.14 (0)	NT	3.08 (20)	2.24 (60)	NE
AMB I.V. 93-2	4.69 (0)	4.70 [4] (0)	2.93 (0)	NT	3.26 (0)	NT	2.23 (100)	NT	Toxic[7]	NT	NE

TOKA #[3] (I.P., b.i.d unless noted)	Sham	0.78	3.13	6.25	12.5	25.0	50.0	Pooled Std Dev.
93-3 P.O.	6.62 (0)	5.09 [1] (0) >100	6.09 (0) -69.90	NT	2.76* (40) -99.99	NT	2.34* (100) -99.99	1.5
93-10 P.O.	5.65 (0)	NT	NT	4.02* (20) -97.63	4.00* (0) -97.78	2.21* (100) -99.96	2.24* (80) -99.96	0.81
94-1 P.O.	7.08 [4] (0)	NT	6.98 (0) -19.83	6.10 (0) -69.62	4.47* (0) -99.76	2.80* (0) -99.99	NT	0.76

1. DBA/2N mice were infected I.V. with 4.8 to 9.4 x 10^6 CFU *C. albicans* MY1055 per mouse. L-743,872 and AMB were administered I.P., b.i.d. unless otherwise noted. Mice received first treatment (route specified above) within 15 to 30 minutes after challenge (except for delayed therapy which was initiated 24 hours after challenge) and were treated for a total of 4 days (8 doses b.i.d. or 4 doses q.d.).
2. Mean log10 CFU/g at 7 days after challenge for paired kidneys (5 mice/group) unless noted [ ]. Percent sterilization indicates number of mice with no detectable yeast where limit of detection was 50 yeast cells per pair of kidneys.
3. Complete Notebook references and page numbers for each experiment can be found in Tables 3 to 10.
4. NT indicates that drug was not tested at this dose in this experiment.
5. NE indicates that values were not estimated.
6. Indicates that values were not processed because no mice in the sample group survived to this time point.
7. No mice in the sample group survived to this time point due to drug related toxicity.
* Denotes that the mean was statistically significantly less than the sham control mean at $\alpha \leq 0.05$.

Table 43

A.

Efficacy of Caspofungin Comparing Routes of Administration Following I.V. Induction of a Disseminated *Candida albicans* MY1055 Infection in DBA/2N Mice[1]

Route of Administration	Mean log10 CFU/g Kidney, Percent Sterilization ( ) and Percent Change From Control at Each Dose (mg/kg/dose)[2]										
	Sham	0.005	0.01	0.02	0.375	1.5	3.13	6.25	12.5	25.0	50.0
I.P.	6.39 (0)	6.42 (0) -71.48	6.01 (0) 58.93	3.50* (80) 99.987	2.22* (80) 99.999	NT[3]	NT	NT	NT	NT	NT
S.C.	6.46 (0)	6.44 (0) -8.62	6.46 (0) 70.89	2.80* (80) 99.999	2.20* (100) 99.999	2.20* (100) 99.999	NT	NT	NT	NT	NT
I.M.	6.09 (0)	6.31 (0) -89.92	6.35 (0) -81.57	3.41* (0) 98.79	2.21* (100) 99.997	2.21* (100) 99.997	NT	NT	NT	NT	NT
I.V.	6.81 (0)	6.84 (0) -36.89	6.49 (0) 93.93	2.34* (20) 99.994	2.16* (100) 99.999	NT	NT	NT	NT	NT	NT
P.O.	6.77 (0)	NT	NT	NT	NT	4.94 (0) 99.47	5.85* (40) 93.55	5.99 (0) 93.09	3.07* (0) 99.91	2.28* (0) 99.997	2.36* (20) 99.995

Experiment 4 Day TOKA 95-12 (11-Sep-1995), Notebook to View 14 (TOKA), pp. 39-41.

1. DBA/2N mice were infected I.V. with 7.2 x 10^6 CFU *C. albicans* MY1055 per mouse. Caspofungin was administered b.i.d. x 2 days (4 total doses). Mice received first treatment (route specified above) within 15 to 30 minutes after challenge.
2. Mean log10 CFU/g at 4 days after challenge for paired kidneys (5 mice/group). Percent sterilization indicates number of mice with no detectable yeast where limit of detection was 50 yeast cells per pair of kidneys.
3. NT indicates that drug was not tested at this dose in this experiment.
* Denotes that the mean was statistically significantly less than the sham control mean at $\alpha = 0.05$ using Dunnett's t-test.

B.

ED50, ED90, and 95% Confidence Interval for Caspofungin in 4 Day Target Organ Kidney Assay (TOKA)[1]

Route of Administration	ED50 (95% C.I.)[2]	ED90 (95% C.I.)[2]
I.P.	0.017 (0.008, 0.038)	0.041 (0.020, 0.084)
S.C.	0.014 (0.004, 0.046)	0.042 (0.014, 0.125)
I.M.	0.028 (0.011, 0.071)	0.088 (0.037, 0.212)
I.V.	0.015 (0.006, 0.036)	0.033 (0.015, 0.074)
P.O.	2.227[3] (0.273, 18.167)	8.012 (1.822, 39.584)

Experiment 4 Day TOKA 95-12 (11-Sep-1995), Notebook to View 14 (TOKA), pp. 39-41.

1. DBA/2N mice were infected I.V. with 7.2 x 10^6 CFU *C. albicans* MY1055 per mouse. Caspofungin was administered b.i.d. x 2 days (4 total doses). Mice received first treatment (route specified above) within 15 to 30 minutes after challenge. Mice were treated with caspofungin, b.i.d. for a total of 2 days either: 1) I.P. or I.V. as fourfold titrated doses of 0.005 to 0.375 mg/kg/dose, 2) S.C. or I.M. at fourfold titrated doses of 0.005 to 1.5 mg/kg/dose, or 3) P.O. as twofold titrated doses of 1.56 to 50.0 mg/kg/dose. For each route of administration, the sham-treated control was sterile distilled water.
2. Effective dose 50% and 95% (ED50 and ED90) values in mg/kg/dose and 95% confidence intervals ( ) were estimated by comparison of mean log10 CFU/g at day 4 after challenge for paired kidneys of treated groups to sham-treated controls by inverse regression analysis to determine which dose reduced organ counts 50 and 90%, respectively (5 mice/group).
3. Some of the values in this confidence interval lie outside of the range of doses tested. Since the ED values are found on linear regression these values are extrapolated under the assumption that the studied habit may in the tested dose range.

In another study (Merck report, 1995, reference # 30), C'5 deficient DBA/2N mice were infected with *C. albicans* (strain MY1055; 4.8 - 7.4 x 10^4 cfu) by the intravenous route. Treatment with MK-0991 was initiated within 30 minutes of infection and given for 1, 2 or 3 days, b.i.d. by either intraperitoneal or oral routes. Animals were sacrificed 4 days after infection and kidneys processed for measurement of fungal burden. The results in Table 45 indicate that administration of MK-0991 at doses ≥ 0.375 mg/kg b.i.d. for 3 days by the intraperitoneal route was effective in reducing the fungal burden. By the oral route, MK-0991 was effective at a much higher dose (30 mg/kg b.i.d.). Fungal burdens in the sham treated animals after 1 or 2 days of treatment were not measured.

Table 44

Efficacy of Caspofungin Compared to Amphotericin B for Reducing *Candida* Tissue Loads in Kidneys, Liver, Spleen and Brain

Mean log_{10} CFU/g tissue (% Clearance)
[percent reduction from sham-treated control] and ED_{90} and ED_{99} values at 4 days after challenge[a]

Caspofungin

mg/kg	Kidneys	Liver	Spleen	Brain
0.375	2.23* (100) [99.99]	2.02* (20) [94.50]	2.77* (30) [89.95]	2.18* (100) [99.90]
0.09	2.44* (40) [99.98]	2.23* (0) [90.92]	2.78* (80) [89.81]	2.28* (40) [99.87]
0.02	4.31* (0) [99.15]	2.59* (0) [79.58]	3.07* (0) [79.77]	3.36* (0) [98.42]
0.005	6.90 (0) [0]	3.10 (0) [33.36]	3.67 (0) [19.85]	5.52 (0) [0]
0	6.37 (0)	3.28 (0)	3.77 (0)	5.18 (0)
ED_{90} (CI)	0.012 (0.004, 0.038)	0.099 (0.039, 0.252)	0.187 (0.037, ∞)	0.014 (0.004, 0.045)
ED_{99} (CI)	0.030 (0.011, 0.083)	>0.375	>0.375	0.053 (0.019, 0.144)

Amphotericin B

mg/kg	Kidneys	Liver	Spleen	Brain
0.375	2.56* (40) [99.98]	1.70* (80) [97.35]	2.63* (80) [92.79]	3.05* (0) [99.26]
0.09	3.41* (0) [99.89]	2.28* (0) [89.81]	3.22 (20) [71.56]	2.76* (0) [99.62]
0.02	6.12 (0) [44.54]	2.98 (0) [49.65]	2.99* (20) [83.33]	4.63 (0) [71.42]
0.005	6.72 (0) [0]	3.22 (0) [11.69]	3.52 (0) [44.28]	5.02 (0) [29.85]
0	6.37 (0)	3.28 (0)	3.77 (0)	5.18 (0)
ED_{90} (CI)	0.023 (0.011, 0.046)	0.090 (0.041, 0.195)	0.292 (0.025, ∞)	0.024 (0.005, 0.108)
ED_{99} (CI)	0.058 (0.030, 0.115)	>0.375	>0.375	0.153 (0.042, ∞)

Experiment 4 Day TOKA 97-24 (20-Nov-1997), Notebook In Vivo 17 (TOKA), pp. 78-81.

a. DBA/2N mice were challenged I.V. with *Candida albicans* MY1055 at 6.2 x 10^4 CFU/mouse. Organs aseptically collected at day 4 after challenge. Mice were treated I.P. and received 4 total doses (b.i.d. for 2 days). Sham-treated control mice received sterile distilled water.
* Significant from sham treated control (p <0.05; Excel t-Test).
∞ stands for infinitely large.

Table 45

Experiment 4 Day TOKA 94-10: Efficacy of L-743,872 administered either I.P. or P.O. b.i.d. for One, Two or Three Days Following I.V. Challenge with *Candida albicans* MY1055[1]

Treatment	Mean log_{10} CFU/g Kidney and Percent Kidney Sterilization () at Each Dose (mg/kg/dose)[2]: 2 Total Doses	4 Total Doses	6 Total Doses
I.P. Sham	NT	NT	6.34 (0)
L-743,872 1.5 mg/kg/dose I.P., b.i.d.	2.15 (100)	2.19 (100)	2.15 (100)
L-743,872 0.375 mg/kg/dose I.P., b.i.d.	2.22 (100)	2.17 (100)	2.13 (100)
L-743,872 0.09 mg/kg/dose I.P., b.i.d.	4.05 (0)	3.14 (0)	2.62 (33)
P.O. Sham	NT	NT	6.96 (0)
L-743,872 30.0 mg/kg/dose P.O., b.i.d.	5.04 (0)	2.58 (67)	2.22 (33)
L-743,872 7.5 mg/kg/dose P.O., b.i.d.	5.62 (0)	5.66 (0)	6.50 (0)
L-743,872 1.88 mg/kg/dose P.O., b.i.d.	6.54 (0)	4.86 [illegible] (50)	6.73 (0)

Notebook: Experiment TOKA 94-10 [illegible], Notebook In Vivo 11 (TOKA), pp. [illegible]

1. DBA/2N mice were infected I.V. with 4.8 x 10^4 CFU *C. albicans* MY1055 per mouse. L-743,872 was administered I.P. or P.O. b.i.d. as noted above. Mice received first treatment within 15 to 30 minutes after challenge and were treated for a total of 1 day (2 doses), 2 days (4 doses), or 3 days (6 doses).
2. Mean log_{10} CFU/g at 4 days after challenge for paired kidneys (3 mice/group unless noted [illegible]). Percent sterilization indicates number of mice with no detectable yeast where limit of detection was 50 yeast cells per pair of kidneys.
3. NT indicates not tested

A single dose of MK-0991 (≥ 0.023 mg/kg) administered by the intraperitoneal route, 30 minutes or 24 hours post-infection, was also effective in reducing the fungal burden (Table 46, Merck report, 1995, reference # 15).

Table 46

Statistical Summary: Single Dose Therapy with L-743,872 and Amphotericin B (AMB) Following I.V. Induction of a Disseminated *Candida albicans* MY1055 Infection in DBA/2N Mice[1]

Mean log_{10} CFU/g Kidney, Percent Kidney Sterilization () and Percent Change From Control for Single Dose (mg/kg)[2]

Treatment	Sham	0.01	0.023	0.046	0.09	0.18	0.375	0.75	1.5	3.0	Pooled Std Dev.
L-743,872 Single I.P. Dose	6.42 (0)	4.67* (0) -98.23	3.31* (0) -97.73	3.49* (0) -99.81	[illegible]	[illegible]	[illegible]	[illegible]	2.13* (100) [illegible]	2.17* (100) [illegible]	0.41
L-743,872 Single I.P. Dose Delayed	6.42 (0)	4.79 (0) >100	3.22* (0) -97.66	3.43* (0) -99.59	4.18* (0) [illegible]	3.60* (0) -99.83	2.39* (40) [illegible]	2.32* (100) -99.95	[illegible]	2.30* (100) [illegible]	0.45
Amphotericin B Single I.P. Dose	6.42 (0)	6.13 (0) -48.94	6.21 (0) -41.85	5.75 (0) -78.72	4.60* (0) [illegible]	4.77* (0) [illegible]	[illegible]	3.37* (0) -99.91	2.92* (20) [illegible]	3.06* (0) [illegible]	0.81
Amphotericin B Single I.P. Dose Delayed	6.42 (0)	5.17 (0) -94.39	6.38 (0) -25.20	5.59 (0) -33.72	5.81 (0) -96.11	4.77* (0) [illegible]	1.89* (0) [illegible]	2.91* (20) [illegible]	1.37* (40) -99.99	1.30* (20) -99.99	0.28

Experiment 7 Day TOKA 94-5 (23 Feb 1994), Notebook In Vivo 12 (TOKA) pp. 85-98.

1. DBA/2N mice were infected I.V. with 3.4 x 10^4 CFU *C. albicans* MY1055 per mouse. L-743,872 and AMB were administered as a single I.P. dose. Mice received first treatment either within 15 to 30 minutes after challenge or 24 hours after challenge for delayed therapy.
2. Mean log_{10} CFU/g at 7 days after challenge for paired kidneys (5 mice/group). Percent sterilization indicates number of mice with no detectable yeast where limit of detection was 50 yeast cells per pair of kidneys.
* Denotes that the mean was statistically significantly less than the sham control mean at α = 0.05.

The effect of discontinuation of treatment with MK-0991, for over 3 weeks, on mycological burden was measured in another study (Merck report, 1995, reference # 7). C'5 deficient mice were infected with *C. albicans* (7.4×10^4 cfu) by the intravenous route, and different doses of MK-0991 administered, b.i.d. for 4 days, starting immediately after infection. Mice were sacrificed at different time points after challenge and kidneys processed for measuring microbial burden. The results in Figure 13 and Table 47 indicate that MK-0991 at doses ≥0.09 mg/kg b.i.d. for 4 days was effective in reducing the fungal burden in a majority of the animals. This effect was maintained for up to a period of 28 days after infection (i.e., 24 days after discontinuation of therapy). On days 35 and 42 post-infection, the fungal burden was also reduced (Table 47).

Figure 13

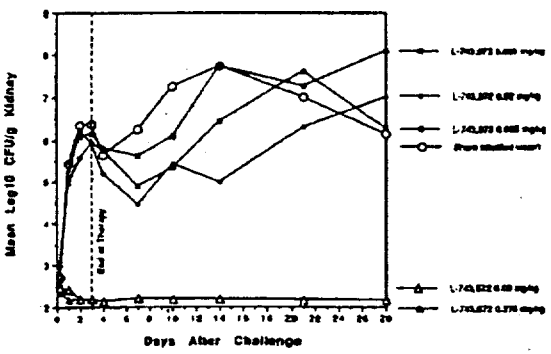


Table 47

Efficacy of L-743,872 at Time Points Following I.V. Induction of a Disseminated *Candida albicans* MY1055 Infection in DBA/2N Mice[1].

Mean Log10 CFU/g Kidney, Percent Sterilization (-) and Percent Change from Sham Mean at Each Dose (mg/kg/dose)[2]

Day after Challenge	Sham	0.001	0.005	0.02	0.09	0.375	Pooled Std Dev.
0.25	2.70 (0)	2.72 (20) 6.66	2.77 (0) 18.58	2.97 (0) 68.80	2.46 (0) -41.66	2.16 (-40) -54.29	0.30
1	5.44 (0)	5.31 (0) -7.33	5.07* (0) -58.12	4.93* (0) -99.24	2.11* (20) -99.91	2.17* (40) -99.95	0.23
2	6.33 (0)	6.08 (0) -42.72	6.23 (0) -20.57	5.37* (0) -82.70	2.19* (100) -99.99	2.18* (100) -99.99	0.28
3	6.37 (0)	6.15 (0) -39.19	5.92* (0) -64.19	5.91* (0) -65.33	2.19* (100) -99.99	2.17* (100) -99.99	0.25
4	5.64 (0)	5.50 (0) -42.56	5.75 (0) -27.06	5.19 (0) -64.68	2.14* (100) -99.97	2.15* (100) -99.97	0.61
7	6.24 (0)	5.82 (0) -75.90	4.89* (0) -95.45	4.25* (0) -98.36	2.21* (100) -99.99	2.21* (100) -99.99	0.98
10	7.24 (0)	6.09 (0) -92.99	5.54 (0) -98.73	5.42 (0) -98.89	2.19* (100) -99.99	2.23* (100) -99.99	1.52
14	7.72 (0)	7.72 (0) 1.66	6.44 (0) -94.70	4.79* (0) -99.81	2.15* (100) -99.99	2.18* (100) -99.99	1.19
21	6.99 (0)	7.25 (0) 84.50	7.61 (0) >100	6.25 (0) -70.77	2.16* (100) -99.99	2.19* (100) -99.99	1.35
28	6.12 (0)	6.10 (0) >100	6.28 (40) 42.89	5.00 (0) >100	2.19* (100) -99.99	2.16* (100) -99.99	1.60
35	8.23 (1)	8.12 (0)	All Dead[3]	4.53 (0)	2.17* (100)	2.16 (100)	NE[4]
42	All Dead[3]	All Dead[3]	All Dead[3]	3.71 (0)	2.15* (100)	2.22 (4) (100)	NE[4]

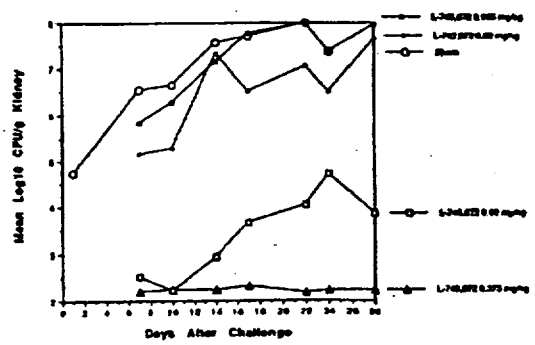
Statistics: Dunnett's Test, TOXA RT-10 (27 Sept. 1995), Statistical Services Group, TOXAL pg. 44-49.

1. DBA/2N mice were infected I.V. with 7.4×10^4 CFU *C. albicans* MY1055 per mouse. L-743,872 was administered I.P., b.i.d. Mice received first treatment within 15 to 30 minutes after challenge and were treated for a total of 4 days (8 doses).
2. Mean log10 CFU/g at time points after challenge for paired kidneys (5 mice/group unless noted ( )). Percent sterilization indicates number of mice with no detectable yeast above limit of detection (50 yeast cells per pair of kidneys).
3. Indicates that values were not presented because no mice in the sample group survived to that time point.
4. NE indicates that statistical analysis were not presented due to an insufficient number of mice surviving in the sham-treated controls.
* Denotes that the mean was statistically significantly less than the sham control mean at $\alpha \leq 0.05$.

Effect of initiating treatment 24 hours post-infection was tested in the same animal model as described above except that MK-0991 was administered once a day for 14 days (Merck report, 1995, reference # 14). Animals were sacrificed at different time points and kidneys processed for measurement of fungal burden. MK-0991 at doses ≥ 0.09 mg/kg were effective in reducing the fungal burden and sterilized the kidneys (Table 48). FCZ was not as effective in sterilization of the kidneys although the fungal burden was reduced compared to the control (Table 49). MK-0991 was most effective at a dose of 0.375 mg/kg and fungal burden remained reduced even 14 days after discontinuation of therapy (Figure 14).

Figure 14

A.



B.

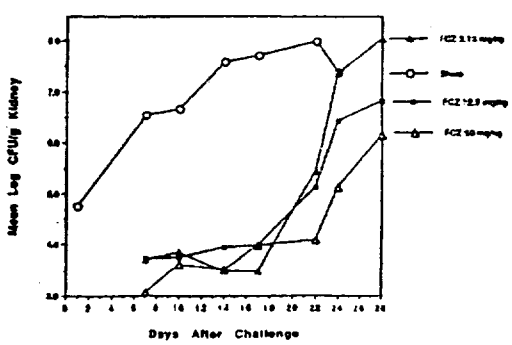


Table 48

Efficacy of Delayed, Prolonged, Once per Day Therapy with L-743,872 at Time Points Following I.V. Induction of a Disseminated *Candida albicans* MY1055 Infection in DBA/2N Mice[1]

Day after Challenge	Sham	0.005	0.02	0.09	0.375	Pooled Std Dev.
	Mean Log_{10} CFU/g Kidney, Percent Kidney Sterilizations ( ) and Percent Change from Sham Mean at Each Dose (mg/kg/dose)[2]					
7	6.54 (0)	5.83 (0) -80.23	5.15* (0) -95.89	2.32* (40) -99.99	2.19* (100) -99.99	0.67
10	6.64 (0)	6.29 (0) -54.71	5.27 [4] (0) -95.67	2.22* (60) -99.99	2.25* (100) -99.99	1.05
14	7.56 (0)	7.14 [4] (0) -62.42	7.32 (0) -42.19	2.94* (20) -99.99	2.23* (100) -99.99	0.70
17	7.70 (0)	7.76 (0) 13.76	6.50* (0) -93.81	3.65* (40) -99.99	2.32* (100) -99.99	0.77
22	7.99 (0)	8.01 (0) 3.27	7.05 (0) -89.04	4.03* (60) -99.99	2.17* (100) -99.99	1.24
24	7.37 (0)	7.36 (0) -1.83	6.49 (0) -86.94	4.72* (20) -99.78	2.22* (100) -99.99	1.68
28	All Dead[4]	7.97 [3] (0)	7.63 (0)	3.66 (40)	2.25 (80)	NE[3]

Notebook: Experiment Foil TOKA 94-4 (31 Jan. 1994), Notebook In Vivo 12 (TOKA), pp. 80-82A.

1. DBA/2N mice were infected I.V. with 4.6×10^5 CFU *C. albicans* MY1055 per mouse. L-743,872 was administered I.P., q.d. Mice received first treatment 24 hours after challenge and were treated for a total of 10 days (14 doses).
2. Mean log_{10} CFU/g at time points after challenge for pooled kidneys (5 mice/group unless noted [ ]). Percent sterilization indicates number of mice with no detectable yeast where the limit of detection was 50 yeast cells per pair of kidneys.
3. NE indicates that statistical analysis were not performed due to an insufficient number of mice surviving in the sham-treated controls.
4. Indicates that values were not performed because no mice in the sample survived to this time point.
* Denotes that the mean was statistically significantly less than the sham control mean at $\alpha \leq 0.05$.

Mean Log_{10} CFU/g Kidneys at Time Points Following I.V. Challenge with *C. albicans* MY1055 and Therapy with L-743,872 I.P., q.d. for 14 days

Table 49

Efficacy of Delayed, Prolonged, Once per Day Therapy with FCZ at Time Points Following I.V. Induction of a Disseminated *Candida albicans* MY1055 Infection in DBA/2N Mice[1]

Day after Challenge	Sham	3.13	12.5	50.0	Pooled Std Dev.
	Mean Log_{10} CFU/g Kidney, Percent Kidney Sterilizations ( ) and Percent Change from Sham Mean at Each Dose (mg/kg/dose)[2]				
7	6.54 (0)	3.71* (0) -99.85	3.74* (0) -99.84	3.08* (0) -99.96	0.38
10	6.64 (0)	3.85* (0) -99.83	3.76* (0) -99.87	3.62* (0) -99.90	0.95
14	7.56 (0)	3.89* (0) -99.99	3.94* (0) -99.98	3.52* (0) -99.98	0.57
17	7.70 (0)	3.48* (0) -99.99	3.98* (0) -99.98	3.97* (0) -99.98	0.56
22	7.99 (0)	5.44* (0) -79.72	5.12* (0) -99.87	4.09* (50) -99.98	0.90
24	7.37 (0)	7.35 (0) -5.81	6.43 (0) -88.52	5.13* (0) -99.42	0.81
28	All Dead[4]	8.04 (0)	6.81 (0)	6.15 (0)	NE[3]

Notebook: Experiment Foil TOKA 94-4 (31 Jan. 1994), Notebook In Vivo 12 (TOKA), pp. 80-82A.

1. DBA/2N mice were infected I.V. with 4.6×10^5 CFU *C. albicans* MY1055 per mouse. FCZ was administered I.P., q.d. Mice received first treatment 24 hours after challenge and were treated for a total of 14 days (14 doses).
2. Mean log_{10} CFU/g at time points after challenge for pooled kidneys (5 mice/group unless noted [ ]). Percent sterilization indicates number of mice with no detectable yeast where the limit of detection was 50 yeast cells per pair of kidneys.
3. NE indicates that statistical analysis were not performed due to an insufficient number of mice surviving in the sham-treated controls.
4. Indicates that values were not performed because no mice in the sample survived to this time point.
* Denotes that the mean was statistically significantly less than the sham control mean at $\alpha \leq 0.05$.

Mean Log_{10} CFU/g Kidneys at Time Points Following I.V. Challenge with *C. albicans* MY1055 and Therapy with FCZ I.P., q.d. for 14 days

The activity of MK-0991 against different species of *Candida* was measured in a C'5 deficient animal model and was compared with ampB and FCZ (Merck report, 1995, reference # 12; Abruzzo *et al.*, 1997, Antimicrob Agents Chemother 41: 2333, reference # 2). The drugs were administered intravenously within 30 minutes of infection, for 4 days. The concentration of inoculum used for infection varied for each species (Table 50). Animals were sacrificed 3 days after discontinuation of treatment. The results in Table 51 indicate that MK-0991 was effective in reducing the fungal burden in the kidneys from mice infected with various *Candida* species, however, the magnitude of reduction in mycological burden varied with the dose. AmpB was effective against different *Candida* species at doses ≥ 0.09 mg/kg (Table 52) although activity against *C. krusei* was not measured. FCZ, on the other hand, was effective against *C. albicans* (at doses ≥ 5.53 mg/kg in reducing cfu but not sterilization) but was not active against *C. krusei* or *C. tropicalis* (Table 53). Activity against other *Candida* species was not measured. It is also of note that FCZ did not cause any sterilization of the kidneys whereas MK-0991 and ampB did at certain doses (Table 53). Due to the variability of responses it is difficult to conclude whether ampB is superior to MK-0991. The residual mycological burden in tissues other than kidneys was not measured.

Higher concentrations of the inoculum of *C. glabrata*, *C. lusitaniae*, *C. parapsilosis* and *C. krusei* were required to achieve acceptable kidney colonization as compared to *C. albicans* and *C. tropicalis*. This could be due to the fact that these organisms are not virulent and lethal in mice. The sponsor has stated that ED_{99} values may be high because of the higher concentration of the inoculum used for infection.

Table 50

Species	MIC (ug/ml)	IV* infectious dose (cfu x 10^4)
C. albicans MY1750	-	4.0×10^4
C. albicans MY1585	0.25	1.68×10^4
C. albicans CLY538	0.25	1.0×10^5
C. tropicalis MY1124	0.25	5.2×10^5
C. tropicalis MY1163	0.25	1.3×10^5
C. tropicalis CLY545	0.125	3.6×10^5
C. parapsilosis MY1943	1.0	1.2×10^7
C. lusitaniae MY1396	0.5	1.32×10^7
C. glabrata MY1381	0.25	1.36×10^8
C. glabrata MY1382	0.25	1.48×10^8
C. krusei CK4935	1.0	8.6×10^7

* IV= intravenous route

Table 51

In vivo antifungal efficacy of L-743,872 against disseminated *Candida* infections (TOKA) in DBA/2N mice[1]

Species/ Strain	Mean Log10 CFU/gm Kidney and Percent Kidney Sterilization ( ) at Each Dose (mg/kg/dose)[2] Sham	1.5	0.375	0.09	0.02	0.005	ED99[3] (mg/kg)	ED90[3] (mg/kg)
C. albicans MY1750 (B311)	6.82 (0)	2.13* (100)	2.18* (100)	3.81* (20)	6.53 (0)	6.79 (3) (0)	0.05 (0.03-0.1)	0.02 (0.01-0.03)
C. albicans MY1585	5.88 (1) (0)	2.22 (100)	2.17 (80)	2.21 (100)	5.75 (0)	4.67 (1) (0)	NE[5]	NE[5]
C. albicans CLY538	7.27 (0)	NT[4]	2.17* (100)	2.19* (100)	4.35* (0)	5.34* (0)	0.006 (0.003-0.009)	0.003 (0.001-0.004)
C. tropicalis MY1124	6.16 (0)	2.19* (60)	2.26* (60)	5.51* (0)	5.97 (0)	6.56 (0)	0.12 (0.09-0.18)	0.055 (0.04-0.08)
C. tropicalis MY1163	5.80 (0)	2.29* (90)	2.25* (100)	4.42* (0)	5.68 (0)	5.99 (0)	0.10 (0.07-0.18)	0.03 (0.02-0.05)
C. tropicalis CLY545 FCZ[6]	6.29 (0)	NT[4]	2.19* (100)	5.02* (0)	5.73* (0)	6.66 (0)	0.30 (0.1-1.5)	0.05 (0.03-0.10)
C. glabrata MY1381	5.65 (0)	3.39* (0)	3.64* (0)	4.83 (0)	5.39 (0)	5.24 (0)	0.82 (0.3-6.2)	0.06 (0.01-0.14)
C. glabrata MY1382	5.48 (0)	3.29* (0)	2.51* (0)	3.70* (0)	5.15 (0)	5.66 (0)	0.12 (0.09-0.16)	0.03 (0.025-0.04)
C. lusitaniae MY1396	5.19 (0)	2.39* (20)	3.89* (0)	4.78 (0)	5.35 (0)	5.27 (0)	0.70 (0.4-1.5)	0.16 (0.10-0.24)
C. parapsilosis MY1943	5.17 (0)	3.90* (0)	4.79 (0)	5.09 (0)	5.25 (0)	5.11 (0)	>1.5	1.0 (0.5-4.8)
C. krusei CK4935 FCZ[6]	4.93 (0)	NT[4]	3.98* (0)	4.96 (0)	4.41 (0)	4.83 (0)	NE[5]	NE[5]

Notebook:
MY1750, MY1585, MY1124, MY1943 - Experiment TOKA 94-3 (25 Jan. 94) Notebook In Vivo 12 (TOKA), pp. 72-82.
MY1396, MY1381, MY1382, MY1163 - Experiment TOKA 94-6 (15 Mar. 94) Notebook In Vivo 13 (TOKA), pp. 1-10.
CLY538, CLY545, CK4935 - Experiment TOKA 94-9 (26 April 94) Notebook In Vivo 13 (TOKA), pp. 32-42.

1. DBA/2N mice were infected I.V. L-743,872 was administered I.P., b.i.d. Mice received first treatment within 15 to 30 minutes after challenge and were treated for a total of 4 days (8 doses).
2. Mean $\log_{10}$ CFU/g at day 7 after challenge for paired kidneys (5 mice/group unless noted [ ]). Percent sterilization indicates number of mice with no detectable yeast where limit of detection was 50 yeast cells per pair of kidneys.
3. Effective dose 90% and 99% (ED_{90} and ED_{99}) values in mg/kg/dose and 95% confidence intervals ( ) were estimated by comparison of mean $\log_{10}$ CFU/g at day 7 after challenge for paired kidneys of treated groups to sham-treated controls by inverse regression analysis to determine which doses reduced kidney counts 90 and 99%, respectively.
4. NT = not tested.
5. NE indicates that ED values were not estimated due to the lack of a significant dose-response relationship over the range of doses tested in this assay.
6. Denotes that the mean was statistically significantly less than the sham control mean at a ≤ 0.05.

Table 52

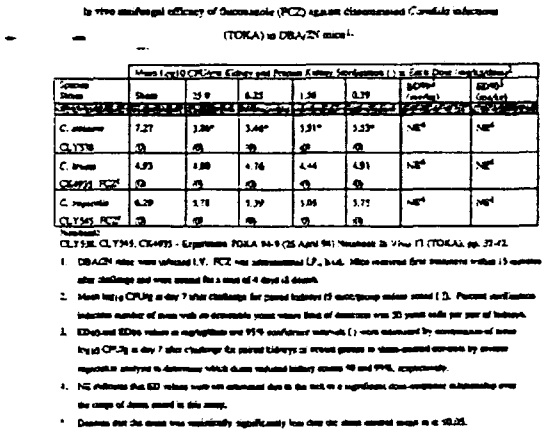
In vivo antifungal efficacy of amphotericin B (AMB) against disseminated *Candida* infections (TOKA) in DBA/2N mice[1].

Species/ Strain	Mean Log10 CFU/gm Kidney and Percent Kidney Sterilization ( ) at Each Dose (mg/kg/dose)[2] Sham	1.5	0.375	0.09	0.02	ED99[3] (mg/kg)	ED90[3] (mg/kg)
C. albicans MY1750 (B311)	6.82 (0)	2.68* (20)	3.85* (60)	3.34* (20)	6.79 (0)	0.08 (0.04-0.08)	0.03 (0.01-0.04)
C. albicans MY1585	5.88 (1) (0)	2.19 (80)	2.39 (60)	2.20 (60)	6.08 (1) (0)	NE[5]	NE[5]
C. tropicalis MY1124	6.16 (0)	2.22* (60)	3.77* (0)	6.60 (0)	6.20 (0)	0.38 (0.22-0.57)	0.20 (0.12-0.28)
C. tropicalis MY1163	5.80 (0)	2.92* (0)	3.77* (0)	6.15 (0)	6.03 (0)	0.56 (0.46-0.71)	0.24 (0.19-0.29)
C. glabrata MY1381	5.65 (0)	2.61* (20)	4.72* (0)	5.58 (0)	5.58 (0)	0.74 (0.46-1.57)	0.17 (0.10-0.25)
C. glabrata MY1382	5.48 (0)	2.17* (100)	2.14* (80)	4.84* (0)	4.96 (0)	0.15 (0.10-0.37)	0.05 (0.03-0.07)
C. parapsilosis MY1943	5.17 (0)	2.56* (20)	2.47* (60)	4.60* (0)	4.93 (0)	0.19 (0.14-0.26)	0.06 (0.04-0.07)
C. lusitaniae MY1396	5.19 (0)	2.77* (0)	2.56* (0)	5.07 (0)	5.00 (0)	0.28 (0.2-0.5)	0.11 (0.09-0.16)

Notebook:
MY1750, MY1585, MY1124, MY1943 - Experiment TOKA 94-3 (25 Jan. 94) Notebook In Vivo 12 (TOKA), pp. 72-82.
MY1396, MY1381, MY1382, MY1163 - Experiment TOKA 94-6 (15 Mar. 94) Notebook In Vivo 13 (TOKA), pp. 1-10.

1. DBA/2N mice were infected I.V. AMB was administered I.P., b.i.d. Mice received first treatment within 15 to 30 minutes after challenge and were treated for a total of 4 days (8 doses).
2. Mean $\log_{10}$ CFU/g at day 7 after challenge for paired kidneys (5 mice/group unless noted [ ]). Percent sterilization indicates number of mice with no detectable yeast where limit of detection was 50 yeast cells per pair of kidneys.
3. Effective dose 90% and 99% (ED_{90} and ED_{99}) values in mg/kg/dose and 95% confidence intervals ( ) were estimated by comparison of mean $\log_{10}$ CFU/g at day 7 after challenge for paired kidneys of treated groups to sham-treated controls by inverse regression analysis to determine which doses reduced kidney counts 90 and 99%, respectively.
4. NE indicates that ED values were not estimated due to the lack of a significant dose-response relationship over the range of doses tested in this assay.
5. Denotes that the mean was statistically significantly less than the sham control mean at a ≤ 0.05.

Table 53



(ii) **Effect on survival**

MK-0991 was shown to improve the survival of immunocompetent CD-1 and DBA/2N C'5 deficient mice infected with 9.4 x 10^5 cfu of *C. albicans* (Merck report, reference # 27; Abruzzo *et al.*, 1997, Antimicrob Agents Chemother 41: 2333, reference # 2). This concentration of the inoculum was higher than that used in the studies described above and was shown to cause mortality in both immunocompetent and C'5 deficient mice, within 24 hours of infection. Treatment was initiated 15 - 30 minutes after infection for 4 days, by the intraperitoneal or oral routes. MK-0991 was effective in improving the survival of infected animals. The ED_{50} values on days 7, 14 and 21 after initiation of infection show MK-0991 administered intraperitoneally to be effective at a lower dose compared to ampB (Table 54). Microbial burden was not measured in this study.

(b) Studies in agranulocytopenic and pancytopenic mice

(i) **Disseminated candidiasis:**

Immunocompetent and agranulocytopenic CD-1 mice were infected with 10^7 and 10^5 cfu, respectively, of *C. albicans*, strain MY 1055 (Merck report, 1995, reference # 17). Agranulocytopenia was induced 24 hours prior to infection by injection of a monoclonal antibody (RB6-8C5) specific for GR-1 receptor, every other day for 10 days by the intraperitoneal route. Such a regimen was shown to maintain depletion of granulocytes. Treatment with MK-0991, FCZ or ampB, b.i.d., for 4 days, was initiated immediately after infection by the intraperitoneal route,. The results in Table 55 show lower MK-0991 ED_{50} values compared to ampB or FCZ. A higher dose of MK-0991 (1.5 mg/kg) was effective in conferring 100% protection in neutropenic mice as compared to a 0.375 mg/kg dose in immunocompetent animals (Figures 15A and 15B). At the doses tested, survival rate was better in neutropenic mice treated with MK-0991 as compared to ampB or FCZ (Figures 15). Microbial burden was not measured.

Table 54

Effective dose 50% (ED_{50}) values and 95% confidence intervals for L-743,872, amphotericin B (AMB), and fluconazole (FCZ) against a disseminated *Candida albicans* MY 1055 infection[1] in C5 deficient DBA/2N and normal outbred CD-1 mice

Treatment[2] (Route)	7 Day ED_{50} (mg/kg/dose) DBA/2N	7 Day ED_{50} (mg/kg/dose) CD-1	14 Day ED_{50} (mg/kg/dose) DBA/2N	14 Day ED_{50} (mg/kg/dose) CD-1	21 Day ED_{50} (mg/kg/dose) DBA/2N	21 Day ED_{50} (mg/kg/dose) CD-1
L-743,872 (I.P.)	0.04 (NE[3])	0.07 (0.04, 0.12)	0.04 (NE[3])	0.08 (0.04, 0.17)	0.04 (NE[3])	0.10 (0.06, 0.17)
L-743,872 (P.O.)	14.80 (8.5, 25.8)	42.70 (21.7, 84.2)	14.80 (8.5, 25.8)	42.70 (21.7, 84.2)	14.80 (8.5, 25.8)	42.70 (21.7, 84.2)
AMB (I.P.)	0.30 (0.14, 0.54)	0.30 (0.14, 0.54)	0.30 (0.14, 0.54)	0.30 (0.14, 0.54)	0.30 (0.14, 0.54)	0.30 (0.14, 0.54)
FCZ (P.O.)	0.52 (0.23, 1.32)	0.24 (0.15, 0.45)	1.03 (0.61, 1.84)	1.11 (0.42, 3.36)	1.03 (0.61, 1.84)	9.56 (2.67, 35.9)

Notebook: Experiment ID$_{50}$ CA 93-6 (13 April 1993), Notebook In Vivo 11, pp. 70-73

1. DBA/2N mice were infected I.V. with 5.4×10^5 cells/mouse *C. albicans* MY 1055. CD-1 mice were infected I.V. with 9.4×10^6 cells/mouse *C. albicans* MY 1055.
2. Mice treated b.i.d. for 4 days. Mice received first treatment within 15 to 30 minutes after infection. Five mice per treatment group. ED_{50} values were estimated at days 7, 14, and 21 after challenge using the method of Random Curtis.
3. 95% confidence interval was unattainable.

Table 55: Antifungal activity of L743,872, amphotericin B (AMB), and FCZ against a disseminated C. albicans MY10555 infection[1] in normal outbred and MO-Ab-induced granulocytopenic CD-1 mice

Treatment[3]	14 Day ED_{50} (mg/kg)[4] Normal	14 Day ED_{50} (mg/kg)[4] Neutropenic	21 Day ED_{50} (mg/kg)[4] Normal	21 Day ED_{50} (mg/kg)[4] Neutropenic
L-743,872	0.04	0.12	0.07	0.12
AMB	0.19	0.42	0.19	0.42
FCZ	1.19	2.68	46.04	NC

Notebook: J.G. Smith, In vivo 13 (ED50), ED50 CA94-2, Notebook # 12257, p. 155, 6Sept94.
[4] Calculated by the ED50.BAS Apl program based on method of Knudsen and Curtis.
NC = not calculable.

1. Non granulocytopenic CD-1 mice infected I.V. with 1.1×10^7 cells/mouse (~3.4 x 7 day LD50 or ~25.6 x 14 day LD50) *C. albicans* MY 1055.
   MoAb-induced granulocytopenic CD-1 mice infected I.V. with 1.1×10^5 cells/mouse (~13.4 x 7 day LD50 or 11.6 x 14 day LD50) *C. albicans* MY 1055.
2. CD-1 mice were made granulocytopenic by the I.P. administration of 500 mg RB6-8C5 monoclonal antibody (specific for Gr-1 epitope on mouse granulocytes) one day prior to infection and then 250 mg RB6-8C5 every two days after challenge through day 10.
3. Mice treated I.P., b.i.d. x 4 days. Mice received first treatment within 15 minutes post infection. Ten mice per treatment group.

In another experiment (Merck report, 1999, study 3, reference # 16), immunocompetent and neutropenfc [induced by administering a monoclonal antibody, IgG2b (RB6-8C5) intraperitoneally on day 1 prior to and 2 days post challenge] CD-1 mice were infected with *C. albicans* (strain MY1055, 1.28×10^4 cfu/mouse) by the intravenous route. Treatment with MK-0991 was administered immediately after challenge. AmpB and FCZ, also administered intraperitoneally, were used as comparators. All drugs were administered q.d. for 4 days. The activity of the drugs was measured by reduction in cfu in the kidneys on day 4. The results in Table 56 show MK-0991 to be effective in reducing mycological burden in both immunocompetent and neutropenic mice. FCZ was effective at a higher dose. Neutrophil cell counts were shown to be low (Table 57).

Table 56

Efficacy of Caspofungin, AmB and FCZ Against a Disseminated *C. albicans* [a] Infection in Normal and Monoclonal Antibody Induced-Granulocytopenic[a] CD

Normal (non-granulocytopenic) CD-1 mice

Mean $\log_{10}$ CFU/g kidneys (% Sterilization)[c] and ED_{90} and ED_{99} values[d] at 4 days after challenge.				
Dose[e] (mg/kg)	Caspofungin	AmB	Dose (mg/kg)	FCZ
0.375	2.09* (80)	2.93* (0)	25.0	3.45* (0)
0.09	2.57* (20)	3.94 (0)	6.25	3.36* (0)
0.02	4.20 (0)	4.59 (0)	1.56	3.78 (20)
0.005	5.43 (0)	4.52 (0)	0.39	4.16 (0)
0	4.48 (0)	4.48 (0)	0	4.48 (0)
ED_{90} (95% CI)	0.048 (0.018, 0.128)[f]	0.168 (0.097, 0.599)	ED_{90} (95% CI)	9.635 NC[g]
ED_{99} (95% CI)	0.166 (0.066, 0.429)	>0.375 NC	ED_{99} (95% CI)	>25.0 NC

Granulocytopenic CD-1 mice

Mean $\log_{10}$ CFU/g kidneys (% Sterilization)[b] and ED_{90} and ED_{99} values[c] at 4 days after challenge.				
Dose (mg/kg)	Caspofungin	AmB	Dose (mg/kg)	FCZ
1.5	2.12* (100)	2.13* (80)	100.0	2.17* (20)
0.375	2.13* (100)	2.34* (60)	25.0	2.24* (40)
0.09	4.76* (0)	5.28 (0)	6.25	3.03* (20)
0.02	5.59 (0)	5.72 (0)	1.56	4.42* (0)
0	5.82 (0)	5.82 (0)	0	5.82 (0)
ED_{90} (95% CI)	0.049 (0.021, 0.114)	0.066 (0.029, 0.148)	ED_{90} (95% CI)	<1.56 NC
ED_{99} (95% CI)	0.148 (0.070, 0.311)	0.188 (0.090, 0.393)	ED_{99} (95% CI)	2.603 (0.517, 13.1)[f]

TOKA 95-16 (13-Oct-1995) Notebook In Vivo 14 (TOKA), pp. 56.

[a] CD-1 mice were made granulocytopenic by the I.P. administration of 500 mg RB6-8C5 mAb (specific for Gr-1 epitope on mouse granulocytes) one day prior to infection and then 250 µg RB6-8C5 on day 2.
[b] Non-granulocytopenic CD-1 mice infected I.V. with 1.28×10^4 CFU/mouse *C. albicans* MY1055. Granulocytopenic (mAb-induced) CD-1 mice infected I.V. with 1.28×10^4 CFU/mouse *C. albicans*. Mice were treated I.P., q.d. x 4 days and received first treatment immediately following infection. Kidneys harvested and CFU/g quantitated at 4 days after challenge. Five mice per treatment group. Percent sterilization indicated the number of mice with no detectable yeast where the limit of detection was 50 yeast cells per pair of kidneys.
[c] Effective dose 90% and 99% (ED_{90} and ED_{99}) values in mg/kg/dose and 95% confidence intervals ( ) were estimated by comparison of mean $\log_{10}$ CFU/g at day 4 after challenge for paired kidneys of treated groups to sham-treated controls by inverse regression analysis to determine which dose reduced organ counts 90 and 99%, respectively.
[d] Not calculable.
[e] Some of the values in this confidence interval lie outside of the range of dose tested. Since the ED values are based on linear regression these values are extrapolated under the assumption that the model holds outside the tested dose range.
[f] Significant from sham treated control ($p < 0.05$; Excel t-test).

Table 57

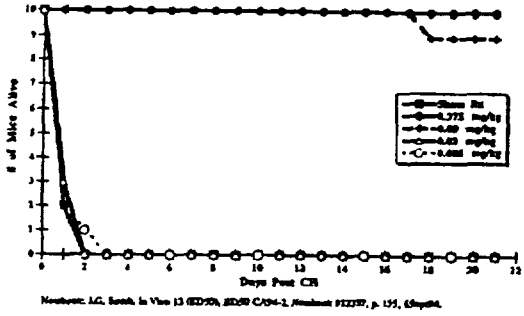
Percent Neutrophils in Peripheral Blood Following I.P. Administration of RB6-8C5 Monoclonal to CD-1 Mice[a]

Time point (day)	Percent neutrophils ± SEM: PBS control	Percent neutrophils ± SEM: RB6-8C5 treated
0	12.53 ± 2.37	0.20 ± 0.05
2	9.67 ± 2.12	0.03 ± 0.03
3	9.97 ± 0.67	0.05 ± 0.03

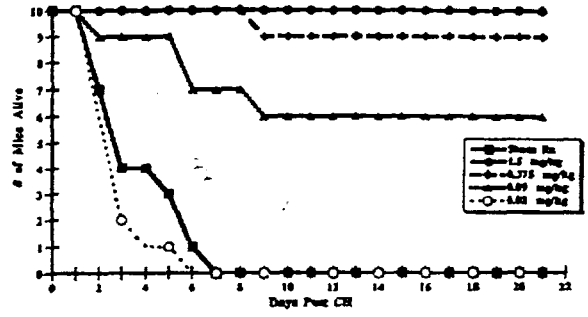
[a] CD-1 mice were made granulocytopenic by the I.P. administration of 500 mg RB6-8C5 mAb (specific for Gr-1 epitope on mouse granulocytes) on day -1 and then 250 µg RB6-8C5 on day 2. Control mice received sterile PBS on the same days. Three mice per sample point.
(RB6AP93-5 (13-Oct-1993) Notebook PMM FACS-2).

Figure 15

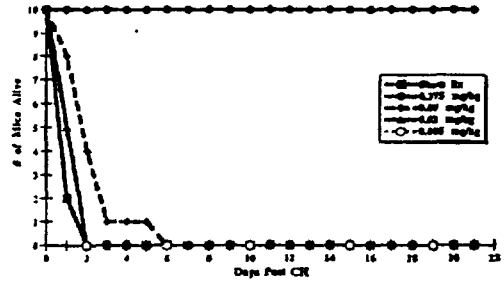
A. Efficacy of L-743,872 in normal CD-1 mice following IV challenge with Candida albicans MY1055



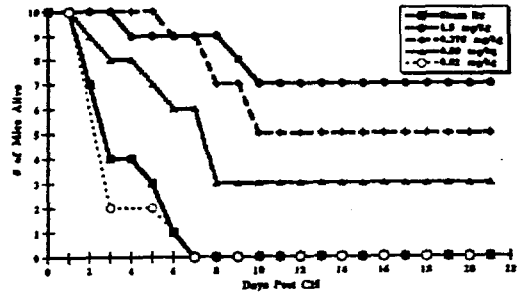
B. Efficacy of L-743,872 in granulocytopenic CD-1 mice following IV challenge with Candida albicans MY1055



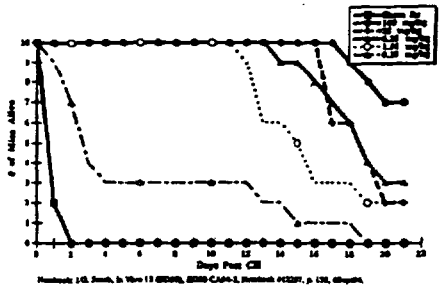
C. Efficacy of Amphotericin B in normal CD-1 mice following IV challenge with Candida albicans MY1055



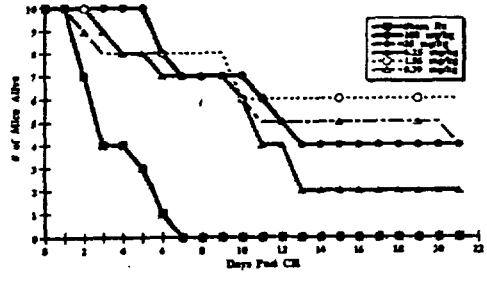
D. Efficacy of Amphotericin B in granulocytopenic CD-1 mice following IV challenge with Candida albicans MY1055



E. Efficacy of Fluconazole in normal CD-1 mice following IV challenge with Candida albicans MY1055



F. Efficacy of Fluconazole in granulocytopenic CD-1 mice following IV challenge with Candida albicans MY1055



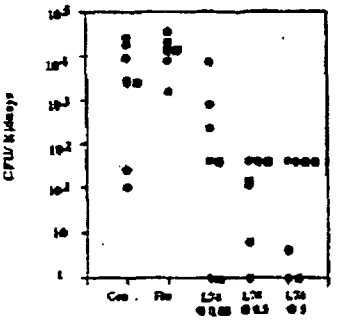
Studies by Graybill *et al.*, 1997 (Antimicrob Agents Chemother 41: 1775) measured the activity of MK-0991 in ICR mice (immunocompetent and neutropenic) infected intravenously with a FCZ resistant strain (FCZ MICs: 32 and > 64 ug/ml at 24 and 48 hours, respectively; MK-0991 MICs: at 24 and 48 hours were ≤ 0.125 ug/ml, at both the timings) of *C. albicans* (isolate # 93-1226: 10^5 cfu/mouse). Treatment with different doses of MK-0991 was initiated intraperitoneally, 24 hours post-infection, for 7 days. FCZ, administered by gavage, was used as a comparator. Mice were followed for survival for up to 30 days post-infection. The results in Table 58 show that MK-0991 was effective in improving survival of immunocompetent and neutropenic mice infected with this FCZ resistant strain of *C. albicans*. Mycological burden was measured in kidneys from a group of mice on day 8. The results in Figure 16 show that MK-0991 at a dose of ≥ 0.5 mg/kg/day was effective in reducing the fungal burden. The authors have stated that true sterilization of tissues was not achieved. FCZ was not effective in improving survival or reducing cfu (Table 58 and Figure 16).

Table 58: Survival of mice after infection and treatment With FCZ or MK-0991

Study no.[a] and group	Dose (mg/kg)	Survival (mean no. of days ± SEM)	P (compared with control)
1			
Control	None	7.3 ± 0.8	
Fluconazole	5 (BID)[b]	8.4 ± 1.3	0.3347
M991	5	31.0 ± 0	0.0001
	2	25.7 ± 3.7	0.0006
	0.5	28.2 ± 2.4	0.0001
2			
Control	None	8.0 ± 0.8	
M991	0.5	28.7 ± 2.3	0.0001
	0.1	27.9 ± 2.5	0.0001
	0.05	22.5 ± 2.0	0.0001
3A			
Control	None	7.5 ± 0.7	
M991	0.05	24.6 ± 2.2	0.0001
	0.025	19.2 ± 2.3	0.0001
	0.0125	16.7 ± 2.4	0.0003
3B			
Control	None	6.5 ± 0.2	
M991	0.05	11.4 ± 2.4	0.0258
	0.025	8.5 ± 0.5	0.1744
	0.0125	9.2 ± 2.6	0.0930

[a] Studies 1, 2, and 3B were performed with neutropenic mice, while study 3A was performed with immunocompetent mice.
[b] BID, twice a day.

Figure 16



L 74 = MK-0991

In another study by Graybill *et al.*, 1997 (Antimicrob Agents Chemother 41: 1937) the activity of MK-0991 was measured in immunocompetent, neutropenic, and/or immunosuppressed ICR mice infected with *C. krusei* (isolates 94-2696 and 94-2501) and *C. glabrata* (isolate 95-1129). The results of the *in vitro* susceptibility shown in Table 59 show that the isolates were sensitive to MK-0991 but not to FCZ. Mice were infected with *C. krusei* (10^8 cfu) or *C. glabrata* (1.4 x 10^8 cfu), a day after induction of neutropenia or immunosuppression. The route of infection was not specified. Administration of MK-0991 or FCZ was initiated immediately after infection for 7 days by the intraperitoneal and oral routes, respectively. Animals were followed for survival and reduction in mycological burden for up to 30 days post-infection. The results in Figure 17 and Table 60 show that MK-0991 was effective in improving survival and reducing mycological burden in the kidneys but not in the spleen of immunosuppressed mice infected with *C. krusei*. The results presented in Figure 17 correspond to study 4 in Table 60. It is of note that the reduction in mycological burden is marginal and varied with the immune status of the host. Although the cell count was not performed in animals it appears that neutropenia or pancytopenia induced would be reversible (for details of the regimens used for inducing neutropenia or pancytopenia see Table 60). Overall, MK-0991 was more effective than FCZ.

Table 59: *In vitro* susceptibility to L-743,872 and fluconazole

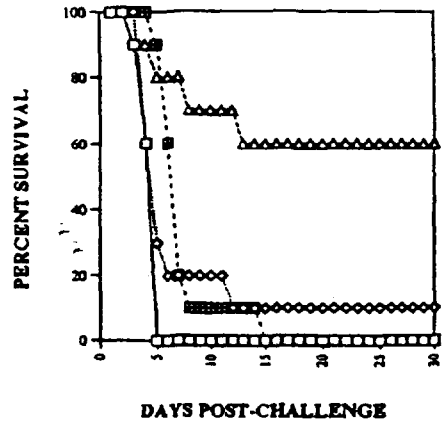
Species or isolate	MIC (μg/ml) at:			
	Fluconazole at:		L-743,872 at:	
	24 h	48 h	24 h	48 h
C. krusei				
94-2696	16	32	0.25	0.25
94-2501	16	64	0.25	0.25
C. glabrata	8	16	0.25	0.25

Table 60:Tissue colony counts of *C. krusei*

Study	Isolate (tissue)	Immune suppression	Inoculum (no. of cells)	No. of mice	Drug (dose [mg/kg])[a]	Log mean CFU	SEM
1	94-2696 (kidney)	None	1.2×10^6	7	None	11.34	0.45
					Flu[b] (5)	9.80	0.29
					L-743,872 (5)	8.13[c]	0.36
					L-743,872 (0.5)	8.07[c]	0.25
2	94-2501 (kidney)	None	9.0×10^6	7	None	8.73	0.63
					Flu (5)	9.79	0.49
					L-743,872 (5)	5.77[c]	0.44
3	94-2696 (kidney)	SFU[d] (150 mg/kg) 24 h prior to infection and SFU (75 mg/kg) on day 5 postinfection	1.4×10^6	7	None	11.41	1.26
					Flu (5)	9.00	1.41
					L-743,872 (5)	7.52[c]	0.98
					L-743,872 (2)	5.35[c]	0.78
					L-743,872 (0.5)	8.49	0.77
4	94-2696 (kidney)	Cortisone (125 mg/kg) 3 consecutive days prior to infection and cyclophosphamide (125 mg/kg) 24 h prior to infection	8.0×10^5	10	None	13.31	0.53
					L-743,872 (5)	10.56[c]	0.49
					L-743,872 (2)	11.36[c]	0.39
					L-743,872 (0.5)	12.75[c]	0.24
5	94-2501 (kidney)	Cortisone (100 mg/kg) 3 consecutive days prior to infection	1.8×10^6	7	None	15.98	1.02
					Flu (5)	15.91	0.69
					L-743,872 (10)	4.99[c]	1.05
					L-743,872 (5)	6.71[c]	0.46
					L-743,872 (2)	10.11[c]	0.74
					L-743,872 (0.5)	15.86	0.76
6	94-2501 (kidney)	None	1.0×10^6	7			
					None	8.92	0.72
					Flu (5)	7.37	0.61
					L-743,872 (5)	5.87[c]	0.37
					L-743,872 (0.5)	5.94	0.49
	94-2501 (spleen)				None	8.83	0.20
					Flu (5)	9.78	0.52
					L-743,872 (5)	10.33	0.63
					L-743,872 (0.5)	9.11	0.26

[a] All fluconazole doses were given twice daily; all L-743,872 doses were given once daily.
[b] Flu, fluconazole.
[c] Significantly different ($P < 0.01$) from control.
[d] SFU, 5-fluorouracil.

Figure 17



Infection with *C. glabrata* in neutropenic mice was shown to be nonlethal. Mice were sacrificed to measure residual mycological burden on day 8 (24 hours after discontinuation of drug). MK-0991 was more effective than FCZ in reducing mycological burden in the kidneys (Table 61). In the spleens neither MK-0991 nor FCZ were effective in reducing mycological burden.

Table 61: Tissue colony counts after i.v. infection with 1.4×10^8 *C. glabrata* cells

Tissue	Drug	Dose (mg/kg)[a]	Log mean CFU	SEM
Kidney	None		14.84	0.7
	Flu[b]	5	12.71[c]	0.58
	L-743,872	5	4.93[d]	0.31
	L-743,872	2	7.24[d]	0.29
	L-743,872	0.5	8.27[d]	0.20
Spleen	None		11.36	0.24
	Flu	5	11.75	0.36
	L-743,872	5	10.80	0.68
	L-743,872	2	11.98	0.18
	L-743,872	0.5	11.24	0.25

[a] All fluconazole doses were given twice daily; all L-743,872 doses were given once daily.
[b] Flu, fluconazole.
[c] $P < 0.05$ compared with control.
[d] $P < 0.001$ compared with either fluconazole or control.

In another study, the effect of MK-0991 on mycological burden was measured in pancytopenic mice (Merck report, 1999, study 4, reference # 18). ICR mice were immunosuppressed with cyclophosphamide 3 days prior to infection. Immunosuppression was maintained by administering an additional dose of cyclophosphamide one day post-infection. Strain MY1055 of *C. albicans* (1.12×10^4 cfu) was injected intravenously and a single dose of MK-0991 administered intraperitoneally, 24 hours post-infection. Mice were sacrificed at different time intervals (before treatment, and after 2, 4, 6, 24, 30, 48, or 72 hours after drug administration) and kidneys

processed for measurement of cfu. Blood was collected for measuring drug levels in plasma by a radioimmunoassay. The results in Table 62A show that mycological burden was reduced in the kidneys after treatment with a single dose of MK-0991. However, the single dose was not effective in sterilization although the plasma concentrations of MK-0991 were above the MIC values (Table 62B).

Table 62

A.

Efficacy of Single Dose Delayed Therapy With Caspofungin (I.P.) Against *Candida albicans* MY1055 in TOKA [a]

CFU/g Kidneys (% Sterilization) [b]
[Mean Percent Reduction] [c]

Time (hr) Post Dosing	Sham-treated	Caspofungin 2.0 mg/kg	Caspofungin 1.0 mg/kg	Caspofungin 0.5 mg/kg	Caspofungin 0.25 mg/kg
0 (pre-treat)	4.33 (0)	-	-	-	-
2	4.64 (0)	3.93 (0) [80.3]	4.10* (0) [70.9]	3.91* (0) [81.2]	4.32 (0) [52.1]
4	4.68 (0)	3.61* (0) [91.4]	3.78* (0) [87.2]	3.71* (0) [89.0]	4.41 (0) [46.3]
6	5.06 (0)	3.80* (0) [94.3]	3.73* (0) [95.1]	3.87* (0) [93.3]	3.97* (0) [91.5]
24	5.40 (0)	3.65* (0) [98.2]	4.17* (0) [94.1]	3.89* (0) [96.9]	4.16* (0) [94.3]
30	5.90 (0)	3.90* (0) [99.0]	4.20* (0) [98.0]	3.99* (0) [98.8]	4.87* (0) [90.5]
48	5.90 (0)	3.56* (0) [99.6]	3.61* (0) [99.5]	4.38* (0) [97.0]	4.89* (0) [90.4]
72	6.18 (0)	3.51* (0) [99.8]	3.20* (0) [99.9]	3.90* (0) [99.5]	5.21* (0) [89.2]

TOKA 97-11 (11-Apr-97) Notebook In vivo 16, pp 99-104.

a. Mice were challenged I.V. with *Candida albicans* MY1055 at 1.12×10^4 cfu/mouse. Kidneys aseptically collected at time points after therapy. Mice were treated delayed therapy (24 hr after challenge) intraperitoneally (I.P.) and received a single dose. Five mice per group.
b. Percent sterilization indicates the number of mice with no detectable yeast, where the limit of detection because of the dilution scheme, was 50 yeast cells per pair of kidneys.
c. Mean percent reduction in CFU/g of kidney for treated groups relative to the mean of the sham-treated control.
* Significant from sham treated control ($p < 0.05$; Excel t-Test).

B.

Plasma Levels of Caspofungin at Time Points Following a Single I.P. Dose in *Candida albicans* MY1055 Infected, Cyclophosphamide-Induced Immune Suppressed Mice [a]

Mean caspofungin concentration (µg/mL) [b]

Time (hr) Post Dosing	Sham-treated	Caspofungin 2.0 mg/kg	Caspofungin 1.0 mg/kg	Caspofungin 0.5 mg/kg	Caspofungin 0.25 mg/kg
0 (pre-treat)	0	-	-	-	-
1	0	4.66	2.64	1.08	0.44
4	0	3.87	1.64	0.61	0.36
8	0	2.91	1.26	0.59	0.39
24	0	0.36	0.20	0.07	0.06
30	0	0.23	0.16	0.07	0.01

TOKA 97-11 (18-Jun-1997; 28-Sep -15 Oct-1997) Notebook: 15777, pp. 359-360, 383-386, 389-392.

a. Mice were challenged I.V. with *Candida albicans* MY1055 at 1.12×10^4 cfu/mouse. Mice were treated delayed therapy (24 hr after challenge) intraperitoneally (I.P.) and received a single dose. Five mice per group.
b. Mean caspofungin concentration was determined by radioimmunoassay.

The effectiveness of MK-0991 in reducing mycological burden and improving survival was also measured in another pancytopenic model study (Merck report, 1999, study 4, reference # 16). ICR mice were immunosuppressed by administering 3 doses of cyclophosphamide (300 mg/kg) intraperitoneally, starting 3 days prior to challenge and administering at a 3-day interval (until 4 days post-infection). Treatment for 7 days, with MK-0991, ABLC or ampB was initiated 24 hours post-challenge, with *C. albicans* MY1055 (2 x 10^4 cfu, intravenous). Animals were followed up to 21 days for survival. A group of mice were sacrificed a day after discontinuation of therapy and kidneys processed for determination of mycological burden. The results in Tables 63 and 64 and Figures 18A-18C show that MK-0991, ampB and ABLC were effective in reducing mycological burden (measured on day 7) and improving survival (measured up to day 21). The total leukocyte count was shown to be low for up to 7 days in immunosuppressed mice (Figure 18D).

Table 63: Efficacy of Delayed Therapy With Caspofungin, AmB and ABLC Against a Disseminated *C. albicans* MY1055 Infection in Cyclophosphamide-Induced Pancytopenic ICR Mice [a]

Mean $\log_{10}$ CFU/g kidneys (% Sterilization) [b] and ED_{90} and ED_{99} values [c] at 8 days after challenge

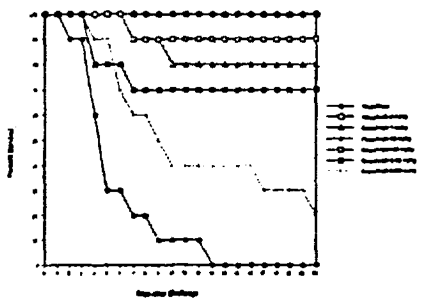
Dose (mg/kg)	Caspofungin	AmB	ABLC
2.0	2.10* (100)	NT [d]	3.59* (0)
1.0	2.14* (100)	2.89* (20)	5.27* (0)
0.5	2.13* (100)	3.48* (0)	4.82 (20)
0.25	2.38* (80)	4.46* (0)	5.91* (0)
0.125	4.62* (0)	4.78* (0)	6.83 (0)
0.063	6.06 (0)	5.61* (0)	7.04 (0)[3]
0	6.47 (0)[3]	6.47 (0)[3]	6.47 (0)[3]
ED_{90} (95% CI)	0.049 (0.014, 0.189)	0.071 (0.028, 0.254)	0.399 (0.121, 1.313)
ED_{99} (95% CI)	0.119 (0.038, 0.374)	0.198 (0.069, 0.571)	1.119 (0.361, ∞)

a. Mice were challenged I.V. with *C. albicans* MY1055 at 2.0×10^4 CFU/mouse. Kidneys aseptically collected at day 8 after challenge. Mice received first treatment 24 hr after challenge (delayed therapy) and were treated I.P., q.d. for 7 days.
b. Mean $\log_{10}$ CFU/g at 8 days after challenge for paired kidneys. Five mice per group unless indicated by superscript *d*. Percent sterilization indicates the number of mice with no detectable yeast where the limit of detection was 50 yeast cells per pair of kidneys.
c. ED values (95% confidence interval) were calculated by Biometrics Research, Rahway and are based on reduction in CFU/g kidneys of treated groups compared to sham-treated control animals.
d. NT = not tested.
* Significant from sham treated control ($p < 0.05$; Excel t-test).
– stands for indefinitely large.

Figure 18

A.

Efficacy of Delayed Therapy With Caspofungin Against a Disseminated *C. albicans* MY1055 Infection in Cyclophosphamide-Treated ICR Mice[a]

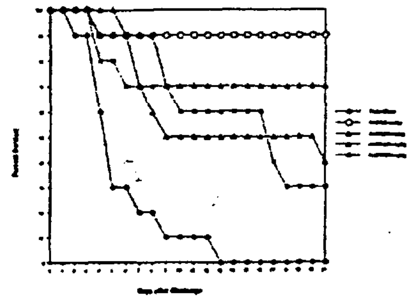


TOKA 97-10 (04-Apr-1997) Notebook In Vivo 16 (TOKA), pp. 89-98.

a. Mice were challenged I.V. with *C. albicans* MY1055 at 2.0 x 10^4 CFU/mouse. Kidneys aseptically collected at day 8 after challenge. Mice received first treatment 24 hr after challenge (delayed therapy) and were treated I.P., q.d. for 7 days.

B.

Efficacy of Delayed Therapy With AmB Against a Disseminated *C. albicans* MY1055 Infection in Cyclophosphamide-Treated ICR Mice[a]

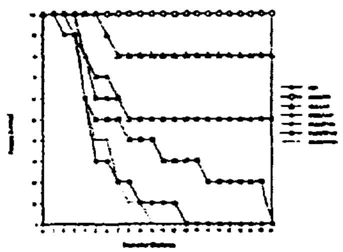


TOKA 97-10 (04-Apr-1997) Notebook In Vivo 16 (TOKA), pp. 89-98.

a. Mice were challenged I.V. with *C. albicans* MY1055 at 2.0 x 10^4 CFU/mouse. Kidneys aseptically collected at day 8 after challenge. Mice received first treatment 24 hr after challenge (delayed therapy) and were treated I.P., q.d. for 7 days.

C.

Efficacy of Delayed Therapy With ABLC Against a Disseminated *C. albicans* MY1055 Infection in Cyclophosphamide-Treated ICR Mice[a]

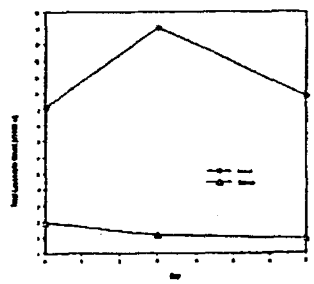


TOKA 97-10 (04-Apr-1997) Notebook In Vivo 16 (TOKA), pp. 89-98.

a. Mice were challenged I.V. with *C. albicans* MY1055 at 2.0 x 10^4 CFU/mouse. Kidneys aseptically collected at day 8 after challenge. Mice received first treatment 24 hr after challenge (delayed therapy) and were treated I.P., q.d. for 7 days.

D.

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



PK 97-1 (21-Mar-1997) Notebook In Vivo 16 (PK), pp. 1-3.

a. ICR mice were immune suppressed with a 300 mg/kg dose of cyclophosphamide administered I.P. on day -3 (time point not shown on figure). Immunosuppression was maintained by 2 additional doses of cyclophosphamide (100 mg/kg, I.P.) on day 1 and 4. Immune suppression was monitored by differential white cell counts performed b[redacted], Rahway on days 0, 3 and 7.

Table 64

Effective Dose 50% (ED_{50}) and 90% (ED_{90}) Values Based on Survival and 95% Confidence Intervals for Caspofungin, AmB and ABLC in a Delayed-Treatment Model of Disseminated Candidiasis in Cyclophosphamide-Treated ICR Mice[a]

ED_{50} values in mg/kg/dose[b]

	Caspofungin	AmB	ABLC
Day 7	0.082 ([illegible])	0.053 ([illegible])	0.109 ([illegible])
Day 14	0.095 ([illegible])	0.144 ([illegible])	0.255 ([illegible])
Day 21	0.113 ([illegible])	0.222 ([illegible])	0.406 ([illegible])

ED_{90} values in mg/kg/dose[b]

	Caspofungin	AmB	ABLC
Day 7	0.267 (0.159, 0.608)	0.596 (0.364, ∞)	1.519 (0.709, ∞)
Day 14	0.342 (0.252, 0.918)	>1.000 (0.672, ∞)	1.177 (0.737, ∞)
Day 21	0.327 (0.273, 0.779)	>1.000 (0.646, ∞)	1.071 (0.708, ∞)

TOKA 97-10 (04-Apr-1997) Notebook In Vivo 16 (TOKA), pp. 89-98

a. Mice were challenged I.V. with *C. albicans* MY1055 at 2.0 x 10^4 CFU/mouse. Kidneys aseptically collected at day 8 after challenge. Mice received first treatment 24 hr after challenge (delayed therapy) and were treated I.P., q.d. for 7 days.

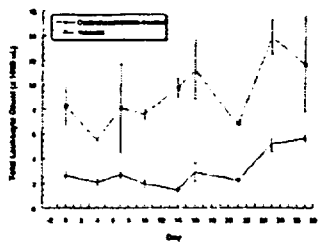
b. ED_{50} and ED_{90} values (95% confidence interval) were calculated by Biometrics Research, Rahway and are based on survival.

∞ stands for infinitely large.

In another experiment conducted in the pancytopenic murine model, immunosuppression was induced by administering 10 doses of cyclophophamide at 3-day intervals (Merck report, 1999, study 5, reference # 16). Mice were challenged intravenously with *C. albicans* strain MY1055 (1.22 x 10^5 cfu/mouse) 3 days after the induction of immunosuppression. Mice were treated 24 hours after challenge, by the intraperitoneal route, q.d. for 7 days. Animals were followed for survival and mycological burden in kidneys for up to 28 days post infection. AmpB and FCZ were used as comparators. The results in Figure 19 show leukocyte counts to be depressed in immunosuppressed mice. No mice died in the uninfected immunosuppressed group. Treatment with MK-0991 or ampB was more effective than FCZ in reducing cfu in kidney (Tables 65 – 66) and improving survival (Table 67 and Figure 20).

Figure 19

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



[a] Total leukocyte count (x 1000 µL) ± SEM. ICR mice were immune suppressed with a 6 mg/mouse dose of cyclophosphamide (CY) administered P.O. on day -3. Immune suppression was maintained by additional doses of CY (2 mg/mouse, P.O.) on days 1, 4, 7, 10, 13, 16, 19, 22, and 25. Immune suppression was monitored by differential white cell counts performed by Laboratory Resources, Ralway on days 0, 4, 7, 10, 14, 16, 21, 25 and 29

Table 66

Effective Dose 90% (ED_{90}) and 99% (ED_{99}) Values[a] Based on Reductions in CFU/g From Sham-Treated Control at Three Points After Challenge for Caspofungin, AmB and FCZ in a Delayed-Treatment Model of Disseminated Candidiasis in Cyclophosphamide-Treated, Chronically Pancytopenic ICR Mice[b]

ED_{90} values in mg/kg/dose[c]

	Caspofungin	AmB	FCZ
Day 4	0.025	0.618	> 80
Day 8	< 0.25	0.086	< 20
Day 14	0.002	0.083	9.865
Day 21	0.044	0.197	NC
Day 28	0.035	0.037	NC

ED_{99} values in mg/kg/dose[c]

	Caspofungin	AmB	FCZ
Day 4	0.186	> 1	> 80
Day 8	< 0.25	0.165	< 20
Day 14	0.011	0.195	NC
Day 21	0.120	0.371	NC
Day 28	0.079	0.141	NC

Pooled Data from TOKA 98-12 (08-Oct-1998) and TOKA 99-3 (12-Mar-1999) Notebook in Vivo 18, pp. 42-50 and 100-110.

a. ED_{90} and ED_{99} values (95% confidence interval) were calculated by Biometrics Research, Ralway and are based on reductions in CFU/g kidneys of treated groups compared to sham-treated control animals.
b. Mice were challenged I.V. with *C. albicans* MY1055 at 5.6 x 10^4 CFU/mouse (TOKA 98-12) and 1.22 x 10^5 CFU/mouse (TOKA 99-3). Kidneys aseptically collected at days 4, 8, 14, 21, 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).
c. NC = Not calculated. 0* stands for infinitely small, ∞ stands for infinitely large.

Table 65

Efficacy of Delayed Therapy With Caspofungin, AmB and FCZ Against a Disseminated *C. albicans* MY1055 Infection in Cyclophosphamide-Induced, Chronically Pancytopenic ICR Mice[a]

		Mean log_{10} CFU/g kidneys (% Sterilization)[b] [percent reduction from control][c] at time points after challenge				
Compound	Dose (mg/kg)	Day 4	Day 8	Day 14	Day 21	Day 28
Caspofungin	1.00	3.04* (0) [99.85]	2.30* (90) [99.98]	2.07* (70) [99.99]	2.07* (100) [99.99]	2.09* (100) [99.99]
	0.50	3.94* (0) [99.30]	2.30* (100) [99.99]	2.06* (100) [99.99]	2.08* (100) [99.99]	2.10* (90) [99.99]
	0.25	4.34* (0) [99.26]	2.10* (90) [99.99]	2.75* (50) [99.971]	3.44* (50) [99.68]	3.80* (40) [99.93]
AmB	1.00	5.09* (0) [95.82]	2.07* (80) [99.99]	2.46* (60) [99.98]	2.37* (80) [99.97]	3.41* (80) [99.97]
	0.50	5.63* (0) [55.99]	2.91* (60) [99.93]	2.93* (50) [99.95]	3.47* (20) [99.66]	4.14* (40) [99.84]
	0.25	6.20 (0) [46.79]	3.58* (10) [99.67]	4.08* (30) [99.23]	4.56 (10) [95.73]	4.44* (20) [99.68]
FCZ	80.00	5.57* (0)[d] [99.53]	3.51* (20)[d] [99.87]	4.37* (0)[d] [99.69]	6.75 (0)[d] [NC][e]	6.01 (20)[d] [NC]
	40.00	5.91* (0)[d] [79.26]	3.45* (0)[d] [99.89]	5.35 (0)[d] [97.11]	6.97 (0)[d] [NC]	6.17 (20)[d] [NC]
	20.00	5.70* (0)[d] [87.12]	2.87* (20)[d] [99.97]	5.22 (0)[d] [97.83]	4.15 (40)[d] [NC]	7.68 (0)[d] [NC]
Sham-Treated		6.47 (0)	6.06 (0)	6.26 (0)[d]	5.94 (0)[d]	6.93 (0)[d]

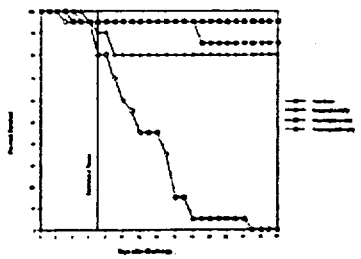
Pooled Data from TOKA 98-12 (02-Oct-1998) and TOKA 99-3 (12-Mar-1999) Notebook in Vivo 18, pp. 42-50 and 100-110.

a. Mice were challenged I.V. with *C. albicans* MY1055 at 5.6 x 10^4 CFU/mouse (TOKA 98-12) and 1.22 x 10^5 CFU/mouse (TOKA 99-3). Kidneys aseptically collected at days 4, 8, 14, 21, 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. Ten mice per group unless indicated by superscript #. Percent sterilization indicates the number of mice with no detectable yeast where the limit of detection was 50 yeast cells per pair of kidneys.
c. Percent reduction calculated by Biometrics Research, Ralway and are based on reduction in CFU/g kidneys of treated groups compared to sham-treated control animals.
d. Not calculated.
* Significant from sham treated control ($p < 0.05$; Excel t-test).

Figure 20

A.

Efficacy of Delayed Therapy With Caspofungin Against a Disseminated *C. albicans* MY1055 Infection in Cyclophosphamide-Treated, Chronically Pancytopenic ICR Mice[a]

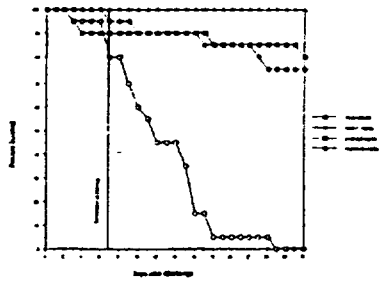


Pooled Data from TOKA 98-12 (02-Oct-1998) and TOKA 99-3 (12-Mar-1999) Notebook in Vivo 18, pp. 42-50 and 109-116.

a. Mice were challenged I.V. with *C. albicans* MY1055 at 5.6×10^4 CFU/mouse (TOKA 98-12) and 1.22×10^5 CFU/mouse (TOKA 99-3). 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.

Efficacy of Delayed Therapy With AmB Against a Disseminated *C. albicans* MY1055 Infection in Cyclophosphamide-Treated, Chronically Pancytopenic ICR Mice[a]

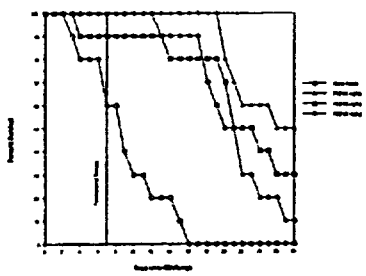


Pooled Data from TOKA 98-12 (02-Oct-1998) and TOKA 99-3 (12-Mar-1999) Notebook in Vivo 18, pp. 42-50 and 109-116.

a. Mice were challenged I.V. with *C. albicans* MY1055 at 5.6×10^4 CFU/mouse (TOKA 98-12) and 1.22×10^5 CFU/mouse (TOKA 99-3). 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).

C.

Efficacy of Delayed Therapy With FCZ Against a Disseminated *C. albicans* MY1055 Infection in Cyclophosphamide-Treated, Chronically Pancytopenic ICR Mice[a]



TOKA 99-3 (12-Mar-1999) Notebook in Vivo 18, pp. 109-116.

a. Mice were challenged I.V. with *C. albicans* MY1055 at 1.22×10^5 CFU/mouse (TOKA 99-3). 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).

Table 67

Day 28 Effective Dose 50% (ED_{50}) and 90% (ED_{90}) Values Based on Survival and 95% Confidence Intervals for Caspofungin, AmB and FCZ in a Delayed-Treatment Model of Disseminated Candidiasis in Cyclophosphamide-Treated, Chronically Pancytopenic ICR Mice[a]

		ED values in mg/kg/dose[b]	
Expt	Compound Administration	ED_{50}	ED_{90}
TOKA 98-12	Caspofungin	0.020 (0+, 0.210)	0.160 (NC)
	AMB	< 0.25 (NC)	< 0.25 (NC)
TOKA 99-03	Caspofungin	< 0.25 (NC)	0.357 (NC)
	AMB	0.293 (0.056, 0.443)	0.508 (0.518, –)
	FCZ	76.317 (45.475, –)	> 80 (NC)

Data from TOKA 98-12 (02-Oct-1998) and TOKA 99-3 (12-Mar-1999) Notebook in Vivo 18, pp. 42-50 and 109-116.

a. Mice were challenged I.V. with *C. albicans* MY1055 at 5.6×10^4 CFU/mouse (TOKA 98-12) and 1.22×10^5 CFU/mouse (TOKA 99-3). 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. ED_{50} and ED_{90} values (95% confidence interval) were calculated by Biometrics Research, Rahway and are based on survival. NC = Not calculated. 0+ stands for infinitely small. – stands for infinitely large.

(ii) Studies in oropharyngeal and gastrointestinal models of candidiasis

CD4 deficient transgenic mice were infected with 2 x 10^7 cfu of *C. albicans* (strain MY1055) by gavage (Merck report, 1995, reference # 45). In addition their oral cavity was swabbed with the yeast suspension (10^8 cfu/ml) and buccal mucosa gently abraded. Treatment with MK-0991 was initiated 3 days after infection either by the intraperitoneal route, b.i.d. for 4 days or by the oral route for 10 days. The residual fungal burden was measured in the oral and fecal samples at different time intervals during treatment and after discontinuation of therapy. The activity of MK-0991 was compared with ampB, nystatin and FCZ. The results in Tables 68A and 68C indicate a reduction in *C. albicans* scores in oral swabs after oral or intraperitoneal treatment with

MK-0991 or FCZ. In fecal samples, treatment with either of the drugs showed a marginal reduction in cfu (Tables 68B and 68D)

Table 68

A. Subjective oral scores of *Candida albicans* MY 1055 per oral swab in CD4+ T-lymphocyte deficient transgenic mice treated with antifungals in the drinking water.

Treatment	Dose Mg/kg	Day After Challenge, Therapy Day 3-13 Day 3	Day 5	Day 7	Day 10	Day 13	Day 14
Sham Rx		4+[a,b]	4+	4+	1+	1+	1+
L-743,872	100	4+	1+[a]	1+[a]	0[a]	0[a]	0[a]
L-743,872	25	4+	1+	1+	1+	0	0
L-743,872	6.25	4+	1+	1+	1+	1+	1+
NYS	100	4+	1+	3+	3+	3+	4+
NYS	25	4+	3+	3+	1+	2+	1+
NYS	6.25	4+[a]	2+[a]	2+[a]	3+[a]	2+[a]	1+[a]
FCZ	100	4+	0	0	0	0	0
FCZ	25	3+	0	0	0	0	0
FCZ	6.25	4+[a]	1+[a]	1+[a]	1+[a]	1+[a]	2+[a]

Notebook: A. Flattery, In Vivo 12 (Esophageal), RC 93-3, NB# 39515, pp. 084-087, 20SEP193.
[a] 0 CFU = 0, 1-49 CFU = 1+, 50-99 CFU = 2+, 100-199 CFU = 3+, ≥200 CFU = 4+
[b] n = 5 unless marked * (n = 4).

B. Log CFU *Candida albicans* MY 1055 per gram feces in CD4+ T-lymphocyte deficient transgenic mice treated with antifungals in the drinking water.

Treatment	Dose Mg/kg	Day After Challenge, Therapy Days 3-13 Day 3	Day 5	Day 7	Day 10	Day 13	Day 14
Sham Rx		6.18 (0.17)[a]	5.46 (0.11)[a]	5.54 (0.09)[a]	5.43 (0.17)[a]	5.61 (0.07)[a]	4.71 (0.07)[a]
L-743,872	100	6.07 (0.36)	2.56[b] (0.06)	2.58 (0.07)	2.34 (0.06)	2.60 (0.04)	2.34 (0.35)
L-743,872	25	5.21 (0.19)	3.23 (0.20)	3.03 (0.17)	3.12 (0.21)	2.74 (0.04)	3.27 (0.35)
L-743,872	6.25	6.06 (0.16)	3.65 (0.20)	3.72 (0.22)	3.26 (0.31)	2.52 (0.21)	3.06 (0.31)
NYS	100	6.17 (0.20)	4.78 (0.26)	4.70 (0.11)	5.64 (0.42)	4.34 (0.56)	5.27 (0.47)
NYS	25	5.56 (0.11)	5.14 (0.09)	5.22 (0.28)	5.41 (0.24)	4.78 (0.12)	5.06 (0.28)
NYS	6.25	5.65 (0.08)[a]	4.86 (0.17)[a]	4.98 (0.22)[a]	4.98 (0.09)[a]	4.50 (0.57)[a]	4.63 (0.27)[a]
FCZ	100	5.75 (0.17)	3.23 (0.27)	2.48 (0.05)	2.66 (0.05)	2.42 (0.06)	2.70 (0.10)
FCZ	25	5.48 (0.16)	2.99 (0.15)	2.83 (0.05)	2.79 (0.06)	2.70 (0.03)	2.59 (0.10)
FCZ	6.25	5.63 (0.10)[a]	3.73 (0.36)[a]	3.81 (0.19)[a]	3.09 (0.39)[a]	2.80 (0.07)[a]	3.31 (0.46)[a]

Notebook: A. Flattery, In Vivo 12 (Esophageal), RC 93-3, NB# 39515, pp. 084-087, 20SEP193.
[a] Numbers in parentheses represent standard error of the mean (SEM), n = 5 unless marked with * (n = 4) or ** (n = 3).
[b] Significant reduction by Student's t test ($p<0.05$) is denoted by numbers in bold.

C. Subjective oral scores of *Candida albicans* MY 1055 per oral swab in CD4+ T-lymphocyte deficient transgenic mice treated I.P. with antifungals.

Treatment	Dose mg/kg	Day After Challenge, Therapy (IP, b.i.d.) Days 3-6 Day 3	Day 4	Day 5	Day 6	Day 7
Sham		3+[a]	3+	4+	4+	4+
L-743,872	0.375	2+	1+	1+	1+	2+
AMB	0.375	4+	5+	4+	4+	4+
FCZ	5.0	4+	2+	1+	1+	2+

Notebook: A. Flattery, In Vivo 13 (Esophageal), RC 93-5, NB# 39515, pp. 091-093, 29NOV93.
[a] 0 CFU = 0, 1-49 CFU = 1+, 50-99 CFU = 2+, 100-199 CFU = 3+, ≥200 CFU = 4+, n = 5.

D. Log CFU *Candida albicans* MY 1055 per gram feces in CD4+ T-lymphocyte deficient transgenic mice treated I.P. with antifungals.

Treatment	Dose mg/kg	Day After Challenge, Therapy (IP, b.i.d.) Days 3-6 Day 3	Day 4	Day 5	Day 6	Day 7
Sham		5.23 (0.15)[a]	5.14 (0.09)	5.31 (0.13)	5.60 (0.13)	5.79 (0.23)
L-743,872	0.375	4.80 (0.37)	4.52 (0.45)	4.35[b] (0.40)	4.16 (0.34)	4.69 (0.27)
AMB	0.375	5.38 (0.16)	5.24 (0.29)	5.40 (0.25)	5.24 (0.32)	5.75 (0.26)
FCZ	5.0	5.15 (0.15)	4.67 (0.27)	4.39 (0.17)	4.07 (0.07)	3.74 (0.31)

Notebook: A. Flattery, In Vivo 13 (Esophageal), RC 93-5, NB# 39515, pp. 091-093, 29NOV93.
[a] Numbers in parentheses represent standard error of the mean (SEM), n = 5.
[b] Significant reduction by Student's t test ($p<0.05$) is denoted by numbers in bold.

In another study (Merck report, 1999, study 2, reference # 18), outbred germ free Swiss Black mice were infected by swabbing the oral cavity and mildly abrading the mucosa with a suspension of *C. albicans* strain MY1055 (1.33×10^9 cfu/ml). The actual volume of the yeast suspension used for infection was not stated. Treatment with MK-0991, ampB, or FCZ by the oral route (in drinking water) was initiated 14 days post-challenge. The lowest dose of MK-0991 (1.56 mg/kg/day) was administered for 22 days followed by a higher dose (50 mg/kg/day) until day 43. The duration of treatment for high and medium dose (25 and 6.25 mg/kg/day) groups of MK-0991, ampB, or FCZ treated mice was not specified. It is possible that all treatments were administered for 43 days. Fecal samples were collected at different time intervals and

processed for measurement of mycological burden (cfu) and *in vitro* susceptibility. The results in Table 69A indicate that MK-0991 at a dose of 25 mg/kg/day reduced the cfu by ≤ 1 log. The reduction in cfu in mice treated with MK-0991 appears to be less than that of ampB or FCZ treated animals. The results of the *in vitro* susceptibility of isolates collected on day 43 show that the MIC values of isolates from MK-0991 treated mice increased 4 to 33 fold compared to the isolates from sham treated mice (Table 69B and 69C). It is of note that the MK-0991 MIC values of isolates from mice treated with ampB or FCZ were similar. The MIC values of isolates from ampB treated mice were similar to that of sham treated mice. The MIC value of isolates from one of the FCZ treated animal was increased when measured at 48 hours, whereas the 24-hour MIC value was similar to the sham treated mice. The MIC values of these isolates to ampB and FCZ were not altered. The differences in MIC values between sham and MK-0991 (medium and high dose) treated mice were very variable on day 51 (Table 69D). The isolates from mice treated with a low dose (1.56 mg/kg/day) followed by a high dose (50 mg/kg/day) of MK-0991 showed higher MICs (10 to 26-fold) than sham treated mice. The MICs for isolates collected from FCZ treated mice on day 60 (17 days after termination) of therapy were increased 8 to 64 fold (Table 69E). It is of note that a small number of mice were tested in each group. Nevertheless, the results do suggest a trend towards an increase in MIC values upon treatment with MK-0991.

Table 69: A. Mean *Candida albicans* Log$_{10}$ CFU/g Feces in Germ-Free Black Swiss Mice Monoassociated With *C. albicans* MY1055[1]

Treatment	Dose Mg/kg	Day After Initiation of Therapy: Day 0	Day 2	Day 4	Day 7	Day 10	Day 14	Day 22	Day 29	Day 36	Day 43	Day 51
Sham-Treated		7.59	7.92	7.97	8.20	7.93	7.93	7.83	7.62	8.08	7.47	7.62
Caspofungin Drinking Water	25	7.87	7.60*	7.46*	7.09*	6.50*	7.33*	7.70	6.93*	7.58	7.58	7.55
Caspofungin Drinking Water	6.25	7.91	8.06	8.33	8.05	7.59*	8.06	7.84	7.70	8.12	ND	7.76
Caspofungin Drinking Water	1.56 (50)[2]	7.93	8.10	8.11	7.87*	8.00	8.01	7.87	7.12*	7.77	ND	7.75
FCZ Drinking Water	25	7.65	7.66	7.18*	6.73*	6.67*	6.39*	5.73*	6.36*	6.50*	6.45	ND
FCZ Drinking Water	6.25	7.71	7.88	7.28*	7.31*	6.70*	6.66*	5.90*	6.63*	6.82*	ND	ND
FCZ Drinking Water	1.56 (50)[2]	7.47	8.01	7.78	7.63*	7.52*	7.97	7.14*	6.40*	6.50*	ND	ND
AmB Drinking Water	25	7.96	7.48*	6.76*	6.57*	5.65*	5.31*	4.94*	4.44*	4.74*	5.03*	ND
AmB Drinking Water	6.25	7.81	8.04	7.96	7.67*	7.44*	7.59*	7.66	6.94*	7.36*	ND	ND
AmB Drinking Water	1.56 (50)[2]	7.54	7.63	7.92	7.75*	7.79	7.78	7.87	5.83*	3.12*	ND	ND
Caspofungin I.P.	2	7.72	7.43*	7.58*	7.66*	7.59*	7.81	ND	ND	ND	ND	ND
Caspofungin I.P.	1	8.01	8.02	8.05	7.79*	7.78	7.92	ND	ND	ND	ND	ND
Caspofungin I.P.	0.5	8.06	7.73	7.98	7.85*	7.84	7.66	ND	ND	ND	ND	ND
Caspofungin I.P.	0.25	7.74	7.77	7.92	7.89	7.93	7.96	7.64	8.18	8.12	ND	ND

Data from Experiment RC96-4 (18-Jul-1996), Notebook la Vivo 16, pp. 11-28.
1. Germ-free Swiss black mice were monoassociated with *C. albicans* MY1055 by oral swabbing with 1.33 x 10^8 cells/mL.
2. Mice were treated with antifungal agents at 1.56 mg/kg/day for 22 days then 50 mg/kg/day until day 43 via the drinking water.
* = Significant from sham treated control ($p < 0.05$ Exact t-Test).

B.

MIC (μg/mL) for Caspofungin, Fluconazole and Amphotericin B Against Pooled Inocula From Day 43 Isolates (End of Therapy) From Monoassociated Mice Treated in the Drinking Water

Treatment	Mouse #	Caspofungin 24 hr	Caspofungin 48 hr	Fluconazole 24 hr	Fluconazole 48 hr	Amphotericin B 24 hr	Amphotericin B 48 hr
C. albicans MY1055		≤0.03	0.06	0.5	0.5	0.25	0.25
Sham-treated	1-1	0.06	0.25	0.25	0.25	0.25	0.5
	1-2	0.06	0.125	0.25	0.25	0.125	0.25
	1-3	0.06	0.06	0.25	0.25	0.125	0.25
Caspofungin 25 mg/kg	2-1	2	2	0.25	0.25	0.125	0.25
	2-2	0.25	1	0.25	0.25	0.125	0.25
	2-3	1	2	0.25	0.25	0.125	0.25
Fluconazole 25 mg/kg	5-1	0.06	0.25	0.5	0.5	0.25	0.5
	5-2	≤0.03	0.06	0.5	0.5	0.125	0.25
	5-3	0.06	0.06	0.5	>64*	0.125	0.25
Amphotericin B 25 mg/kg	8-1	0.06	0.06	0.25	0.25	0.125	0.25
	8-2	0.06	0.06	0.25	0.25	0.125	0.25
	8-3	0.06	0.06	0.25	0.25	0.125	0.25

Data from Experiment RC96-4 (18-Jul-1996), Notebook la Vivo 16, pp. 11-28.
a. Trailing endpoint beyond 0.5 μg/mL.

C.

MIC (μg/mL) for Caspofungin Against Clones From Day 43 Isolates (End of Therapy) From Monoassociated Mice Treated With Caspofungin at 25 mg/kg in the Drinking Water

Mouse Treatment	Mouse #	Clone #	ID	Caspofungin 24 hr	Caspofungin 48 hr
Caspofungin 25 mg/kg	2-1	1	Clone 2-1 #1 Day 43	1	4
		2	Clone 2-1 #2 Day 43	2	4
		3	Clone 2-1 #3 Day 43	0.125	0.25
	2-2	1	Clone 2-2 #1 Day 43	0.5	1
		2	Clone 2-2 #2 Day 43	0.5	2
		3	Clone 2-2 #3 Day 43	0.5	2
	2-3	1	Clone 2-3 #1 Day 43	0.5	2
		2	Clone 2-3 #2 Day 43	0.5	2
		3	Clone 2-3 #3 Day 43	0.5	2

Data from Experiment RC96-4 (18-Jul-1996), Notebook la Vivo 16, pp. 11-28.

Table 69 (continued)

D.

MIC (μg/mL) for Caspofungin Against Pooled Inocula and Clones From Day 51 Isolates (8 Days After Termination of Therapy) From Monoassociated Mice Treated With Caspofungin in the Drinking Water

Mouse Treatment	Mouse #	Clone #	ID	Caspofungin 24 hr	Caspofungin 48 hr
C. albicans MY1055				0.125	0.5
Sham-treated	1-1		Pool 1-1 Day 51	0.06	0.125
	1-2		Pool 1-2 Day 51	0.06	0.125
	1-3		Pool 1-3 Day 51	0.125	0.25
	1-4		Pool 1-4 Day 51	0.06	0.125
	1-5		Pool 1-5 Day 51	0.06	0.125
Caspofungin 25 mg/kg	2-1	1	Clone 2-1 #1 Day 51	0.06	0.25
		2	Clone 2-1 #2 Day 51	0.06	0.25
		3	Clone 2-1 #3 Day 51	0.125	1
	2-2	1	Clone 2-2 #1 Day 51	0.125	1
		2	Clone 2-2 #2 Day 51	0.25	1
		3	Clone 2-2 #3 Day 51	0.125	1
	2-3	1	Clone 2-3 #1 Day 51	0.25	2
		2	Clone 2-3 #2 Day 51	0.25	1
		3	Clone 2-3 #3 Day 51	0.25	1
	2-4	1	Clone 2-4 #1 Day 51	0.25	1
		2	Clone 2-4 #2 Day 51	≤0.03	0.125
		3	Clone 2-4 #3 Day 51	0.125	1
	2-5	1	Clone 2-5 #1 Day 51	0.25	1
		2	Clone 2-5 #2 Day 51	0.25	1
		3	Clone 2-5 #3 Day 51	0.06	0.125
Caspofungin 6.25 mg/kg	3-1		Pool 3-1 Day 51	0.06	0.25
	3-2		Pool 3-2 Day 51	0.06	0.125
	3-3		Pool 3-3 Day 51	0.06	0.125
	3-4		Pool 3-4 Day 51	0.06	0.125
	3-5		Pool 3-5 Day 51	0.06	0.125
Caspofungin 1.56 then 50 mg/kg	4-1		Pool 4-1 Day 51	2	4
	4-2		Pool 4-2 Day 51	1	2
	4-3		Pool 4-3 Day 51	2	4
	4-4		Pool 4-4 Day 51	2	4
	4-5		Pool 4-5 Day 51	2	4

Data from Experiment EC96-4 (18-Jul-1996), Notebook In Vivo 16, pp. 11-28.

E.

MIC (μg/mL) for Fluconazole Against Pooled Inocula From Day 60 Isolates (17 Days After Termination of Therapy) From Monoassociated Mice Treated With Fluconazole in the Drinking Water

Mouse Treatment	Mouse #	Fluconazole 24 hr	Fluconazole 48 hr
MY1055 Control		0.5	0.5
Fluconazole 25 mg/kg	5-1	0.5	1
	5-2	0.5	1
	5-3	0.5	1
	5-4	0.5	1
	5-5	0.5	1
Fluconazole 6.25 mg/kg	6-1	1	1
	6-2	0.5	1
	6-3	0.5	1
	6-4	0.5	1
	6-5	0.5	1
Fluconazole 1.56 then 50 mg/kg	7-1	0.5	32
	7-2	0.5	16
	7-3	0.5	8
	7-4	0.5	8
	7-5	0.5	4

Data from Experiment EC96-4 (18-Jul-1996), Notebook In Vivo 16, pp. 11-28.

Isolates collected on day 43 from mice treated with a dose of 25 mg/kg of MK-0991 were cloned and tested for virulence by inoculating intravenously to DBA/2 mice at different yeast cell concentrations (1.16×10^8 – 9.6×10^2). Naïve strain MY1055 of *C. albicans* was used as a comparator. Mice were followed for survival for up to 21 days. The actual survival rates on different days were not shown. The results in Table 70 show that the virulence of the 3 clones tested was similar to the naive MY1055 strain.

Table 70

Lethal Dose 50% (LD_{50})[1] for *Candida* Strains at 4, 7, 14 and 21 Days After I.V. Challenge in DBA/2N Mice[2]

Strain	CFU/Mouse 4 Day	7 Day	14 Day	21 Day
C. albicans MY1055	4.9×10^5	8.7×10^4	7.0×10^4	7.0×10^4
C. albicans Clone2-1 #1	3.7×10^5	3.7×10^5	3.7×10^5	3.7×10^5
C. albicans Clone 2-1 #2	3.7×10^5	1.6×10^5	1.3×10^5	1.3×10^5
C. albicans Clone 2-1 #3	5.4×10^4	2.3×10^4	1.0×10^4	1.0×10^4

Data from Experiment TOKA-Ca 96-22 (10-Oct-1996), Notebook In Vivo 15, pp. 124-134.

1. LD_{50} determined using Knudson Curtis method (5).
2. DBA/2 mice were infected I.V. with *C. albicans*; MY1055 at 7.2×10^6 CFU/mouse, MY1055 at 9.6×10^5, 9.6×10^4, 9.6×10^3, and 9.6×10^2 CFU/mouse, Isolate 2-1 #1at 1.16×10^8, 1.16×10^7, 1.16×10^6, 1.16×10^5, 1.16×10^4, and 1.16×10^3 CFU/mouse, Isolate 2-1 #2 at 1.16×10^8, 1.16×10^7, 1.16×10^6, 1.16×10^5, 1.16×10^4, and 1.16×10^3 CFU/mouse, and Isolate 2-1 #3 at 7.4×10^7, 7.4×10^6, 7.4×10^5, 7.4×10^4, 7.4×10^3, and 7.4×10^2 CFU/mouse.

The *in vivo* activity of MK-0991 against the 3 clones (2 of the 3 clones showed higher MIC values) was measured. For this, groups of mice infected either with 9.6 x 10^4 cfu of the 3 clones or the naive strain MY1055 were treated with MK-0991 by the intraperitoneal route, b.i.d. for 2 days. Treatment was initiated within 15 to 30 minutes of infection. The experimental conditions were the same as described above. Mice were sacrificed 2 days after discontinuation of treatment and kidneys processed for measurement of cfu. The results in Table 71 show that mice infected with the clones (2-1#1 and 2-1#2: 24 and 48 hour MIC of 1 to 2 ug/ml and 4 ug/ml, respectively) with higher *in vitro* MICs to MK-0991 were refractory to therapy with MK-0991. The mice infected with the susceptible clone (2-1#3 24 and 48 hour MIC of 0.125 ug/ml and 1 ug/ml, respectively) or the naive strain MY1055 showed reduction in CFU and sterilization of the kidney upon treatment with MK-0991. This preliminary data suggests that a potential for development of resistance to MK-0991 *in vivo* exist.

Table 71

Efficacy of Caspofungin Against *Candida albicans* MY1055 and Strains Isolated From Monoassociated Mice in TOKA[1]

	CFU/g Kidneys (% Sterilization)			
Dose (mg/kg)	*C. albicans* MY1055	*C. albicans* Clone 2-1#1	*C. albicans* Clone 2-1#2	*C. albicans* Clone 2-1#3
Sham	6.40 (0)	4.79 (0)	5.29 (0)	6.90 (0)
6.0	NT[2]	3.92 (0)	3.99* (0)	2.20* (80)
1.5	NT	4.61 (0)	4.87 (0)	2.26* (100)
0.375	2.26* (100)	5.04 (0)	5.27 (0)	2.25* (100)
0.09	2.24* (80)	4.27 (0)	4.91 (0)	2.68* (40)
0.02	5.92 (0)	4.82 (0)	5.57 (0)	6.60 (0)
0.005	6.71 (0)	NT	NT	NT
ED_{90}	0.016 (0.007, 0.04)	>6.0	5.932 (0.399, ∞)	0.006 (0, 0.143)
ED_{99}	0.038 (0.016, 0.087)	>6.0	>6.0	0.027 (0.002, 0.359)

Data from Experiment TOKA-Ca 96-22 (10-Oct-1996), Notebook In Vivo 15, pp. 124-134.

1. DBA/2 mice were infected I.V. with *C. albicans*: MY1055 at 7.2 x 10^4 CFU/mouse, Strain 2-1-#1 at 1.16 x 10^5 CFU/mouse, Strain 2-1 #2 at 1.16 x 10^5 CFU/mouse, and Strain 2-1 #3 at 7.4 x 10^4 CFU/mouse. Caspofungin was administered I.P. b.i.d. x 2 days (4 total doses) at fourfold titrated doses of 0.005 to 6.0 mg/kg/dose. Mice received first dose within 15 to 30 minutes after challenge. The sham-treated control received sterile distilled water. Effective dose 90% and 99% (ED_{90} and ED_{99}) values in mg/kg/dose and 95% confidence intervals ( ) were estimated by comparison of mean log_{10} CFU/g at day 4 after challenge for paired kidneys of treated groups to sham-treated controls by inverse regression analysis to determine which doses reduced organ counts 90 and 99%, respectively (5 mice/group).
2. NT = not tested

In vivo* activity of MK-0991 against *Cryptococcus

C'5 deficient DBA/2N mice were infected with *C. neoformans* (MY 2061 - 4 x 10^5 cfu) by the intravenous route (IND report # 24). Treatment with MK-0991 was initiated 24 hours before infection, by the intraperitoneal route, b.i.d., for 8 days. The activity of MK-0991 was compared with ampB. Animals were sacrificed on the last day of treatment and fungal burden measured in the spleen and brain. MK-0991 at a dose of 10 mg/kg (the highest dose tested) was not effective in reducing the fungal burden (Table 72).

Table 72: Efficacy of L-743,872 and Amphotericin B (AMB) Following I.V. Induction of a Disseminated *Cryptococcus neoformans* MY2061 Infection in DBA/2N Mice[1]

		Mean log_{10} CFU/g Kidney, and Percent Sterilization () for Each Dose (mg/kg)[2]					
Treatment	Organ	Sham	0.31	1.25	2.5	5.0	10.0
L-743,872 I.P. b.i.d.	Brain	5.34 (0)	NT[3]	6.35 (0)	6.04 (0)	5.85 (0)	5.92 (0)
L-743,872 I.P. b.i.d.	Spleen	5.52 (0)	NT[3]	5.39 (0)	5.60 (0)	5.21 (0)	5.52 (0)
Amphotericin B I.P. b.i.d.	Brain	5.84 (0)	2.13 (100)	NT[3]	NT[3]	NT[3]	NT[3]
Amphotericin B I.P. b.i.d.	Spleen	5.52 (0)	2.81 (100)	NT[3]	NT[3]	NT[3]	NT[3]

Experiment: 7 Day TOKSA 93-4 (25 Oct 1993), Notebook In Vivo 12 (TOKA) pp. 1-4.

1. DBA/2N mice were infected I.V. with 4.0 x 10^5 CFU *C. neoformans* MY2061 per mouse. L-743,872 and AMB were administered I.P., b.i.d. for 8 days (16 total doses). Mice received first treatment one day prior challenge and were treated until day 6 after challenge.
2. Mean log_{10} CFU/g at 7 days after challenge for brain and spleen (5 mice/group). Percent sterilization indicated the number of mice with no detectable yeast where limit of detection was 50 yeast cells per organ.

Similar studies were conducted using 2 other stains of *C. neoformans* (MY1051 and MY1146) as well as MY2061 (IND report # 25). In this study, however, treatment was initiated immediately after infection by intraperitoneal route, b.i.d. for 4 days. Here again, MK-0991 was not effective in improving the survival of infected mice as compared to the sham treated group.

Resistance

Serial passaging of a strain of *C. albicans* strain MY1055 was conducted 20 times *in vitro* in the presence of subinhibitory concentrations of the drug (Merck report, 1995, reference # 21). After 20 passages about a 4-fold increase in MICs and MFCs was observed (Table 73). MK-0991 did not significantly alter the susceptibility of *C. albicans*. The morphology of *C. albicans* was also not altered by such a treatment.

Table 73

	L-743,872	
passage#	MIC	MFC
1	0.004	0.030
2	0.008	0.030
3	0.008	0.030
4	0.004	0.016
5	0.008	0.016
6	0.008	0.016
7	0.004	0.016
8	0.004	0.016
9	0.008	0.030
10	0.008	0.016
11	0.008	0.016
12	0.016	0.030
13	0.008	0.030
14	0.008	0.030
15	0.008	0.060
16	0.008	0.060
17	0.008	0.125
18	0.008	0.125
19	0.008	0.125
20	0.008	0.125

In another study, the potential for development of resistance by *C. albicans*, strain MY1055, to MK-0991 (500 ug/ml concentration) was examined in a serial plating system using spiral concentration gradients (reference # 73: MRL report, 2000). 5-FC (25 mg/ml concentration), known to exhibit a high frequency of resistance development, was used as a comparator. The cultures were inoculated on to a series of spiral culture gradients. The radial growth zone or spiral gradient end point was measured after each passage and a loop full of yeast cells at the edge of the growth/no growth zone was used to inoculate fresh medium and cultures grown for 6 to 8 hours. The concentration of the yeast cells was adjusted spectrophotometrically to 1.0 at OD_{600}. A total of 12 or 6 such serial passages were performed for MK-0991 and 5-FC respectively. At the end of each passage the cultures were stored and also plated on to [redacted] agar plates. Passages (P) 13 (2000 ug/ml), 14 (5000 ug/ml), and 15 (2000 ug/ml) were performed at higher concentrations of MK-0991. The identification of *C. albicans* was performed at P15 for MK-0991, and P6 for 5-FC using [redacted] method. Both control or drug exposed cells formed green colonies. *In vitro* susceptibility testing of control and drug exposed yeast cells was performed by the microdilution technique. The results in Table 74A and Figure 21A show that 5-FC induced rapid resistance by 5 serial passages leading to an increase in MIC values by >60-fold. MK-0991 induced an increase in zone diameter, which was evident at the 12th passage (Table 74B and Figure 21B). These results suggest a very low potential for development of resistance to MK-0991.

Table 74: Spiral gradient endpoints obtained with 5-FC and caspofungin

A. 5-FC

Passage #	Stock Concentration* (mg/mL)	Growth Zone (mm)	Change from P_1 (mm)	Reference Notebook/ page #
P_1	25	19	0	N99370/p81
P_2	25	21[b]	2	N99370/p82,97
P_3	25	23[b]	4	N99370/p99
P_4	25	32[b]	13	N99370/p103,104
P_5	25	60 (max)[c]	41	N99370/p116
P_6	25	60 (max)[c]	41	N99370/p118

[a] Concentration of inhibitor dispensed to form gradient
[b] Breakthrough colonies present
[c] Max = Confluent growth over entire area of application
Study Period: May to Jun-1999

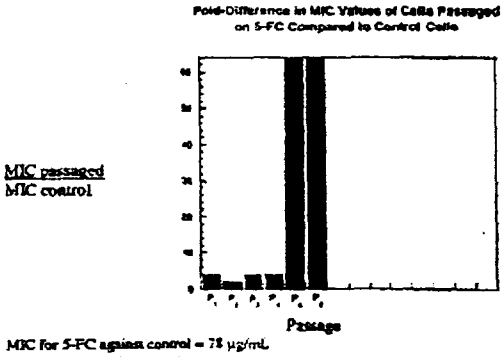
B. Caspofungin

Passage Number	Stock Concentration* (µg/mL)	Growth Zone (mm)	Change from P_1 (mm)	Reference Notebook/ page number
P_1	500	25	0	N99370/p81
P_2	500	26	1	N99370/p82,97
P_3	500	29	4	N99370/p99
P_4	500	32	7	N99370/p103
P_5	500	32	7	N99370/116
P_6	500	32	7	N99370/p118
P_7	500	36	11	N99370/p120
P_8	500	34	9	N99370/p126
P_9	500	38	13	N99370/p127
P_{10}	500	39[b]	14	N99370/p133
P_{11}	500	36[b]	11	N99370/p138
P_{12}	500	60 (max)[c]	45	N99370/p141
P_{13} (control)	2000	15	NA[d]	N99370/p146
P_{13}	2000	45[b]	30	N99370/p146
P_{14} (control)	5000	9	NA[d]	N99760/p8,10
P_{14}	5000	no growth	NA[d]	N99760/p8,10

[a] Concentration of inhibitor dispensed to form gradient
[b] Breakthrough colonies present
[c] Max = Confluent growth over entire area of application
[d] NA = Not applicable
P_{13} = Control cells plated on a spiral plate generated with a 2000 µg/mL stock concentration
P_{14} = Control cells plated on a spiral plate generated with a 5000 µg/mL stock concentration
Batch Number: L-743872-003M024
Study Period: May to Jun-1999

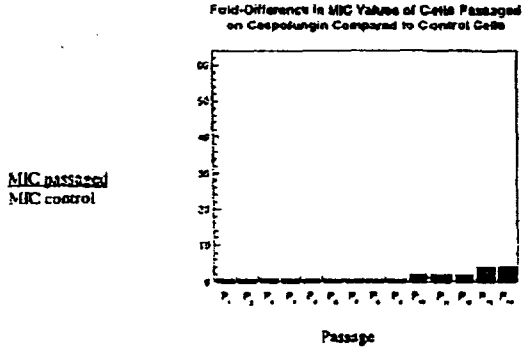
Figure 21

A



MIC for 5-FC against control = 78 µg/mL
Notebook # 99760 pages 46-48
Study Period: May to Aug-1999

B



MIC for caspofungin against control cells = 0.4 µg/mL
Batch Number: L-743872-003M024
Notebook # 99760 pages 46-48
Study Period: May to Aug-1999

The effect of MK-0991 on 6 multi drug resistant (MDR) genes of *C. albicans* was tested *in vitro* using mutant strains (Merck report, 2000, reference # 68). These MDR genes are considered to be important in influencing susceptibility/resistance to some antimicrobial and antitumor agents. In *C. albicans*, CDR1 gene (a member of the family of MDR genes) has been implicated in resistance to FCZ. In this study the strains were obtained from 2 separate sources [redacted] and grown as specified by the respective source. The *in vitro* susceptibility was measured against ampB, FCZ and MK-0991. The characteristics of the strains tested and the 24 hour MIC values are shown in Tables 75A and 76A. MIC1 represents no growth and MIC2 represents partial growth inhibition (since complete inhibition was not observed). It is of note that MK-0991 MIC1 or MIC2 values were not significantly altered for various strains (Tables 75B and 76B). Also, the FCZ MIC1 values were only 4-fold higher for the FCZ resistant strains compared to the susceptible ones. FCZ was not used as a comparator for the [redacted] strains.

Table 75

[redacted] strains.

[redacted]	Fluconazole susceptibility	Relevant Genotype
CAI-4	wild type	CDR1/CDR1 CDR2/CDR2 BEN/BEN
DSY654	hypersusceptible	Scer1/Scer1 Scer2/Scer2
DSY465	wild type	ΔBEN/ΔBEN
CAP1-1	wild type	CDR1/CDR1 CDR2/CDR2 BEN/BEN
CAP4-2	wild type	CDR1/CDR1 CDR2/CDR2 BEN/BEN
DSY448	hypersusceptible	Scer1/Scer1
DSY468	hypersusceptible	Scer1/Scer1 ΔBEN/ΔBEN
DSY653	wild type	Scer2/Scer2

CAI-4 is another wild type strain obtained from Dr. M. Kurtz (Merck)

b: MIC1 results (ug/ml) in CDR1, CDR2, [redacted] strains BEN disrupt and wild type [redacted] strains

[redacted] Medium

Compound	Result type	CAI-4	DSY654	DSY653	CAP1-1	CAP4-2	DSY448	DSY468
Amphotericin B	MIC1	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Fluconazole	MIC2	2	0.5	2	2	2	0.5	2
Caspofungin	MIC2	0.06	0.06	0.06	0.06	0.06	0.06	0.06

[redacted] Medium

Compound	Result type	CAI-4	DSY654	DSY653	CAP1-1	CAP4-2	DSY448	DSY468
Amphotericin B	MIC1	0.25	0.125	0.125	0.125	0.125	0.125	0.125
Fluconazole	MIC2	0.5	0.125	0.5	0.25	0.5	0.125	0.125	0.5
Caspofungin	MIC1	0.125	0.25	0.25	0.25	0.125	0.25	0.25	0.25

Notebook/page: 52462/117-121
Study Period: Dec-1999
Batch No.: caspofungin (L-743872-0003/0011)

Table 76

[redacted] strains

Strain	Relevant Genotype	Fluconazole phenotype
CAI-4	FCR1/FCR1	wild type
CAI4 [pMIC22]	FCR1/FCR1	wild type
FM7	ΔCR1/ΔCR1	resistant
FM7 [pVEC]	FM7 + vector control	resistant
FM7 [pVEC-FCR1]	FM7 + FCR1 plasmid	wild type
CJD21	cap1Δ/cap1Δ	wild type
CJD21 [pMIC22]	CJD21 + vector control	wild type
CJD21 [pMIC22-CAP1]	CJD21 + CAP1 plasmid	wild type
CJD21 [pMIC22-CAP1TR]	CJD21 + CAP1TR Plasmid	resistant
CIB4	cdr3Δ::hisG/cdr3Δ::hisG	wild type
CIB4 [YPB-ADHpi]	CIB4+ vector control	wild type
CIB4 [YPB-ADHpi-CDR3]	CIB4+ vector + CDR3	wild type

b: Echinocandin MIC2 (ug/ml)

[redacted] strains)

Strain	L-733560	Caspofungin
CAI-4	0.25	0.25
CAI4 [pMIC22]	0.125	0.25
FM7	0.25	0.25
FM7 [pVEC]	0.25	0.25
FM7 [pVEC-FCR1]	0.125	0.25
CJD21	0.25	0.25
CJD21 [pMIC22]	0.25	0.25
CJD21 [pMIC22-CAP1]	0.25	0.25
CJD21 [pMIC22-CAP1TR]	0.125	0.25
CIB4	0.125	0.25
CIB4 [YPB-ADHpi]	0.25	0.25
CIB4 [YPB-ADHpi-CDR3]	0.25	0.50

Notebook/page: 52462/140-146, 166
Study Period: Feb to May-2000
Batch No.: caspofungin (L-743872-0003/0011), L-733560-0018/016

The preliminary data (Merck report, 1999, study 2, reference # 18) discussed on page 56 suggests that a potential for development of resistance to MK-0991 *in vivo* exist.

Drug combination

(a) *In vitro*

A combination of MK-0991 with ampB was found to exhibit additive to synergistic activity against *A. fumigatus* and *C. neoformans in vitro* with fractional inhibitory concentrations (FICs) varying from 0.39 to 0.66 (Merck report, 1995, reference # 10; Bartizal et al., 1997, Antimicrob Agents Chemother 41: 2326, reference # 1). Against *C. albicans*, ampB did not alter the *in vitro* susceptibility of MK-0991 (FICs varied from 0.74 to 0.90, Table 77). It was stated that FICs of ≤ 0.5 indicated synergy and > 1 antagonism. No antagonism was observed. It is of note that these results are based on 2 isolates/strains of each species.

Table 77

Fractional fungicidal concentration (FFC averages against *C. albicans* and *C. neoformans* and fractional inhibitory concentration (FIC) averages against *A. fumigatus* strains for L-743,872 combined with AMB.

Organism	L-743,872 plus Amphotericin B FFC or FIC[a]
C. albicans MY1055[b]	0.74
C. albicans MY1750	0.90
C. neoformans MY1051	0.66
C. neoformans MY2061	0.39
A. fumigatus MF5668	0.52
A. fumigatus MF5669	0.39

Notebook: EVAL IV-CP, pp. 11-23, March 31-April 2, 1993.
[a]FIC or FFC: Synergistic: X ≤ 0.5, Additive: 0.5 < X < 4, Antagonistic: X ≥ 4.
[b]For *C. albicans* and *C. neoformans*, MFC values were used to calculate the FFCs and for *A. fumigatus* MIC values were used to calculate FICs.

Similar observations were made in another study by Franzot and Casadevall, 1997 (Antimicrob Agents Chemother 41: 331, reference # 20) using 18 strains of *C. neoformans* var. *neoformans*, and *C. neoformans* var. *gatii*. No antagonistic reaction was observed.

In an abstract (F-35) by Vazquez *et al.*, 1996 (ICAAC, p106, reference # 41), it was stated that "in contrast to the synergistic action of FCZ when exposed to MK-0991, preexposure to FCZ was antagonistic, reducing both the rate and total level of killing by MK-0991." However, the complete details of the experimental design and results were not provided and were requested from the sponsor in October 2000. Additional details provided by the sponsor on December 15, 2000 state that cultures of *C. albicans* were incubated in the absence or presence of FCZ (64 ug/ml) for 5 hours followed by overnight incubation with or without MK-0991 (1 ug/ml). Pre-exposure of some of the strains (number not specified) of *C. albicans* reduced the *in vitro* susceptibility to MK-0991. The actual data were not submitted for review. *C. dubliniensis* was stated to be less susceptible to MK-0991 and prior exposure to FCZ had no effect on the *in vitro* susceptibility to MK-0991. In comparison, *C. stellatoidea* was stated to be susceptible to MK-0991 and *in vitro* susceptibility to MK-0991 was not altered by prior FCZ exposure. It is of note that these cultures were performed at pH 5.5 and therefore the relevance of that finding to the activity *in vivo* is unclear.

(b) ***In vivo:***

In vivo, the effect of a combination of MK-0991 with ampB or FCZ was measured in C'5 deficient DBA/2N mice infected with *C. albicans* (strain MY1055), *A. fumigatus* (strain MF5668), or *C. neoformans* (strain MY2061) (Merck report, 1999, study 7, reference # 16). In a disseminated candidiasis model, 5.8 x 10^4 cfu of the yeast cell suspension were inoculated intravenously and treatment with MK-0991 and/or ampB or FCZ was initiated within 15 minutes of challenge for 4 days. Mice were sacrificed 7 days post infection and kidneys processed for measurement of mycological burden. The results in Table 78 show that treatment with any of the drugs (MK-0991, ampB or FCZ) was effective in reducing the CFU. A combination of MK-0991 with either ampB or FCZ did not show antagonism. There appears to be improved survival in the group administered a combination of low doses of MK-0991 and ampB (0.008 and 0.125 mg/kg, respectively) compared to either drug alone at the same dose. Whether the activity was really synergistic could not be determined from this experiment. The survival was not measured.

Table 78

A. Combination Therapy With Caspofungin and AmB Against a Disseminated *C. albicans* Infection (TOKA) in DBA/2N Mice[1]

Mean Log_{10} CFU/g Kidneys (% Sterilization)[2]

Caspofungin (mg/kg)	AmB (mg/kg)				
	0	0.5	0.125	0.031	0.008
0	6.75 (0)	3.81* (0)	3.10* (20)	4.72* (0)	6.12 (0)
2.0	2.25* (100)	2.22* (100)	2.22* (100)	2.18* (100)	2.22* (100)
0.5	2.19* (100)	2.18* (100)	2.24* (100)	2.19* (100)	2.22* (100)
0.125	2.20* (100)	2.20* (100)	2.20* (100)	2.23* (100)	2.37* (80)
0.031	4.62* (0)	3.09* (20)	3.08* (0)	3.08* (0)	3.16* (40)
0.008	5.92 (0)	3.54* (0)	2.83* (20)	3.39* (0)	3.71* (0)

7D TOKA-Ca 96-8 (04-Mar-1996) Notebook 16 Vivo 15 pg. 47-52.

1. DBA/2N mice (5 mice/group) were infected I.V. with 5.8×10^4 CFU of *C. albicans* MY1055. Mice were treated with either caspofungin alone, AmB alone or both drugs (combination therapy) administered I.P., q.d. Mice received their first treatment within 15 min after challenge and were treated for a total of 4 days (4 total doses of each drug).
2. Mean log_{10} CFU/g at day 7 after challenge for paired kidneys. Percent sterilization indicates the number of mice with no detectable yeast where the limit of detection was 50 cells per pair of kidneys.
* Denotes that the mean was significantly less than the mean control at $p < 0.05$ (Excel t-test).

B. Combination Therapy With Caspofungin and FCZ Against a Disseminated *C. albicans* Infection (TOKA) in DBA/2N Mice[1]

Mean Log_{10} CFU/g Kidneys (% Sterilization)[2]

Caspofungin (mg/kg)	FCZ (mg/kg)				
	0	5.0	1.25	0.31	0.08
0	6.75 (0)	3.99* (0)	3.28* (0)	4.01* (0)	5.20* (0)
2.0	2.25* (100)	2.19* (100)	2.23* (100)	2.23* (100)	2.18* (100)
0.5	2.19* (100)	2.20* (100)	2.21* (100)	2.19* (100)	2.21* (100)
0.125	2.20* (100)	2.25* (100)	2.22* (100)	2.28* (80)	2.18* (100)
0.031	4.62* (0)	4.31* (0)	3.68* (0)	3.12* (0)	3.26* (20)
0.008	5.92 (0)	3.69* (0)	3.03* (0)	4.03* (0)	4.99* (0)

7D TOKA-Ca 96-8 (04-Mar-1996) Notebook 16 Vivo 15 pg. 47-52.

1. DBA/2N mice (5 mice/group) were infected I.V. with 5.8×10^4 CFU of *C. albicans* MY1055.
2. Mice were treated with either caspofungin alone, FCZ alone or both drugs (combination therapy) administered I.P., q.d. Mice received their first treatment within 15 min after challenge and were treated for a total of 4 days (4 total doses of each drug).
3. Mean log_{10} CFU/g at day 7 after challenge for paired kidneys. Percent sterilization indicates the number of mice with no detectable yeast where the limit of detection was 50 cells per pair of kidneys.
* Denotes that the mean was significantly less than the mean control at $p < 0.05$ (Excel t-test).

In the disseminated Aspergillosis infection model, 10^6 cfu of the conidial suspension of *A. fumigatus* were administered intravenously. Treatment with MK-0991 and/or ampB was initiated within 15 minutes of challenge by the intraperitoneal route, for 5 days. Mice were followed for survival for up to day 28 post-challenge. Like in the candidiasis model, no antagonism was observed. There appears to be improved survival in the group administered a combination of low doses of MK-0991 and ampB (0.008 and 0.125 mg/kg, respectively) compared to either drug alone at the same dose (Table 79). Effect on mycological burden was not measured.

Table 79

A. In Vivo Antifungal Efficacy Against a Disseminated *A. fumigatus* MF5668 Infection[1] in DBA/2N Mice at Day 28 After Challenge. Comparison of Therapy With Caspofungin Alone and in Combination With AmB[2]

Percent Survival

Caspofungin (mg/kg/dose)	AmB (mg/kg/dose) 0	0.5	0.125	0.031	0.008
0	0	20	40	10	30
2.0	80	90	100	90	50
0.5	90	100	70	80	50
0.125	70	70	70	50	80
0.031	40	80	90	90	50
0.008	0	90	90	50	0

ED50 AF 96-6 (13-May-1996) Notebook In Vivo 15 pg. 8-15.

1. DBA/2N mice were infected I.V. with 1.0×10^6 conidia/mouse.
2. Mice were treated I.P., q.d. x 5 days. Mice received first treatment within 15 min after challenge. 10 mice/treatment group.

B. Effective Dose 50% (ED_{50}) and 90% (ED_{90}) Values Based on Survival and 95% Confidence Intervals for Caspofungin and AmB Against a Disseminated *A. fumigatus* MF5668 Infection[1] in DBA/2N Mice

ED_{50} values in mg/kg/dose[2]

	Caspofungin	AmB
Day 21	0.056 (0.006, 0.128)	0.148 (0.062, ∞)
Day 28	0.075 (0.030, 0.160)	0.127 (0.052, ∞)

ED_{90} values in mg/kg/dose[2]

	Caspofungin	AmB
Day 21	0.602 (0.236, ∞)	>0.500 (NC)
Day 28	0.619 (0.252, ∞)	>0.500 (NC)

ED50 AF 96-6 (13-May-1996) Notebook In Vivo 15 pg. 8-15.

1. DBA/2N mice were infected I.V. with 1.0×10^6 conidia/mouse. Mice were treated I.P., q.d. x 5 days. Mice received first treatment within 15 min after challenge. 10 mice/treatment group.
2. ED_{50} and ED_{90} values (95% confidence interval) were calculated by Biometrics Research, Rahway and are based on survival. NC = Not calculated. ∞ stands for infinitely large.

In the disseminated *C. neoformans* model, 3.8 to 9 x 10^5 cfu of yeast cells were administered intravenously. Treatment with MK-0991 and/or ampB or FCZ was initiated within 15 minutes of challenge by the intraperitoneal route for 4 days. Mice were sacrificed on day 7 of challenge and brains and spleens processed for measurement of mycological burden. The results in Table 80 show that MK-0991 alone was not effective in reducing cfu. A combination of MK-0991 with ampB did not show any improved activity compared to either drug alone. The effect of a combination of MK-0991 with FCZ also showed no beneficial effect over FCZ alone. No antagonism was observed.

Table 80

A. Combination Therapy With Caspofungin and AmB Against a Disseminated *C. neoformans* Infection (TOBSA) in DBA/2N Mice[1]

Mean Log_{10} CFU/g Brain (% Sterilization)[2]

Caspofungin (mg/kg)	AmB (mg/kg) 0	0.375	0.09	0.02	0.005
0	6.28 (0)	2.13* (10)	4.18* (20)	6.01 (0)	6.39 (0)
24.0	6.73 (0)	2.12* (10)	4.15* (0)	6.41 (0)	6.69 (0)
12.0	6.42 (0)	2.13* (10)	4.67* (0)	6.36 (0)	6.43 (0)
6.0	6.60 (0)	2.25* (10)	3.88* (0)	6.21 (0)	6.79 (0)

Mean Log_{10} CFU/g Spleen (% Sterilization)[2]

Caspofungin (mg/kg)	AmB (mg/kg) 0	0.375	0.09	0.02	0.005
0	4.10 (20)	2.96* (100)	3.75 (20)	4.27 (0)	4.51 (0)
24.0	4.90 (0)	2.81* (100)	3.16* (20)	4.30 (0)	5.19 (0)
12.0	4.31 (0)	2.81* (100)	3.18* (20)	4.22 (0)	4.44 (0)
6.0	4.40 (0)	2.88* (100)	3.38* (20)	3.90* (0)	4.60 (0)

TO TOBSA-Ca 95-5 (24-Oct-1995) Notebook In Vivo 14 pg. 1-4.

1. DBA/2N mice (5 mice/group) were infected I.V. with 9.0 ± 10^5 CFU of *C. neoformans* MY2041.
2. Mice were treated with either caspofungin alone, AmB alone or both drugs (combination therapy) administered I.P., b.i.d. Mice received their first treatment within 15 min after challenge and were treated for a total of 4 days (8 total doses of each drug).
3. Mean log_{10} CFU/g at day 7 after challenge for organs (brain or spleen). Percent sterilization indicates the number of mice with no detectable yeast where the limit of detection was 50 cells per organ.
4. Denotes that the mean was significantly less than the mean control at $p < 0.05$ (Dunnett t-test).

B. Combination Therapy With Caspofungin and FCZ Against a Disseminated *C. neoformans* Infection (TOBSA) in DBA/2N Mice[1]

Mean Log_{10} CFU/g Brain (% Sterilization)[2]

Caspofungin (mg/kg)	FCZ (mg/kg) 0	20.0	5.0	1.25	0.31
0	5.02 (0)	4.29* (0)	4.81 (0)	4.57* (0)	4.96 (0)
20.0	5.63 (0)	3.95* (0)	4.86 (0)	5.37 (0)	5.06 (0)
5.0	5.18 (0)	4.22* (0)	4.31* (0)	4.79* (0)	4.82 (0)
1.25	5.43 (0)	4.34* (0)	4.48* (0)	4.67 (0)	4.48* (0)

Mean Log_{10} CFU/g Spleen (% Sterilization)[2]

Caspofungin (mg/kg)	FCZ (mg/kg) 0	20.0	5.0	1.25	0.31
0	4.57 (0)	3.71* (0)	4.74 (0)	4.07 (0)	3.81* (0)
20.0	4.70 (0)	3.37* (20)	4.74 (0)	4.55 (0)	4.45 (0)
5.0	4.30 (0)	3.83* (0)	4.09 (0)	3.87* (0)	4.06 (0)
1.25	4.85 (0)	4.29 (20)	3.95* (0)	3.90 (0)	4.13 (0)

TO TOBSA-Ca 96-1 (26-Mar-1996) Notebook In Vivo 15 pg. 1-6.

1. DBA/2N mice (5 mice/group) were infected I.V. with 3.8 ± 10^5 CFU of *C. neoformans* MY2041.
2. Mice were treated with either caspofungin alone, FCZ alone or both drugs (combination therapy) administered I.P., q.d. Mice received their first treatment within 15 min after challenge and were treated for a total of 4 days (4 total doses of each drug).
3. Mean log_{10} CFU/g at day 7 after challenge for organs (brain or spleen). Percent sterilization indicates the number of mice with no detectable yeast where the limit of detection was 50 cells per organ.
4. Denotes that the mean was significantly less than the mean control at $p < 0.05$ (Dunnett t-test).

The Label (proposed by the sponsor):

MICROBIOLOGY

Mechanism of Action

Caspofungin acetate, the active ingredient of CANCIDAS, inhibits the synthesis of β (1,3)-D-glucan, an essential component of the cell wall of filamentous fungi and yeast. β (1,3)-D-glucan is not present in mammalian cells.

Activity in vitro

Susceptibility testing was performed according to the National Committee for Clinical Laboratory Standards (NCCLS) method M38-P Standardized susceptibility testing methods for β (1,3)-D-glucan synthesis inhibitors have not been established, and results of susceptibility studies do not correlate with clinical outcome.

Activity in vivo

Drug Interactions

Studies *in vitro* and *in vivo* of caspofungin in combination with amphotericin B no antagonism of antifungal activity against *A. fumigatus*

The clinical significance of these results are unknown.

CONCLUSIONS:

Mechanism of Action:

MK-0991 inhibits the activity of the enzyme glucan synthase from *Aspergillus fumigatus* with a 50% inhibitory concentration (IC_{50}) value of 9.6 nM. The IC_{50} values against *Candida albicans* and *Cryptococcus neoformans* were 0.6 nM and 2.5 μM, respectively. The enzyme glucan synthase, is important in the synthesis of β-(1,3)-D-glucan from glucose. Glucan is an important constituent of the cell wall of many fungi. The proportion of this polysaccharide in the walls of different fungi varies which may explain the variable effect of the drug against various species. Even though MK-0991's ultimate activity is specific for the glucan component of the fungal cell wall, this is accomplished by the drug's more immediate action on a cell membrane enzyme modulated by a FKS1 gene.

It is also of note that cilofungin, an analogue of echinocandin, was shown to decrease ergosterol content by 55 – 60 % and glucan content by >70% after incubation of *C. albicans* with the drug for 18 hours. There was a minimal effect (4-13% reduction) on lanosterol. Chitin and mannan content were increased. It is unclear whether the changes in chitin, mannan and ergosterol are a direct effect of cilofungin or a consequence of a alteration in glucan content leading to dysregulation of carbohydrate synthesis that leads to changes in the integrity of the cell membrane. Whether MK-0991 or other echinocandins will have a similar effect is unknown.

Activity against *Aspergillus in vitro*:
MK-0991, like other echinocandins, does not show traditional inhibition of growth against *Aspergillus* species. The minimum effective concentration (MEC) which altered the morphology of the hyphae of various species, varied from 0.06 to >2 µg/ml (Table 81).

Table 81

Species	Incubation (Hours)	MEC (µg/ml)					
		Arikan *et al.*, 1999			Espinel-Ingroff, 2000		
		Range	(GM)	N	Range	(GM)	N
A. fumigatus	24		(0.29)	26		(0.29)	55
	48		(0.3)	26			
	72		(0.28)	26			
A. flavus	24		(0.27)	27		(0.24)	13
	48		(0.31)	27			
	72		(0.26)	27			
A. niger	24		(0.41)	17		(0.16)	10
	48		(0.42)	17			
	72		(0.42)	17			
A. terreus	24		(0.5)	9		(0.12)	11
	48		(0.5)	9			
	72		(0.5)	9			
A. nidulans	24					(0.42)	13
	48		(0.5)	3			
	72		(0.5)	3			

GM = geometric mean

Staining with fluorescein dyes which penetrate the cell based on viability and non viability (CFDA, and $DiBAC_4(3)$, respectively] showed that MK-0991 (0.3 µg/ml, for 6 hours) was lytic at the selective areas of the fungal cell 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 was attributed to ampB's disruption of membrane activity.

Standard broth dilution methods utilized for bacteria and yeasts are technically difficult to perform for filamentous fungi, therefore, *in vitro* susceptibility testing relies on a qualitative visual scale to quantitate growth inhibition. Using the NCCLS proposed method (M-38P), a substantial reduction in growth was used as an end-point. This was termed as MIC-2 (≥ 50% inhibition of growth) or MIC-80 (≥ 80% inhibition of growth). The *in vitro* susceptibility was measured in 5 different laboratories including the sponsor's laboratory. The results of studies from 2 different laboratories are summarized in Table 82. Over all, 220 clinical isolates of *A. fumigatus*, 94 of *A. flavus*, 41 of *A. niger*, 51 of *A. terreus*, and 20 of *A. nidulans* were

tested (Table 83). These include over 90 clinical isolates collected from 36 patients enrolled in the study 019 [*A. fumigatus* (80), *A. flavus* (H), *A. niger* (4), and *A. terreus* (3)]. It is of note that the *in vitro* activity of the drug varied with the medium, the concentration of the conidial suspension, and the incubation time. The usefulness of the MIC-2 or MIC-80 values in predicting clinical outcome has not been established.

Table 82: *In vitro* susceptibility of *Aspergillus* species isolates to MK-0991 in 2 different laboratories†

Species	Espinel-Ingroff, 2000 (MIC-2, μg/ml)				Arikan *et al.*, 1999 (MIC-80, μg/ml)	
	Range	(n)	MIC_{90}	Geometric Mean	Range (n)	Geometric Mean
A. fumigatus	[redacted]	(56)	0.5	0.25	[redacted] (26)	0.7
A. flavus		(13)	0.2	0.2	(27)	2.7
A. niger		(10)	0.2	0.14	(17)	0.4
A. terreus		(11)	0.2	0.12	(9)	0.5
A. nidulans		(13)	0.5	0.44	(3)*	0.6*

† NCCLS M-38P protocol using RPMI medium (24 hour read, 35°C. MIC in the Espinel-Ingroff study represent ≥50% inhibition in growth (MIC-2) and in Arikan *et al.* study ≥80% inhibition of growth (MIC-80); MIC_{90} represents concentration of the drug effective in inhibiting 90% of the isolates

*represent 48 and 72 hour values; no growth observed at 24 hours

Table 83: A summary of the total number of isolates for various *Aspergillus* species.
The results represent number of isolates (MIC_{90} or geometric mean*).

Reference	*A. fumigatus*	*A. flavus*	*A. niger*	*A. terreus*	*A. nidulans*
Arikan *et al.*, 1999*	26 (0.7)	27 (2.7)	17 (0.4)	9 (0.5)	3 (0.6)
Espinel-Ingroff, 1998*	13 (2.2)	11 (0.5)	-	2 (0.5)	-
Espinel-Ingroff, 2000	52 (0.5)	12 (0.2)	10 (0.2)	12 (0.2)	12 (0.5)
Del Poeta *et al.*, 1997*	8 (≤0.09)	8 (0.2)	-	-	-
Rinaldi, 2000	41 (≤0.125)	25 (≤0.125)	10 (≤0.125)	25 (≤0.125)	5 (≤0.125)
Merck, 2000	80 (0.5)	11 (>64)	4 (≤0.03-0.125)#	3 (0.06-0.5)#	-
Total	220	94	41	51	20

represents range since number of isolates was too low (<10) to calculate MIC_{90}

The patients enrolled in the clinical trial were refractory to or intolerant of therapies presently approved for the treatment of Aspergillosis. For details about the criteria used for appropriateness of categorizing patients as refractory to previous antifungal therapy, see Medical Officer's review. It is of note that *in vitro* susceptibility of the clinical isolates at baseline, during or after treatment did not correlate with clinical outcome.

Activity against *Aspergillus* in *vivo*:
The *in vivo* activity of MK-0991 against *Aspergillus* species (*A. fumigatus* and *A. flavus*) was measured in immunocompromised animals.

Activity against *A. fumigatus* was measured in mice, rats, and rabbits. Strain MF5668 was used for infecting C'5 deficient, neutropenic, and pancytopenic mice with disseminated or pulmonary aspergillosis. In a study done in pancytopenic rats and rabbits with pulmonary aspergillosis, strain H11-20 and NIH isolate # 4215, respectively, were used for infection.

In C'5 deficient mice, administration of MK-0991 was initiated either at the time of challenge or 24 hours later. The activity was measured by following the survival rate. The results show that MK-0991 was effective in improving the survival rate and reducing mycological burden.

In neutropenic rabbits infected with *A. fumigatus*, MK-0991 at a dose of 1, 3, or 6 mg/kg/day for 12 days were not effective in reducing mycological burden in the lung. At a low dose (1 mg/kg/day), MK-0991 was effective in reducing the lung weight and the infarct score. The higher doses of MK-0991 were less effective in reducing lung infarction. AmpB was, however, most effective in reducing mycological burden, infarct score or the lung weight although none of the rabbits treated with ampB survived the 12 days of treatment. The mean survival time was 10.4 days in MK-0991 (1mg/kg/day) treated rabbits as compared to 6.9 and 8.8 days in the untreated control and ampB treated rabbits, respectively. These comments are based on a preliminary report at the present time.

In neutropenic mice with pulmonary aspergillosis, both MK-0991 and ampB were not effective in improving survival. The difference between the results of this study compared to the remaining 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.

In immunosuppressed rats with pulmonary aspergillosis, MK-0991 was effective in improving survival. The activity of MK-0991 was comparable to ampB. Additional testing in animals infected with different concentrations of the inoculum (10^4 to 10^8) and initiating treatment 24 to 48 hours post-infection would have been helpful in understanding the activity of the drug.

The effect on mycological burden was measured in only kidney tissues from mice with disseminated aspergillosis. MK-0991 was effective in reducing mycological burden (based on cfu and histological findings) on days 4, 15 and 28. The results of mycological burden on day 8 are less clear since one untreated animal was negative for cfu but had histological evidence of infection in 2 of the 4 sections screened. The correlation between cfu and histological evidence of infection is not clear. This may be limited by the lower limit of detection of the plating assay and/or the evaluation of a very small portion of the tissue (approximately 4 sections) for histological evidence of infection. Overall, the activity of MK-0991 was comparable to ampB.

There are limitations to culture and plating methods for filamentous fungi and therefore the measurements of cfu are not precise. Efforts are being made to improve such methods. In a recent preliminary study conducted in C'5 deficient mice, the mycological burden was measured by standard culture techniques as well as Taqman® assay. TaqMan® assay is based on the detection of 18S ribosomal RNA (rRNA) of *A. fumigatus*. MK-0991, like ampB, was effective in reducing cfu and rRNA. Whether similar effects will be observed in severely infected and/or immunocompromised animals is not known.

Against *A. flavus* infection in C'5 deficient mice, MK-0991 at a dose of 0.31 mg/kg/day for 5 days was marginally more protective than AmpB. The effect of MK-0991 on mycological burden in animals infected

with *A. flavus* was not measured. It is unclear whether the strain of *A. flavus* used for infection was more susceptible to MK-0991. The activity of MK-0991 *in vivo* against other species of *Aspergillus* (*A. niger*, *A.nidulans*, and *A. terreus*) was not examined.

Overall, the studies *in vitro* and *in vivo* show that MK-0991 is effective in inhibiting the areas of fungal cell with active cell growth. However, the activity against *Aspergillus* species is not cidal. It is possible that the selective activity of MK-0991 against the areas of active cell growth may allow the regions of less active growth to grow. Such an effect would lead to an improved survival in animals infected with *A. fumigatus* but not reducing the mycological burden. Due to the limitations of the plating methods for filamentous fungi the effect on mycological burden was not established. In addition, the fact that at the concentrations achievable in plasma MK-0991, unlike ampB, does not exhibit complete inhibition of growth *in vitro* shows MK-0991 to be less active than ampB.

Activity against *Candida in vitro:*

As stated above (page 2), 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 NCCLS. The usefulness of these methods in predicting clinical outcome for a new class of antifungal agent is currently under investigation.

In vitro, MK-0991 was shown to be effective against some of the *Candida* species. The MIC values against various species of *Candida* varied with the period of incubation, the medium used, and the concentration of the inoculum. The results show lower MK-0991 MICs in [redacted] medium as compared to [redacted] for various isolates of *Candida* species. The relevance of such an observation to the activity *in vivo* is unknown. It is hypothesized that [redacted] medium supplemented with [redacted] is hypertonic and may protect the yeast cells from lysis by MK-0991.

The results of *in vitro* susceptibility testing against various *Candida* species were available from 4 different laboratories and are summarized in Table 84. The results show lowest MICs (≤ 2 ug/ml) against *C. albicans, C. lusitaniae, C. krusei, C. kefyr, C. glabrata,* and *C. lipolytica*. MICs against *C. guilliermondii* and some of the isolates of *C. tropicalis* were high (> 8 ug/ml). It is also of note that the number of isolates tested for all the non albicans species is <100. No correlation was observed between clinical outcome and *in vitro* susceptibility.

A panel of *C. albicans* isolates resistant to other anti-fungal agents was shown to be susceptible to MK-0991. The relevance to the activity *in vivo* is currently under investigation.

The MFCs against various species were found comparable to MIC values. The time to kill *C. albicans*, *C. tropicalis* or *C. glabrata* was shown to be slower for MK-0991 as compared to ampB. Also, the fungicidal effect was shown to vary with the concentration of the drug, time of incubation, the species and the isolate tested.

The PAEF of MK-0991 at a concentration of ≥ 1X MIC was comparable to that of ampB at 0.25-0.5X MIC. A 1 hour of exposure to MK-0991 or ampB at the concentrations specified above exhibited a PAEF of > 12 hours. Incubations of less than an hour were less effective. FCZ did not exhibit any PAEF.

MK-0991 is a protein-binding compound. Presence of human or mouse serum between the concentrations of 0 - 50% did not significantly alter the MIC values of MK-0991 against *C. albicans*.

Table 84: Summary of in vitro susceptibility data from different studies. Results represent number of isolates of various *Candida* species (MIC_{90} – ug/ml)*

Reference	*C. albicans*	*C. tropicalis*	*C. parapsilosis*	*C. lusitaniae*	*C.guilliermondii*
Bartizal *et al.*, 1997 (#1, 6)	40 (0.5)	20 (1.0)	20 (0.5)	20 (0.5)	20 (2.0)
Espinel-Ingroff, 1998 (#11)	20 (1.0)	12 (1.0)	12 (2.0)	12 (2)	ND
Vazquez *et al.*, 1997 (#8)	50 (0.4)	10 (0.4)	7 (0.2)	5 (0.8)	5 (1.6)
Merck, 2000					
Protocol 003 (# 69)	283 (1.0)	6 (0.25->8)**	5 (1-2)**	ND	12 (>64)
Protocol 004 (#70)	173 (1.0)	3 (1->8)	3 (>8)	1 (1.0)	14 (>8)
Protocol 007 (#78)	20 (1.0)	ND	ND	ND	ND
Total:	586	51	47	38	51
Merck, 2000 (#62) (Protocol 003, 004, 007, 014, 020)	771 (1.0)	31 (2.0)	16 (>8)	1 (1.0)	44 (>8)

Table 84 continued:

Reference	*C. krusei*	*C. pseudotropicalis*	*C. glabrata*	*C. kefyr*	*C. lipolytica*
Bartizal *et al.*, 1997 (#1, 6)	20 (2.0)	20 (0.5)	20 (1.0)	ND	ND
Espinel-Ingroff, 1998 (#11)	13 (2.0)	ND	12 (1.0)	ND	ND
Vazquez *et al.*, 1997 (#8)	5 (0.4–0.8)	ND	21 (0.4)	5 (0.05-0.4)	ND
Merck, 2000					
Protocol 003 (# 69)	8 (1-2)	ND	7 (0.5-2)	1 (0.5)	2 (2)
Protocol 004 (#70)	4 (2.0)	ND	12 (1-2)	ND	2 (2)
Protocol 007 (#78)	ND	ND	1 (2.0)	ND	ND
Total:	50	20	73	6	4
Merck, 2000 (#62) (Protocol 003, 004, 007, 014, 020)	18 (2.0)	ND	74 (2.0)	1 (0.5)	7 (0.5-2)

Pfaller *et al.*, 1999 (#9) : 71 isolates of ***C. dubliensis*** were tested with MIC_{90} of 0.5 ug/ml
ND not done
*The MIC_{90} values were determined if the number of isolates≥ 10.
** represents range of MICs for isolates where MIC_{90} values were not determined.

Activity against *Candida in vivo:*

Activity against *Candida* was measured *in vivo* in immunocompetent and immunocompromised (complement C'5 deficient, neutropenic, and CD4 deficient) mice by measurement of survival and/or mycological burden. The measurement of mycological burden was limited to kidney tissues in a majority of the studies.

In C'5 deficient mice, MK-0991 was shown to be effective in improving survival and reducing fungal burden in the kidneys. In one of the studies, tissues other than kidneys were processed for mycological burden. A dose of 0.375 mg/kg/day was more effective than ampB in sterilization of kidney and brain tissues whereas ampB was more effective than MK-0991 in sterilization of liver and spleen tissues. MK-0991 was found to be effective at a lower dose (0.09 mg/kg) when administered by the intraperitoneal route as compared to the oral route (12.5 mg/kg). Intravenous route of drug administration at the doses tested (0.375 mg/kg) was

effective in reducing the microbial burden. MK-0991 also improved the survival of mice infected with a higher dose of *C. albicans*, which would otherwise cause mortality within 24 hours of infection.

The effectiveness of MK-0991 against other species of *Candida*, which includes *C. tropicalis*, *C. lustitanae*, *C. krusei* and *C. parapsilosis* was measured in the C'5 deficient murine model. The activity of MK-0991 against different *Candida* species showed variable activity (Table 85). For example, MK-0991 was effective in reducing the mycological burden with ≥ 60% sterilization of the kidneys from mice with either *C. albicans* or *C. tropicalis* infection. On the other hand, in *C. lustitaniae*, *C. krusei*, *C. glabrata*, or *C. parapsilosis* infected mice, MK-0991 was effective in reducing fungal burden but not in sterilization. This could be due to differences in virulence of the isolates or a reflection of a variable glucan contents among various fungal species.

Table 85

Species	Activity *in vtro* MIC (ug/ml)	IV* infectious dose (cfu x 10^4)	Activity *in vivo* MK-0991	AmpB	FCZ
C. albicans MY1750	-	4.0×10^4	+	±	ND
C. albicans MY1585	0.25	1.68×10^4	+	+	ND
C. albicans CLY538	0.25	1.0×10^5	+	ND	±
C. tropicalis MY1124	0.25	5.2×10^5	+	+	ND
C. tropicalis MY1163	0.25	1.3×10^5	+	±	ND
C. tropicalis CLY545	0.125	3.6×10^5	+	ND	-
C. parapsilosis MY1943	1.0	1.2×10^7	±	±	ND
C. lusitaniae MY1396	0.5	1.32×10^7	±	±	ND
C. glabrata MY1381	0.25	1.36×10^8	±	±	ND
C. glabrata MY1382	0.25	1.48×10^8	±	+	ND
C. krusei CK4935	1.0	8.6×10^7	±	ND	-

* IV= intravenous route ND = not done
+ = ↓ mycological burden in kidneys and ≥60 % sterilization;
± = ↓ mycological burden but no sterilization;
- = no activity as measured by mycological burden and sterilization

Studies in neutropenic mice infected with *C. albicans* indicate that MK-0991 was effective in improving survival and reducing mycological burden in the kidneys. Survival rate was better in neutropenic mice treated with MK-0991 as compared to ampB or FCZ. Organs other than kidneys were not tested. In comparison to immunocompetent mice, a higher dose of MK-0991 was effective in conferring protection in neutropenic mice.

In immunosuppressed mice infected with a FCZ resistant strain of *C. albicans*, MK-0991 was effective in improving survival and reducing mycological burden in the kidneys. In immunosuppressed mice infected with *C. krusei*, MK-0991 was effective in improving survival and reducing mycological burden in the kidneys but not in the spleen. Also, the reduction in mycological burden varied with the immune status of the host. In immunocompromised mice infected with *C. glabrata*, MK-0991 was effective in reducing mycological burden in kidneys but not in the spleen. No mortality was observed in the infected mice.

In CD4 deficient mice with oropharyngeal and gastrointestinal candidiasis treatment with MK-0991 demonstrated a reduction in residual *C. albicans* burden in the fecal samples and oral swabs. In this model, the oral route of drug administration was found to be more effective than the intraperitoneal route.

No study was done to measure the activity of MK-0991 against *C. guilliermondii* in animals.

Activity against *Cryptococcus in vitro* and *in vivo:*
MK-0991 exhibited no activity against *Cryptococcus neoformans in vitro.* The *in vitro* MIC values varied from 4 - 16 ug/ml. *In vivo, the* activity against *Cryptococcus* was measured in C'5 deficient mice. MK-0991 did not improve the survival rate nor was effective in reducing the fungal burden.

Activity against other fungal species:
Activity of MK-0991 against some of the other fungal species (*Fusarium, Histoplasma, Pseudallescheria, Trichosporum,* and *Blastomyces*) was measured *in vitro* only against a very small number of isolates (see Tables 6, 7, 10, and 28). The results show minimal to no effect against these species.

Resistance:
A potential for development of resistance was examined using a strain of *C. albicans* (1) by serial passaging 20 times *in vitro* in the presence of subinhibitory concentrations of the drug, and (2) using serial plating and spiral concentration gradients. After 20 serial passages *in vitro* the MK-0991 MICs and MFCs were not significantly altered. Studies using the serial plating and spiral concentration gradient technique showed a slight increase in zone diameter after 12 passages. Such an increase was very small compared to 5-FC. The relevance of this observation to the development of resistance is unclear.

Preliminary studies *in vivo* suggest that MK-0991 does have a potential for inducing resistance. It is possible that the mutations of FKS1 gene can reduce the synthesis of β (1,3)-D-glucan and cause resistance to echinocandins.

Cross-resistance:
A panel of clinical isolates of *C. albicans*, which were resistant to other anti-fungal agents (ampB, 5-FC, and/or FCZ), were shown to be susceptible to MK-0991. It is of note that a majority of the isolates were resistant to FCZ. Very few isolates resistant to ampB, or 5-FC were tested.

Activity of MK-0991 was also tested against laboratory derived echinocandin resistant isolates of *C. albicans* and *C(T). glabrata.* The echinocandin resistant isolates were less susceptible to MK-0991. However, it is unclear which echinocandin was used for inducing resistance.

Isolates collected from mice treated with MK-0991 were shown to be susceptible to FCZ and ampB.

Drug combination:
A combination of MK-0991 with ampB was found to exhibit additive activity against *C. albicans* and synergistic effects against *A. fumigatus* and *C. neoformans in vitro.* Studies *in vivo* show that a combination of MK-0991 with ampB or FCZ did not exhibit any antagonism. However, no synergism was observed in mice infected with *C. albicans* or *A. fumigatus.*

Cidal vs. static effect:
Against *Candida* the activity of MK-0991 may be fungicidal or fungistatic depending on the species, isolate and test conditions. Against *Aspergillus* species, however, MK-0991 is not cidal.

The Label (changes recommended):

The clinical indication being pursued at the present time is only for the treatment of refractory or intolerant Aspergillosis. This review includes a description of studies conducted to measure the activity of the drug against fungal species other than *Aspergillus*. As mentioned above (page 5), these studies are helpful in understanding the antifungal effect of MK-0991. However, the correlation of activity *in vitro* with clinical outcome for the treatment of candidiasis is currently under investigation. A description of the activity against *Candida* species in the label at the present time would promote off-label use. Therefore, no reference to the activity of MK-0991 against species other than *Aspergillus* should be made in the label.

At the present time the criteria used for listing of species is limited to species that are clinically indicated and also the number of isolates to be tested should be about 100. Based on the information discussed above, it is clear that the number of isolates for all the *Aspergillus* species other than *A. fumigatus* is less than 100. For *A. flavus*, the number of isolates tested was close to 100 (see Table 83). Therefore, *Aspergillus niger, Aspergillus nidulans, and Aspergillus terreus* should not be listed in the microbiology section of the label.

Studies show that that MK-0991is effective in regions of active growth of the hyphae of *A. fumigatus*. The activity of caspofungin against *A. fumigatus* is not cidal. Such a statement should be made in either the 'Mechanism of Action' or the 'Activity *in vitro*' sections in the Microbiology section of the label.

RECOMMENDATIONS:

This NDA is approvable pending an accepted version of the label.

/S/

Shukal Bala
Microbiologist, HFD-590

CONCURRENCES:

/S/

HFD-590/Deputy Dir. _____ Signature 1/12/01 Date
HFD-590/SMicro /S/ Signature 1/12/01 Date
CC:
HFD-590/Original NDA
HFD-590/Division File
HFD-590/MO
HFD-590/Pharm
HFD-590/Chem
HFD-590/Review Micro/BalaS
HFD-590/CSO/ChanL

REVIEW TO HFD-590
OFFICE OF NEW DRUG CHEMISTRY
- MICROBIOLOGY STAFF/HFD-805
MICROBIOLOGY REVIEW #1 OF NDA

8 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: na
5. ASSIGNED FOR REVIEW: June 23, 2000
6. SUPPORTING/RELATED DOCUMENTS:

C. REMARKS: This application was submitted electronically. This review was performed using a pre-submission hard copy of the Sterility Assurance section. The electronic version of the NDA was examined to ensure that it did not differ from the pre-submission hard copy as well as to review the stability protocol.

D. CONCLUSIONS: This submission is approvable pending resolution of microbiology deficiencies. Please see "Microbiologist's List of Deficiencies" at the end of this review.

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.