CENTER FOR DRUG EVALUATION AND RESEARCH

Approval Package for:

APPLICATION NUMBER:

21-227 / S-014
21-227 / S-011

Trade Name: **Cancidas**

Generic Name: **Casofugin acetate**

Sponsor: **Merck**

Approval Date: **March 17, 2004**

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-227 / S-014
21-227 / S-011

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CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-227 / S-014
21-227 / S-011

APPROVAL LETTER

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20857

NDA 21-227/S-011, S-014

Merck & Co., Inc.
Attention: Tamra L. Goodrow, Ph.D.
Director, Regulatory Affairs
BLA-20
P.O. Box 4
West Point, PA 19486-0004

Dear Dr. Goodrow:

Please refer to your supplemental new drug application (S-011) dated June 5, 2003, received June 6, 2003, submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for Cancidas™ (caspofungin acetate) Injection, 50 mg/vial, 70 mg/vial.

We acknowledge receipt of your submissions dated December 8, 2003 and March 2, 2004.

Your submission of January 29, 2004 constitutes a complete response to our December 4, 2003 letter.

This supplemental new drug application provides for the following revision to the package insert (additions are double underlined and deletions are ~~strikethrough~~):

MICROBIOLOGY

- The *Drug Resistance* subsection was revised to read:

Drug Resistance
~~A study in mice infected with *C. albicans* and treated with orally administered doses of caspofungin suggests that there is a potential for resistance development to occur. *In vitro* resistance development to caspofungin by *Aspergillus* species has not been studied. In limited clinical experience, drug resistance in patients with invasive aspergillosis has not been observed~~. The incidence of drug resistance by various clinical isolates of *Candida* and *Aspergillus* species is unknown.

Drug Resistance
Mutants of *Candida* with reduced susceptibility to caspofungin have been identified in some patients during treatment. Similar observations were made in a study in mice infected with *C. albicans* and treated with orally administered doses of caspofungin. MIC values for caspofungin should not be used to predict clinical outcome, since a correlation

between MIC values and clinical outcome has not been established. The incidence of drug resistance by various clinical isolates of *Candida* and *Aspergillus* species is unknown.

Please refer also to your supplemental new drug application (S-014) dated February 11, 2004, received February 12, 2004, submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for Cancidas™ (capsofungin acetate) for Injection, 50 mg/vial, 70 mg/vial.

This supplemental new drug application provides for the following revision to the package insert (additions are double underlined and deletions are ~~strikethrough~~):

ADVERSE REACTIONS

- The *General* subsection was revised to read:

  General

  Possible histamine-mediated symptoms have been reported including ~~isolated~~ reports of rash, facial swelling, pruritus, sensation of warmth, or bronchospasm. Anaphylaxis has been reported during administration of CANCIDAS.

We have completed the review of these supplemental applications, as amended, and have concluded that adequate information has been presented to demonstrate that the drug product is safe and effective for use as recommended in the final printed label submitted on March 2, 2004 (enclosed). Accordingly, these supplemental applications are approved effective on the date of this letter.

If a letter communicating important information about this drug product (i.e., a "Dear Health Care Professional" letter) is issued to physicians and others responsible for patient care, we request that you submit a copy of the letter to this NDA and a copy to the following address:

MEDWATCH, HF-2
FDA
5600 Fishers Lane
Rockville, MD 20857

We remind you that you must comply with the requirements for an approved NDA set forth under 21 CFR 314.80 and 314.81.

If you have any questions, call Robin Anderson, R.N., M.B.A., Labeling Reviewer, at (301) 827-2127.

Sincerely,

{See appended electronic signature page}

Renata Albrecht, M.D.
Director
Division of Special Pathogen and Immunologic Drug Products
Office of Drug Evaluation IV
Center for Drug Evaluation and Research

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/s/

Renata Albrecht
3/17/04 10:45:51 PM

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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APPROVABLE LETTER

Within 10 days after the date of this letter, you are required to amend this supplemental application, notify us of your intent to file an amendment, or follow one of your other options under 21 CFR 314.110. In the absence of any such action FDA may proceed to withdraw this supplemental application. Any amendment should respond to all the deficiencies listed. We will not process a partial reply as a major amendment nor will the review clock be reactivated until all deficiencies have been addressed.

This product may be considered to be misbranded under the Federal Food, Drug, and Cosmetic Act if it is marketed with these changes prior to approval of this supplemental application.

If you have any questions, call Robin Anderson, Labeling Reviewer, at (301) 827-2127.

Sincerely,

{See appended electronic signature page}

Renata Albrecht, M.D.
Director
Division of Special Pathogen and Immunologic Drug Products
Office of Drug Evaluation IV
Center for Drug Evaluation and Research

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/s/

Renata Albrecht
12/4/03 04:01:44 PM

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-227 / S-014
21-227 / S-011

LABELING

INTRAVENOUS INFUSION (not for IV Bolus Injection)

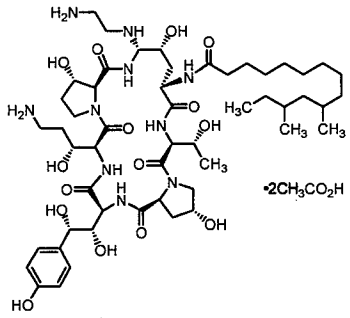
CANCIDAS®

(caspofungin acetate) FOR INJECTION

DESCRIPTION

CANCIDAS* is a sterile, lyophilized product for intravenous (IV) infusion that contains a semisynthetic lipopeptide (echinocandin) compound synthesized from a fermentation product of *Glarea lozoyensis.* CANCIDAS is the first of a new class of antifungal drugs (glucan synthesis inhibitors) that inhibit the synthesis of β (1,3)-D-glucan, an integral component of the fungal cell wall.

CANCIDAS (caspofungin acetate) is 1-[(4*R*,5*S*)-5-[(2-aminoethyl)amino]-N^2-(10,12-dimethyl-1-oxotetradecyl)-4-hydroxy-L-ornithine]-5-[(3*R*)-3-hydroxy-L-ornithine] pneumocandin B_0 diacetate (salt). In addition to the active ingredient caspofungin acetate, CANCIDAS contains the following inactive ingredients: sucrose, mannitol, acetic acid, and sodium hydroxide. Caspofungin acetate is a hygroscopic, white to off-white powder. It is freely soluble in water and methanol, and slightly soluble in ethanol. The pH of a saturated aqueous solution of caspofungin acetate is approximately 6.6. The empirical formula is $C_{52}H_{88}N_{10}O_{15}•2C_2H_4O_2$ and the formula weight is 1213.42. The structural formula is:



CLINICAL PHARMACOLOGY

Pharmacokinetics

Distribution

Plasma concentrations of caspofungin decline in a polyphasic manner following single 1-hour IV infusions. A short α-phase occurs immediately postinfusion, followed by a β-phase (half-life of 9 to 11 hours) that characterizes much of the profile and exhibits clear log-linear behavior from 6 to 48 hours postdose during which the plasma concentration decreases 10-fold. An additional, longer half-life phase, γ-phase, (half-life of 40-50 hours), also occurs. Distribution, rather than excretion or biotransformation, is the dominant mechanism influencing plasma clearance. Caspofungin is extensively bound to albumin (~97%), and distribution into red blood cells is minimal. Mass balance results showed that approximately 92% of the administered radioactivity was distributed to tissues by 36 to 48 hours after a single 70-mg dose of [^{3}H] caspofungin acetate. There is little excretion or biotransformation of caspofungin during the first 30 hours after administration.

Metabolism

Caspofungin is slowly metabolized by hydrolysis and N-acetylation. Caspofungin also undergoes spontaneous chemical degradation to an open-ring peptide compound, L-747969. At later time points (≥5 days postdose), there is a low level (≤7 picomoles/mg protein, or ≤1.3% of administered dose) of covalent binding of radiolabel in plasma following single-dose administration of [^{3}H] caspofungin acetate, which may be due to two reactive intermediates formed during the chemical degradation of caspofungin to L-747969. Additional metabolism involves hydrolysis into constitutive amino acids and their degradates, including dihydroxyhomotyrosine and N-acetyl-dihydroxyhomotyrosine. These two tyrosine derivatives are found only in urine, suggesting rapid clearance of these derivatives by the kidneys.

*Registered trademark of MERCK & CO., Inc.

Excretion

Two single-dose radiolabeled pharmacokinetic studies were conducted. In one study, plasma, urine, and feces were collected over 27 days, and in the second study plasma was collected over 6 months. Plasma concentrations of radioactivity and of caspofungin were similar during the first 24 to 48 hours postdose; thereafter drug levels fell more rapidly. In plasma, caspofungin concentrations fell below the limit of quantitation after 6 to 8 days postdose, while radiolabel fell below the limit of quantitation at 22.3 weeks postdose. After single intravenous administration of [^{3}H] caspofungin acetate, excretion of caspofungin and its metabolites in humans were 35% of dose in feces and 41% of dose in urine. A small amount of caspofungin is excreted unchanged in urine (~1.4% of dose). Renal clearance of parent drug is low (~0.15 mL/min) and total clearance of caspofungin is 12 mL/min.

Special Populations

Gender

Plasma concentrations of caspofungin in healthy men and women were similar following a single 70-mg dose. After 13 daily 50-mg doses, caspofungin plasma concentrations in women were elevated slightly (approximately 22% in area under the curve [AUC]) relative to men. No dosage adjustment is necessary based on gender.

Geriatric

Plasma concentrations of caspofungin in healthy older men and women (≥65 years of age) were increased slightly (approximately 28% AUC) compared to young healthy men after a single 70-mg dose of caspofungin. In patients with candidemia or other *Candida* infections (intra-abdominal abscesses, peritonitis, or pleural space infections), a similar modest effect of age was seen in older patients relative to younger patients. No dosage adjustment is necessary for the elderly (see PRECAUTIONS, *Geriatric Use*).

Race

Regression analyses of patient pharmacokinetic data indicated that no clinically significant differences in the pharmacokinetics of caspofungin were seen among Caucasians, Blacks, and Hispanics. No dosage adjustment is necessary on the basis of race.

Renal Insufficiency

In a clinical study of single 70-mg doses, caspofungin pharmacokinetics were similar in volunteers with mild renal insufficiency (creatinine clearance 50 to 80 mL/min) and control subjects. Moderate (creatinine clearance 31 to 49 mL/min), advanced (creatinine clearance 5 to 30 mL/min), and end-stage (creatinine clearance <10 mL/min and dialysis dependent) renal insufficiency moderately increased caspofungin plasma concentrations after single-dose administration (range: 30 to 49% for AUC). However, in patients with invasive aspergillosis, candidemia, or other *Candida* infections (intra-abdominal abscesses, peritonitis, or pleural space infections) who received multiple daily doses of CANCIDAS 50 mg, there was no significant effect of mild to end-stage renal impairment on caspofungin concentrations. No dosage adjustment is necessary for patients with renal insufficiency. Caspofungin is not dialyzable, thus supplementary dosing is not required following hemodialysis.

Hepatic Insufficiency

Plasma concentrations of caspofungin after a single 70-mg dose in patients with mild hepatic insufficiency (Child-Pugh score 5 to 6) were increased by approximately 55% in AUC compared to healthy control subjects. In a 14-day multiple-dose study (70 mg on Day 1 followed by 50 mg daily thereafter), plasma concentrations in patients with mild hepatic insufficiency were increased modestly (19 to 25% in AUC) on Days 7 and 14 relative to healthy control subjects. No dosage adjustment is recommended for patients with mild hepatic insufficiency. Patients with moderate hepatic insufficiency (Child-Pugh score 7 to 9) who received a single 70-mg dose of CANCIDAS had an average plasma caspofungin increase of 76% in AUC compared to control subjects. A dosage reduction is recommended for patients with moderate hepatic insufficiency (see DOSAGE AND ADMINISTRATION). There is no clinical experience in patients with severe hepatic insufficiency (Child-Pugh score >9).

Pediatric Patients

CANCIDAS has not been adequately studied in patients under 18 years of age.

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 susceptible *Aspergillus* species and *Candida* species.

β (1,3)-D-glucan is not present in mammalian cells. Caspofungin has shown activity against *Candida* species and in regions of active cell growth of the hyphae of *Aspergillus fumigatus*.

Activity in vitro

Caspofungin exhibits *in vitro* activity against *Aspergillus* species (*Aspergillus fumigatus, Aspergillus flavus*, and *Aspergillus terreus*) and *Candida* species (*Candida albicans, Candida glabrata, Candida guilliermondii, Candida krusei, Candida parapsilosis,* and *Candida tropicalis*). Susceptibility testing was performed according to the National Committee for Clinical Laboratory Standards (NCCLS) method M38-A (for *Aspergillus* species) and M27-A (for *Candida* species). Standardized susceptibility testing methods for β (1,3)-D-glucan synthesis inhibitors have not been established for yeasts and filamentous fungi, and results of susceptibility studies do not correlate with clinical outcome.

Activity in vivo

Caspofungin was active when parenterally administered to immunocompetent and immunosuppressed mice as long as 24 hours after disseminated infections with *C. albicans,* in which the endpoints were prolonged survival of infected mice and reduction of *C. albicans* from target organs. Caspofungin, administered parenterally to immunocompetent and immunosuppressed rodents, as long as 24 hours after disseminated or pulmonary infection with *Aspergillus fumigatus*, has shown prolonged survival, which has not been consistently associated with a reduction in mycological burden.

Drug Resistance

Mutants of *Candida* with reduced susceptibility to caspofungin have been identified in some patients during treatment. Similar observations were made in a study in mice infected with *C. albicans* and treated with orally administered doses of caspofungin. MIC values for caspofungin should not be used to predict clinical outcome, since a correlation between MIC values and clinical outcome has not been established. The incidence of drug resistance by various clinical isolates of *Candida* and *Aspergillus* species is unknown.

Drug Interactions

Studies *in vitro* and *in vivo* of caspofungin, in combination with amphotericin B, suggest no antagonism of antifungal activity against either *A. fumigatus* or *C. albicans*. The clinical significance of these results is unknown.

CLINICAL STUDIES

Candidemia and the following other Candida infections: intra-abdominal abscesses, peritonitis and pleural space infections

In a Phase III randomized, double-blind study, patients with a proven diagnosis of invasive candidiasis received daily doses of CANCIDAS (50 mg/day following a 70-mg loading dose on Day 1) or amphotericin B deoxycholate (0.6 to 0.7 mg/kg/day for non-neutropenic patients and 0.7 to 1.0 mg/kg/day for neutropenic patients). Patients were stratified by both neutropenic status and APACHE II score. Patients with *Candida* endocarditis, meningitis, or osteomyelitis were excluded from this study.

Patients who met the entry criteria and received one or more doses of IV study therapy were included in the primary (modified intention-to-treat [MITT]) analysis of response at the end of IV study therapy. A favorable response at this time point required both symptom/sign resolution/improvement and microbiological clearance of the *Candida* infection.

Two hundred thirty-nine patients were enrolled. Patient disposition is shown in Table 1.

TABLE 1
Disposition in Candidemia and Other *Candida* Infections
(Intra-abdominal abscesses, peritonitis, and pleural space infections)

	CANCIDAS*	Amphotericin B
Randomized patients	114	125
Patients completing study**	63 (55.3%)	69 (55.2%)
DISCONTINUATIONS OF STUDY**		
All Study Discontinuations	51 (44.7%)	56 (44.8%)
Study Discontinuations due to clinical adverse events	39 (34.2%)	43 (34.4%)
Study Discontinuations due to laboratory adverse events	0 (0%)	1 (0.8%)
DISCONTINUATIONS OF STUDY THERAPY		
All Study Therapy Discontinuations	48 (42.1%)	58 (46.4%)
Study Therapy Discontinuations due to clinical adverse events	30 (26.3%)	37 (29.6%)
Study Therapy Discontinuations due to laboratory adverse events	1 (0.9%)	7 (5.6%)
Study Therapy Discontinuations due to all drug-related*** adverse events	3 (2.6%)	29 (23.2%)

*Patients received CANCIDAS 70 mg on Day 1, then 50 mg daily for the remainder of their treatment.
**Study defined as study treatment period and 6-8 week follow-up period.
***Determined by the investigator to be possibly, probably, or definitely drug-related.

Of the 239 patients enrolled, 224 met the criteria for inclusion in the MITT population (109 treated with CANCIDAS and 115 treated with amphotericin B). Of these 224 patients, 186 patients had candidemia (92 treated with CANCIDAS and 94 treated with amphotericin B). The majority of the patients with candidemia were non-neutropenic (87%) and had an APACHE II score less than or equal to 20 (77%) in both arms. Most candidemia infections were caused by *C. albicans* (39%), followed by *C. parapsilosis* (20%), *C. tropicalis* (17%), *C. glabrata* (8%), and *C. krusei* (3%).

At the end of IV study therapy, CANCIDAS was comparable to amphotericin B in the treatment of candidemia in the MITT population. For the other efficacy time points (Day 10 of IV study therapy, end of all antifungal therapy, 2-week post-therapy follow-up, and 6- to 8-week post-therapy follow-up), CANCIDAS was as effective as amphotericin B.

Outcome, relapse and mortality data are shown in Table 2.

TABLE 2

Outcomes, Relapse, & Mortality in Candidemia and Other *Candida* Infections (Intra-abdominal abscesses, peritonitis, and pleural space infections)

	CANCIDAS*	Amphotericin B	% Difference** after adjusting for strata (Confidence Interval)***
Number of MITT† patients	109	115	
FAVORABLE OUTCOMES (MITT) AT THE END OF IV STUDY THERAPY			
All MITT patients	81/109 (74.3%)	78/115 (67.8%)	7.5 (-5.4, 20.3)
Candidemia	67/92 (72.8%)	63/94 (67.0%)	7.0 (-7.0, 21.1)
Neutropenic	6/14 (43%)	5/10 (50%)	
Non-neutropenic	61/78 (78%)	58/84 (69%)	
Endophthalmitis	0/1	2/3	
Multiple Sites	4/5	4/4	
Blood / Pleural	1/1	1/1	
Blood / Peritoneal	1/1	1/1	
Blood / Urine	-	1/1	
Peritoneal / Pleural	1/2	-	
Abdominal / Peritoneal	-	1/1	
Subphrenic / Peritoneal	1/1	-	
DISSEMINATED INFECTIONS, RELAPSES AND MORTALITY			
Disseminated Infections in neutropenic patients	4/14 (28.6%)	3/10 (30.0%)	
All relapses††	7/81 (8.6%)	8/78 (10.3%)	
Culture-confirmed relapse	5/81 (6%)	2/78 (3%)	
Overall study††† mortality in MITT	36/109 (33.0%)	35/115 (30.4%)	
Mortality during study therapy	18/109 (17%)	13/115 (11%)	
Mortality attributed to *Candida*	4/109 (4%)	7/115 (6%)	

* Patients received CANCIDAS 70 mg on Day 1, then 50 mg daily for the remainder of their treatment.

** Calculated as CANCIDAS – amphotericin B

*** 95% CI for candidemia, 95.6% for all patients

† Modified intention-to-treat

†† Includes all patients who either developed a culture-confirmed recurrence of *Candida* infection or required antifungal therapy for the treatment of a proven or suspected *Candida* infection in the follow-up period.

††† Study defined as study treatment period and 6-8 week follow-up period.

In this study, the efficacy of CANCIDAS in patients with intra-abdominal abscesses, peritonitis and pleural space *Candida* infections was evaluated in 19 non-neutropenic patients. Two of these patients had concurrent candidemia. *Candida* was part of a polymicrobial infection that required adjunctive surgical drainage in 11 of these 19 patients. A favorable response was seen in 9 of 9 patients with peritonitis, 3 of 4 with abscesses (liver, parasplenic, and urinary bladder abscesses), 2 of 2 with pleural space infections, 1 of 2 with mixed peritoneal and pleural infection, 1 of 1 with mixed abdominal abscess and peritonitis, and 0 of 1 with *Candida* pneumonia.

Overall, across all sites of infection included in the study, the efficacy of CANCIDAS was comparable to that of amphotericin B for the primary endpoint.

In this study, the efficacy data for CANCIDAS in neutropenic patients with candidemia were limited. In a separate compassionate use study, 4 patients with hepatosplenic candidiasis received prolonged therapy with CANCIDAS following other long-term antifungal therapy; three of these patients had a favorable response.

Esophageal Candidiasis (and information on oropharyngeal candidiasis)

The safety and efficacy of CANCIDAS in the treatment of esophageal candidiasis was evaluated in one large, controlled, noninferiority, clinical trial and two smaller dose-response studies.

In all 3 studies, patients were required to have symptoms and microbiological documentation of esophageal candidiasis; most patients had advanced AIDS (with CD4 counts <50/mm^3).

Of the 166 patients in the large study who had culture-confirmed esophageal candidiasis at baseline, 120 had *Candida albicans* and 2 had *Candida tropicalis* as the sole baseline pathogen whereas 44 had mixed baseline cultures containing *C. albicans* and one or more additional *Candida* species.

In the large, randomized, double-blind study comparing CANCIDAS 50 mg/day versus intravenous fluconazole 200 mg/day for the treatment of esophageal candidiasis, patients were treated for an average of 9 days (range 7-21 days). The primary endpoint was favorable overall response at 5 to 7 days following discontinuation of study therapy, which required both complete resolution of symptoms and significant endoscopic improvement. The definition of endoscopic response was based on severity of disease at baseline using a 4-grade scale and required at least a two-grade reduction from baseline endoscopic score or reduction to grade 0 for patients with a baseline score of 2 or less.

The proportion of patients with a favorable overall response for the primary endpoint was comparable for CANCIDAS and fluconazole as shown in Table 3.

TABLE 3
Favorable Response Rates for Patients with Esophageal Candidiasis

	CANCIDAS	Fluconazole	% Difference* (95% CI)
Day 5-7 post-treatment	66/81 (81.5%)	80/94 (85.1%)	-3.6 (-14.7, 7.5)

* calculated as CANCIDAS – fluconazole

The proportion of patients with a favorable symptom response was also comparable (90.1% and 89.4% for CANCIDAS and fluconazole, respectively). In addition, the proportion of patients with a favorable endoscopic response was comparable (85.2% and 86.2% for CANCIDAS and fluconazole, respectively).

As shown in Table 4, the esophageal candidiasis relapse rates at the Day 14 post-treatment visit were similar for the two groups. At the Day 28 post-treatment visit, the group treated with CANCIDAS had a numerically higher incidence of relapse, however, the difference was not statistically significant.

TABLE 4
Relapse Rates at 14 and 28 Days Post-Therapy in Patients with Esophageal Candidiasis at Baseline

	CANCIDAS	Fluconazole	% Difference* (95% CI)
Day 14 post-treatment	7/66 (10.6%)	6/76 (7.9%)	2.7 (-6.9, 12.3)
Day 28 post-treatment	18/64 (28.1%)	12/72 (16.7%)	11.5 (-2.5, 25.4)

* calculated as CANCIDAS – fluconazole

In this trial, which was designed to establish noninferiority of CANCIDAS to fluconazole for the treatment of esophageal candidiasis, 122 (70%) patients also had oropharyngeal candidiasis. A favorable response was defined as complete resolution of all symptoms of oropharyngeal disease and all visible oropharyngeal lesions. The proportion of patients with a favorable oropharyngeal response at the 5- to 7-day post-treatment visit was numerically lower for CANCIDAS, however, the difference was not statistically significant. The results are shown in Table 5.

TABLE 5
Oropharyngeal Candidiasis Response Rates at 5 to 7 Days Post-Therapy in Patients with Oropharyngeal and Esophageal Candidiasis at Baseline

	CANCIDAS	Fluconazole	% Difference* (95% CI)
Day 5-7 post-treatment	40/56 (71.4%)	55/66 (83.3%)	-11.9 (-26.8, 3.0)

* calculated as CANCIDAS – fluconazole

As shown in Table 6, the oropharyngeal candidiasis relapse rates at the Day 14 and the Day 28 post-treatment visits were statistically significantly higher for CANCIDAS than for fluconazole.

TABLE 6
Oropharyngeal Candidiasis Relapse Rates at 14 and 28 Days Post-Therapy in Patients with Oropharyngeal and Esophageal Candidiasis at Baseline

	CANCIDAS	Fluconazole	% Difference* (95% CI)
Day 14 post-treatment	17/40 (42.5%)	7/53 (13.2%)	29.3 (11.5, 47.1)
Day 28 post-treatment	23/39 (59.0%)	18/51 (35.3%)	23.7 (3.4, 43.9)

* calculated as CANCIDAS – fluconazole

The results from the two smaller dose-ranging studies corroborate the efficacy of CANCIDAS for esophageal candidiasis that was demonstrated in the larger study.

CANCIDAS was associated with favorable outcomes in 7 of 10 esophageal *C. albicans* infections refractory to at least 200 mg of fluconazole given for 7 days, although the *in vitro* susceptibility of the infecting isolates to fluconazole was not known.

Invasive Aspergillosis

Sixty-nine patients between the ages of 18 and 80 with invasive aspergillosis (IA) were enrolled in an open-label, noncomparative study to evaluate the safety, tolerability, and efficacy of CANCIDAS. Enrolled patients had previously been refractory to or intolerant of other antifungal therapy(ies). Refractory patients were classified as those who had disease progression or failed to improve despite therapy for at least 7 days with amphotericin B, lipid formulations of amphotericin B, itraconazole, or an investigational azole with reported activity against *Aspergillus*. Intolerance to previous therapy was defined as a doubling of creatinine (or creatinine ≥2.5 mg/dL while on therapy), other acute reactions, or infusion-related toxicity. To be included in the study, patients with pulmonary disease must have had definite (positive tissue histopathology or positive culture from tissue obtained by an invasive procedure) or probable (positive radiographic or computed tomography evidence with supporting culture from bronchoalveolar lavage or sputum, galactomannan enzyme-linked immunosorbent assay, and/or polymerase chain reaction) invasive aspergillosis. Patients with extrapulmonary disease had to have definite invasive aspergillosis. The definitions were modeled after the Mycoses Study Group Criteria.[1] Patients were administered a single 70-mg loading dose of CANCIDAS and subsequently dosed with 50 mg daily. The mean duration of therapy was 33.7 days, with a range of 1 to 162 days.

An independent expert panel evaluated patient data, including diagnosis of invasive aspergillosis, response and tolerability to previous antifungal therapy, treatment course on CANCIDAS, and clinical outcome.

A favorable response was defined as either complete resolution (complete response) or clinically meaningful improvement (partial response) of all signs and symptoms and attributable radiographic findings. Stable, nonprogressive disease was considered to be an unfavorable response.

Among the 69 patients enrolled in the study, 63 met entry diagnostic criteria and had outcome data; and of these, 52 patients received treatment for >7 days. Fifty-three (84%) were refractory to previous antifungal therapy and 10 (16%) were intolerant. Forty-five patients had pulmonary disease and 18 had extrapulmonary disease. Underlying conditions were hematologic malignancy (N=24), allogeneic bone marrow transplant or stem cell transplant (N=18), organ transplant (N=8), solid tumor (N=3), or other conditions (N=10). All patients in the study received concomitant therapies for their other underlying conditions. Eighteen patients received tacrolimus and CANCIDAS concomitantly, of whom 8 also received mycophenolate mofetil.

Overall, the expert panel determined that 41% (26/63) of patients receiving at least one dose of CANCIDAS had a favorable response. For those patients who received >7 days of therapy with CANCIDAS, 50% (26/52) had a favorable response. The favorable response rates for patients who were either refractory to or intolerant of previous therapies were 36% (19/53) and 70% (7/10), respectively. The response rates among patients with pulmonary disease and extrapulmonary disease were 47% (21/45) and 28% (5/18), respectively. Among patients with extrapulmonary disease, 2 of 8 patients who also had definite, probable, or possible CNS involvement had a favorable response. Two of these 8 patients had progression of disease and manifested CNS involvement while on therapy.

There is substantial evidence that CANCIDAS is well tolerated and effective for the treatment of invasive aspergillosis in patients who are refractory to or intolerant of itraconazole, amphotericin B, and/or lipid formulations of amphotericin B. However, the efficacy of CANCIDAS has not been evaluated in concurrently controlled clinical studies, with other antifungal therapies.

INDICATIONS AND USAGE

CANCIDAS is indicated for the treatment of:

- Candidemia and the following *Candida* infections: intra-abdominal abscesses, peritonitis and pleural space infections. CANCIDAS has not been studied in endocarditis, osteomyelitis, and meningitis due to *Candida*.
- Esophageal Candidiasis (see CLINICAL STUDIES).

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[1] Denning DW, Lee JY, Hostetler JS, et al. NIAID Mycoses Study Group multicenter trial of oral itraconazole therapy for invasive aspergillosis. *Am J Med* 1994; 97:135-144.

- Invasive Aspergillosis in patients who are refractory to or intolerant of other therapies (i.e., amphotericin B, lipid formulations of amphotericin B, and/or itraconazole). CANCIDAS has not been studied as initial therapy for invasive aspergillosis.

CONTRAINDICATIONS

CANCIDAS is contraindicated in patients with hypersensitivity to any component of this product.

WARNINGS

Concomitant use of CANCIDAS with cyclosporine is not recommended unless the potential benefit outweighs the potential risk to the patient. In one clinical study, 3 of 4 healthy subjects who received CANCIDAS 70 mg on Days 1 through 10, and also received two 3 mg/kg doses of cyclosporine 12 hours apart on Day 10, developed transient elevations of alanine transaminase (ALT) on Day 11 that were 2 to 3 times the upper limit of normal (ULN). In a separate panel of subjects in the same study, 2 of 8 who received CANCIDAS 35 mg daily for 3 days and cyclosporine (two 3 mg/kg doses administered 12 hours apart) on Day 1 had small increases in ALT (slightly above the ULN) on Day 2. In both groups, elevations in aspartate transaminase (AST) paralleled ALT elevations, but were of lesser magnitude (see ADVERSE REACTIONS). Hence, concomitant use of CANCIDAS with cyclosporine is not recommended until multiple-dose use in patients is studied.

PRECAUTIONS

General

The efficacy of a 70-mg dose regimen in patients with invasive aspergillosis who are not clinically responding to the 50-mg daily dose is not known. Limited safety data suggest that an increase in dose to 70 mg daily is well tolerated. For candidiasis, see CLINICAL STUDIES. The safety and efficacy of doses above 70 mg have not been adequately studied in patients. However, CANCIDAS was generally well tolerated at a dose of 100 mg once daily for 21 days when administered to 15 healthy subjects.

The safety information on treatment durations longer than 2 weeks is limited, however, available data suggest that CANCIDAS continues to be well tolerated with longer courses of therapy (112 patients received from 15 to 60 days of therapy; 14 patients received from 61 to 162 days of therapy).

Drug Interactions

Studies *in vitro* show that caspofungin acetate is not an inhibitor of any enzyme in the cytochrome P450 (CYP) system. In clinical studies, caspofungin did not induce the CYP3A4 metabolism of other drugs. Caspofungin is not a substrate for P-glycoprotein and is a poor substrate for cytochrome P450 enzymes.

Clinical studies in healthy volunteers show that the pharmacokinetics of CANCIDAS are not altered by itraconazole, amphotericin B, mycophenolate, nelfinavir, or tacrolimus. CANCIDAS has no effect on the pharmacokinetics of itraconazole, amphotericin B, or the active metabolite of mycophenolate.

CANCIDAS reduced the blood $AUC_{0\text{-}12}$ of tacrolimus (FK-506, Prograf®[2]) by approximately 20%, peak blood concentration (C_{max}) by 16%, and 12-hour blood concentration (C_{12hr}) by 26% in healthy subjects when tacrolimus (2 doses of 0.1 mg/kg 12 hours apart) was administered on the 10th day of CANCIDAS 70 mg daily, as compared to results from a control period in which tacrolimus was administered alone. For patients receiving both therapies, standard monitoring of tacrolimus blood concentrations and appropriate tacrolimus dosage adjustments are recommended.

In two clinical studies, cyclosporine (one 4 mg/kg dose or two 3 mg/kg doses) increased the AUC of caspofungin by approximately 35%. CANCIDAS did not increase the plasma levels of cyclosporine. There were transient increases in liver ALT and AST when CANCIDAS and cyclosporine were co-administered (see WARNINGS and ADVERSE REACTIONS).

A drug-drug interaction study with rifampin in healthy volunteers has shown a 30% decrease in caspofungin trough concentrations. Patients on rifampin should receive 70 mg of CANCIDAS daily. In addition, results from regression analyses of patient pharmacokinetic data suggest that co-administration of other inducers of drug clearance (efavirenz, nevirapine, phenytoin, dexamethasone, or carbamazepine) with CANCIDAS may result in clinically meaningful reductions in caspofungin concentrations. It is not known which drug clearance mechanism involved in caspofungin disposition may

[2] Registered trademark of Fujisawa Healthcare, Inc.

be inducible. When CANCIDAS is co-administered with inducers of drug clearance, such as efavirenz, nevirapine, phenytoin, dexamethasone, or carbamazepine, use of a daily dose of 70 mg of CANCIDAS should be considered.

Carcinogenesis, Mutagenesis, and Impairment of Fertility

No long-term studies in animals have been performed to evaluate the carcinogenic potential of caspofungin.

Caspofungin did not show evidence of mutagenic or genotoxic potential when evaluated in the following *in vitro* assays: bacterial (Ames) and mammalian cell (V79 Chinese hamster lung fibroblasts) mutagenesis assays, the alkaline elution/rat hepatocyte DNA strand break test, and the chromosome aberration assay in Chinese hamster ovary cells. Caspofungin was not genotoxic when assessed in the mouse bone marrow chromosomal test at doses up to 12.5 mg/kg (equivalent to a human dose of 1 mg/kg based on body surface area comparisons), administered intravenously.

Fertility and reproductive performance were not affected by the intravenous administration of caspofungin to rats at doses up to 5 mg/kg. At 5 mg/kg exposures were similar to those seen in patients treated with the 70-mg dose.

Pregnancy

Pregnancy Category C. CANCIDAS was shown to be embryotoxic in rats and rabbits. Findings included incomplete ossification of the skull and torso and an increased incidence of cervical rib in rats. An increased incidence of incomplete ossifications of the talus/calcaneus was seen in rabbits. Caspofungin also produced increases in resorptions in rats and rabbits and periimplantation losses in rats. These findings were observed at doses which produced exposures similar to those seen in patients treated with a 70-mg dose. Caspofungin crossed the placental barrier in rats and rabbits and was detected in the plasma of fetuses of pregnant animals dosed with CANCIDAS. There are no adequate and well-controlled studies in pregnant women. CANCIDAS should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nursing Mothers

Caspofungin was found in the milk of lactating, drug-treated rats. It is not known whether caspofungin is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when caspofungin is administered to a nursing woman.

Patients with Hepatic Insufficiency

Patients with mild hepatic insufficiency (Child-Pugh score 5 to 6) do not need a dosage adjustment. For patients with moderate hepatic insufficiency (Child-Pugh score 7 to 9), CANCIDAS 35 mg daily is recommended. However, where recommended, a 70-mg loading dose should still be administered on Day 1 (see DOSAGE AND ADMINISTRATION). There is no clinical experience in patients with severe hepatic insufficiency (Child-Pugh score >9).

Pediatric Use

Safety and effectiveness in pediatric patients have not been established.

Geriatric Use

Clinical studies of CANCIDAS did not include sufficient numbers of patients aged 65 and over to determine whether they respond differently from younger patients. Although the number of elderly patients was not large enough for a statistical analysis, no overall differences in safety or efficacy were observed between these and younger patients. Plasma concentrations of caspofungin in healthy older men and women (≥65 years of age) were increased slightly (approximately 28% in AUC) compared to young healthy men. A similar effect of age on pharmacokinetics was seen in patients with candidemia or other *Candida* infections (intra-abdominal abscesses, peritonitis, or pleural space infections). No dose adjustment is recommended for the elderly; however, greater sensitivity of some older individuals cannot be ruled out.

ADVERSE REACTIONS

General

Possible histamine-mediated symptoms have been reported including reports of rash, facial swelling, pruritus, sensation of warmth, or bronchospasm. Anaphylaxis has been reported during administration of CANCIDAS.

Clinical Adverse Experiences

The overall safety of caspofungin was assessed in 876 individuals who received single or multiple doses of caspofungin acetate. There were 125 patients with candidemia and/or intra-abdominal

abscesses, peritonitis, or pleural space infections, including 4 patients with chronic disseminated candidiasis; 285 patients with esophageal and/or oropharyngeal candidiasis; and 72 patients with invasive aspergillosis enrolled in phase II and phase III clinical studies. The remaining 394 individuals were enrolled in phase I studies. The majority of the patients with *Candida* infections had serious underlying medical conditions (e.g., hematologic or other malignancy, recent major surgery, HIV) requiring multiple concomitant medications. Patients in the noncomparative *Aspergillus* study often had serious predisposing medical conditions (e.g., bone marrow or peripheral stem cell transplants, hematologic malignancy, solid tumors or organ transplants) requiring multiple concomitant medications.

In the randomized, double-blinded invasive candidiasis study, patients received either CANCIDAS 50 mg/day (following a 70-mg loading dose) or amphotericin B 0.6 to 1.0 mg/kg/day. Drug-related clinical adverse experiences occurring in ≥2% of the patients in either treatment group are presented in Table 7.

TABLE 7
Drug-Related* Clinical Adverse Experiences Among Patients with Candidemia or other *Candida* Infections**

Incidence ≥2% for at least one treatment group by Body System

	CANCIDAS 50 mg*** N=114 (percent)	Amphotericin B N=125 (percent)
Body as a Whole		
Chills	5.3	26.4
Fever	7.0	23.2
Cardiovascular System		
Hypertension	1.8	6.4
Hypotension	0.9	2.4
Tachycardia	1.8	10.4
Peripheral Vascular System		
Phlebitis/thrombophlebitis	3.5	4.8
Digestive System		
Diarrhea	2.6	0.8
Jaundice	0.9	3.2
Nausea	1.8	5.6
Vomiting	3.5	8.0
Metabolic/Nutritional/Immune		
Hypokalemia	0.9	5.6
Nervous System & Psychiatric		
Tremor	1.8	2.4
Respiratory System		
Tachypnea	0.0	10.4
Skin & Skin Appendage		
Erythema	0.0	2.4
Rash	0.9	3.2
Sweating	0.9	3.2
Urogenital System		
Renal insufficiency	0.9	5.6
Renal insufficiency, acute	0.0	5.6

* Determined by the investigator to be possibly, probably, or definitely drug-related.
** Intra-abdominal abscesses, peritonitis and pleural space infections
*** Patients received CANCIDAS 70 mg on Day 1, then 50 mg daily for the remainder of their treatment.

The incidence of drug-related clinical adverse experiences was significantly lower among patients treated with CANCIDAS (28.9%) than among patients treated with amphotericin B (58.4%). Also, the proportion of patients who experienced an infusion-related adverse event was significantly lower in the group treated with CANCIDAS (20.2%) than in the group treated with amphotericin B (48.8%).

Drug-related laboratory adverse experiences occurring in ≥2% of the patients in either treatment group are presented in Table 8.

TABLE 8
Drug-Related* Laboratory Adverse Experiences Among Patients with Candidemia or other *Candida* Infections**

Incidence ≥2% for at least one treatment group by Laboratory Test Category

	CANCIDAS 50 mg*** N=114 (percent)	Amphotericin B N=125 (percent)
Blood Chemistry		
ALT increased	3.7	8.1
AST increased	1.9	9.0
Blood urea increased	1.9	15.8
Direct serum bilirubin increased	3.8	8.4
Serum alkaline phosphatase increased	8.3	15.6
Serum bicarbonate decreased	0.0	3.6
Serum creatinine increased	3.7	22.6
Serum phosphate increased	0.0	2.7
Serum potassium decreased	9.9	23.4
Serum potassium increased	0.9	2.4
Total serum bilirubin increased	2.8	8.9
Hematology		
Hematocrit decreased	0.9	7.3
Hemoglobin decreased	0.9	10.5
Urinalysis		
Urine protein increased	0.0	3.7

* Determined by the investigator to be possibly, probably, or definitely drug-related.
** Intra-abdominal abscesses, peritonitis and pleural space infections
*** Patients received CANCIDAS 70 mg on Day 1, then 50 mg daily for the remainder of their treatment.

The incidence of drug-related laboratory adverse experiences was significantly lower among patients receiving CANCIDAS (24.3%) than among patients receiving amphotericin B (54.0%).

The percentage of patients with either a drug-related clinical adverse experience or a drug-related laboratory adverse experience was significantly lower among patients receiving CANCIDAS (42.1%) than among patients receiving amphotericin B (75.2%). Furthermore, a significant difference between the two treatment groups was observed with regard to incidence of discontinuation due to drug-related clinical or laboratory adverse experience; incidences were 3/114 (2.6%) in the group treated with CANCIDAS and 29/125 (23.2%) in the group treated with amphotericin B.

To evaluate the effect of CANCIDAS and amphotericin B on renal function, nephrotoxicity was defined as doubling of serum creatinine relative to baseline or an increase of ≥1 mg/dL in serum creatinine if baseline serum creatinine was above the upper limit of the normal range. In a subgroup of patients whose baseline creatinine clearance was >30 mL/min, the incidence of nephrotoxicity was significantly lower in the group treated with CANCIDAS than in the group treated with amphotericin B.

Drug-related clinical adverse experiences occurring in ≥2% of patients with esophageal and/or oropharyngeal candidiasis are presented in Table 9.

TABLE 9
Drug-Related Clinical Adverse Experiences Among Patients with Esophageal and/or Oropharyngeal Candidiasis*
Incidence ≥2% for at least one treatment dose (per comparison) by Body System

	CANCIDAS 50 mg** N=83 (percent)	Fluconazole IV 200 mg** N=94 (percent)	CANCIDAS 50 mg*** N=80 (percent)	CANCIDAS 70 mg*** N=65 (percent)	Amphotericin B 0.5 mg/kg*** N=89 (percent)
Body as a Whole					
Asthenia/fatigue	0.0	0.0	0.0	0.0	6.7
Chills	0.0	0.0	2.5	1.5	75.3
Edema/swelling	0.0	0.0	0.0	0.0	5.6
Edema, facial	0.0	0.0	0.0	3.1	0.0
Fever	3.6	1.1	21.3	26.2	69.7
Flu-like illness	0.0	0.0	0.0	3.1	0.0
Malaise	0.0	0.0	0.0	0.0	5.6
Pain	0.0	0.0	1.3	4.6	5.6
Pain, abdominal	3.6	2.1	2.5	0.0	9.0
Warm sensation	0.0	0.0	0.0	1.5	4.5
Peripheral Vascular System					
Infused vein complication	12.0	8.5	2.5	1.5	0.0
Phlebitis/thrombophlebitis	15.7	8.5	11.3	13.8	22.5
Cardiovascular System					
Tachycardia	0.0	0.0	1.3	0.0	4.5
Vasculitis	0.0	0.0	0.0	0.0	3.4
Digestive System					
Anorexia	0.0	0.0	1.3	0.0	3.4
Diarrhea	3.6	2.1	1.3	3.1	11.2
Gastritis	0.0	2.1	0.0	0.0	0.0
Nausea	6.0	6.4	2.5	3.1	21.3
Vomiting	1.2	3.2	1.3	3.1	13.5
Hemic & Lymphatic System					
Anemia	0.0	0.0	3.8	0.0	9.0
Metabolic/Nutritional/Immune					
Anaphylaxis	0.0	0.0	0.0	0.0	2.2
Musculoskeletal System					
Myalgia	1.2	0.0	0.0	3.1	2.2
Pain, back	0.0	0.0	0.0	0.0	2.2
Pain, musculoskeletal	0.0	0.0	1.3	0.0	4.5
Nervous System & Psychiatric					
Dizziness	0.0	2.1	0.0	1.5	1.1
Headache	6.0	1.1	11.3	7.7	19.1
Insomnia	1.2	0.0	0.0	0.0	2.2
Paresthesia	0.0	0.0	1.3	3.1	1.1
Tremor	0.0	0.0	0.0	0.0	7.9
Respiratory System					
Tachypnea	0.0	0.0	1.3	0.0	4.5
Skin & Skin Appendage					
Erythema	1.2	0.0	1.3	1.5	7.9
Induration	0.0	0.0	0.0	3.1	6.7
Pruritus	1.2	0.0	2.5	1.5	0.0
Rash	0.0	0.0	1.3	4.6	3.4
Sweating	0.0	0.0	1.3	0.0	3.4

*Relationship to drug was determined by the investigator to be possibly, probably or definitely drug-related.
** Derived from a Phase III comparator-controlled clinical study.
*** Derived from Phase II comparator-controlled clinical studies.

Laboratory abnormalities occurring in ≥2% of patients with esophageal and/or oropharyngeal candidiasis are presented in Table 10.

TABLE 10
Drug-Related Laboratory Abnormalities Reported Among Patients with Esophageal and/or Oropharyngeal Candidiasis*

Incidence ≥2% (for at least one treatment dose) by Laboratory Test Category

	CANCIDAS 50 mg** N=163 (percent)	CANCIDAS 70 mg*** N=65 (percent)	Fluconazole IV 200 mg** N=94 (percent)	Amphotericin B 0.5 mg/kg*** N=89 (percent)
Blood Chemistry				
ALT increased	10.6	10.8	11.8	22.7
AST increased	13.0	10.8	12.9	22.7
Blood urea increased	0.0	0.0	1.2	10.3
Direct serum bilirubin increased	0.6	0.0	3.3	2.5
Serum albumin decreased	8.6	4.6	5.4	14.9
Serum alkaline phosphatase increased	10.5	7.7	11.8	19.3
Serum bicarbonate decreased	0.9	0.0	0.0	6.6
Serum calcium decreased	1.9	0.0	3.2	1.1
Serum creatinine increased	0.0	1.5	2.2	28.1
Serum potassium decreased	3.7	10.8	4.3	31.5
Serum potassium increased	0.6	0.0	2.2	1.1
Serum sodium decreased	1.9	1.5	3.2	1.1
Serum uric acid increased	0.6	0.0	0.0	3.4
Total serum bilirubin increased	0.0	0.0	3.2	4.5
Total serum protein decreased	3.1	0.0	3.2	3.4
Hematology				
Eosinophils increased	3.1	3.1	1.1	1.1
Hematocrit decreased	11.1	1.5	5.4	32.6
Hemoglobin decreased	12.3	3.1	5.4	37.1
Lymphocytes increased	0.0	1.6	2.2	0.0
Neutrophils decreased	1.9	3.1	3.2	1.1
Platelet count decreased	3.1	1.5	2.2	3.4
Prothrombin time increased	1.3	1.5	0.0	2.3
WBC count decreased	6.2	4.6	8.6	7.9
Urinalysis				
Urine blood increased	0.0	0.0	0.0	4.0
Urine casts increased	0.0	0.0	0.0	8.0
Urine pH increased	0.8	0.0	0.0	3.6
Urine protein increased	1.2	0.0	3.3	4.5
Urine RBCs increased	1.1	3.8	5.1	12.0
Urine WBCs increased	0.0	7.7	0.0	24.0

*Relationship to drug was determined by the investigator to be possibly, probably or definitely drug-related.
** Derived from Phase II and Phase III comparator-controlled clinical studies.
*** Derived from Phase II comparator-controlled clinical studies.

In the open-label, noncomparative aspergillosis study, in which 69 patients received CANCIDAS (70-mg loading dose on Day 1 followed by 50 mg daily), the following drug-related clinical adverse experiences were observed with an incidence of ≥2%: fever (2.9%), infused-vein complications (2.9%), nausea (2.9%), vomiting (2.9%) and flushing (2.9%).

Also reported infrequently in this patient population were pulmonary edema, ARDS, and radiographic infiltrates.

Drug-related laboratory abnormalities reported with an incidence ≥2% in patients treated with CANCIDAS in the noncomparative aspergillosis study were: serum alkaline phosphatase increased (2.9%), serum potassium decreased (2.9%), eosinophils increased (3.2%), urine protein increased (4.9%), and urine RBCs increased (2.2%).

Post Marketing Experience:

The following postmarketing adverse events have been reported:

Hepatobiliary: Rare cases of clinically significant hepatic dysfunction

Cardiovascular: swelling and peripheral edema

Metabolic: hypercalcemia

Concomitant Therapy

In one clinical study, 3 of 4 subjects who received CANCIDAS 70 mg daily on Days 1 through 10, and also received two 3 mg/kg doses of cyclosporine 12 hours apart on Day 10, developed transient elevations of ALT on Day 11 that were 2 to 3 times the upper limit of normal (ULN). In a separate panel of subjects in the same study, 2 of 8 subjects who received CANCIDAS 35 mg daily for 3 days and cyclosporine (two 3 mg/kg doses administered 12 hours apart) on Day 1 had small increases in ALT (slightly above the ULN) on Day 2. In another clinical study, 2 of 8 healthy men developed transient ALT elevations of less than 2X ULN. In this study, cyclosporine (4 mg/kg) was administered on Days 1 and 12, and CANCIDAS was administered (70 mg) daily on Days 3 through 13. In one subject, the ALT elevation

occurred on Days 7 and 9 and, in the other subject, the ALT elevation occurred on Day 19. These elevations returned to normal by Day 27. In all groups, elevations in AST paralleled ALT elevations but were of lesser magnitude. In these clinical studies, cyclosporine (one 4 mg/kg dose or two 3 mg/kg doses) increased the AUC of caspofungin by approximately 35% (see WARNINGS).

OVERDOSAGE

In clinical studies the highest dose was 210 mg, administered as a single dose to 6 healthy subjects. This dose was generally well tolerated. In addition, 100 mg once daily for 21 days has been administered to 15 healthy subjects and was generally well tolerated. Caspofungin is not dialyzable. The minimum lethal dose of caspofungin in rats was 50 mg/kg, a dose which is equivalent to 10 times the recommended daily dose based on relative body surface area comparison.

ANIMAL PHARMACOLOGY AND TOXICOLOGY

In one 5-week study in monkeys at doses which produced exposures approximately 4 to 6 times those seen in patients treated with a 70-mg dose, scattered small foci of subcapsular necrosis were observed microscopically in the livers of some animals (2/8 monkeys at 5 mg/kg and 4/8 monkeys at 8 mg/kg); however, this histopathological finding was not seen in another study of 27 weeks duration at similar doses.

DOSAGE AND ADMINISTRATION

Do not mix or co-infuse CANCIDAS with other medications, as there are no data available on the compatibility of CANCIDAS with other intravenous substances, additives, or medications. DO NOT USE DILUENTS CONTAINING DEXTROSE (α-D-GLUCOSE), as CANCIDAS is not stable in diluents containing dextrose.

Candidemia and other Candida infections (see CLINICAL STUDIES)

A single 70-mg loading dose should be administered on Day 1, followed by 50 mg daily thereafter. CANCIDAS should be administered by slow IV infusion over approximately 1 hour. Duration of treatment **should be dictated by the patient's** clinical and microbiological response. In general, antifungal therapy should continue for at least 14 days after the last positive culture. Patients who remain persistently neutropenic may warrant a longer course of therapy pending resolution of the neutropenia.

Esophageal Candidiasis

50 mg daily should be administered by slow IV infusion over approximately 1 hour. Because of the risk of relapse of oropharyngeal candidiasis in patients with HIV infections, suppressive oral therapy could be considered (see CLINICAL STUDIES). A 70-mg loading dose has not been studied with this indication.

Invasive Aspergillosis

A single 70-mg loading dose should be administered on Day 1, followed by 50 mg daily thereafter. CANCIDAS should be administered by slow IV infusion over approximately 1 hour. Duration of treatment should be based upon the severity of the patient's **underlying disease, recovery from** immunosuppression, and clinical response. The efficacy of a 70-mg dose regimen in patients who are not clinically responding to the 50-mg daily dose is not known. Limited safety data suggests that an increase in dose to 70 mg daily is well tolerated. The safety and efficacy of doses above 70 mg have not been adequately studied.

Hepatic Insufficiency

Patients with mild hepatic insufficiency (Child-Pugh score 5 to 6) do not need a dosage adjustment. For patients with moderate hepatic insufficiency (Child-Pugh score 7 to 9), CANCIDAS 35 mg daily is recommended. However, where recommended, a 70-mg loading dose should still be administered on Day 1. There is no clinical experience in patients with severe hepatic insufficiency (Child-Pugh score >9).

Concomitant Medication with Inducers of Drug Clearance

Patients on rifampin should receive 70 mg of CANCIDAS daily. Patients on nevirapine, efavirenz, carbamazepine, dexamethasone, or phenytoin may require an increase in dose to 70 mg of CANCIDAS daily (see PRECAUTIONS, *Drug Interactions*).

Preparation of CANCIDAS for use:

Do not mix or co-infuse CANCIDAS with other medications, as there are no data available on the compatibility of CANCIDAS with other intravenous substances, additives, or medications. DO NOT USE

DILUENTS CONTAINING DEXTROSE (α-D-GLUCOSE), as CANCIDAS is not stable in diluents containing dextrose.

Preparation of the 70-mg infusion

1. Equilibrate the refrigerated vial of CANCIDAS to room temperature.
2. Aseptically add 10.5 mL of 0.9% Sodium Chloride Injection, Sterile Water for Injection, Bacteriostatic Water for Injection with methylparaben and propylparaben, or Bacteriostatic Water for Injection with 0.9% benzyl alcohol to the vial.[a] This reconstituted solution may be stored for up to one hour at ≤25°C (≤77°F).[b]
3. Aseptically transfer 10 mL[c] of reconstituted CANCIDAS to an IV bag (or bottle) containing 250 mL 0.9%, 0.45%, or 0.225% Sodium Chloride **Injection, or Lactated Ringer's Injection. This infusion solution** must be used within 24 hours if stored at ≤25°C (≤77°F) or within 48 hours if stored refrigerated at 2 to 8°C (36 to 46°F). (If a 70-mg vial is unavailable, see below: *Alternative Infusion Preparation Methods, Preparation of 70-mg Day 1 loading dose from two 50-mg vials.*)

Preparation of the daily 50-mg infusion

1. Equilibrate the refrigerated vial of CANCIDAS to room temperature.
2. Aseptically add 10.5 mL of 0.9% Sodium Chloride Injection, Sterile Water for Injection, Bacteriostatic Water for Injection with methylparaben and propylparaben, or Bacteriostatic Water for Injection with 0.9% benzyl alcohol to the vial.[a] This reconstituted solution may be stored for up to one hour at ≤25°C (≤77°F).[b]
3. Aseptically transfer 10 mL[c] of reconstituted CANCIDAS to an IV bag (or bottle) containing 250 mL 0.9%, 0.45%, or 0.225% Sodium Chloride **Injection, or Lactated Ringer's Injection. This infusion solution** must be used within 24 hours if stored at ≤25°C (≤77°F) or within 48 hours if stored refrigerated at 2 to 8°C (36 to 46°F). (If a reduced infusion volume is medically necessary, see below: *Alternative Infusion Preparation Methods, Preparation of 50-mg daily doses at reduced volume.*)

Alternative Infusion Preparation Methods

Preparation of 70-mg dose from two 50-mg vials

Reconstitute two 50-mg vials with 10.5 mL of diluent each (see *Preparation of the daily 50-mg infusion*). Aseptically transfer a total of 14 mL of the reconstituted CANCIDAS from the two vials to 250 mL of 0.9%, 0.45%, or 0.225% Sodium Chloride Injection, **or Lactated Ringer's Injection.**

Preparation of 50-mg daily doses at reduced volume

When medically necessary, the 50-mg daily doses can be prepared by adding 10 mL of reconstituted CANCIDAS to 100 mL of 0.9%, 0.45%, or 0.225% **Sodium Chloride Injection, or Lactated Ringer's** Injection (see *Preparation of the daily 50-mg infusion*).

Preparation of a 35-mg daily dose for patients with moderate Hepatic Insufficiency

Reconstitute one 50-mg vial (see above: *Preparation of the daily 50-mg infusion*). Aseptically transfer 7 mL of the reconstituted CANCIDAS from the vial to 250 mL or, if medically necessary, to 100 mL of 0.9%, 0.45%, or 0.225% Sodium Chloride Injection, **or Lactated Ringer's Injection.**

Preparation notes:

a The white to off-white cake will dissolve completely. Mix gently until a clear solution is obtained.

b Visually inspect the reconstituted solution for particulate matter or discoloration during reconstitution and prior to infusion. Do not use if the solution is cloudy or has precipitated.

c CANCIDAS is formulated to provide the full labeled vial dose (70 mg or 50 mg) when 10 mL is withdrawn from the vial.

TABLE 11
CANCIDAS Concentrations

Dose	Reconstituted Solution Concentration	Infusion Volume	Infusion Solution Concentration
70-mg initial dose	7.2 mg/mL	260 mL	0.28 mg/mL
50-mg daily dose	5.2 mg/mL	260 mL	0.20 mg/mL
70-mg initial dose* (from two 50 mg vials)	5.2 mg/mL	264 mL	0.28 mg/mL
50-mg daily dose* (reduced volume)	5.2 mg/mL	110 mL	0.47 mg/mL
35-mg daily dose* (from one 50 mg vial) for Moderate Hepatic Insufficiency	5.2 mg/mL or 5.2 mg/mL	257 mL or 107 mL	0.14 mg/mL or 0.34 mg/mL

*See preceding text for these special situations.

HOW SUPPLIED

No. 3822 — CANCIDAS 50 mg is a white to off-white powder/cake for infusion in a vial with a red aluminum band and a plastic cap.

NDC 0006-3822-10 supplied as one single-use vial.

No. 3823 — CANCIDAS 70 mg is a white to off-white powder/cake for infusion in a vial with a yellow/orange aluminum band and a plastic cap.

NDC 0006-3823-10 supplied as one single-use vial.

Storage

Vials

The lyophilized vials should be stored refrigerated at 2° to 8°C (36° to 46°F).

Reconstituted Concentrate

Reconstituted CANCIDAS may be stored at ≤25°C (≤77°F) for one hour prior to the preparation of the patient infusion solution.

Diluted Product

The final patient infusion solution in the IV bag or bottle can be stored at ≤25°C (≤77°F) for 24 hours or at 2 to 8°C (36 to 46°F) for 48 hours.

MERCK & CO., INC., Whitehouse Station, NJ 08889, USA

Issued ~~December 2003~~
Printed in USA

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-227 / S-014
21-227 / S-011

MICROBIOLOGY REVIEW(S)

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

NDA #: 21-227

REVIEWER	: Kalavati Suvarna
CORRESPONDENCE DATE	: 03-28-03; 06-05-03; 09-30-03
CDER RECEIPT DATE	: 04-01-03; 06-09-03; 10-02-03
REVIEW ASSIGN DATE	: 04-11-03; 06-19-03; 11-12-03
REVIEW COMPLETE DATE	:11-14-03

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

SUBMISSION REVIEWED: 4P, SLR-011 (original, B1)

DRUG CATEGORY: Anti-fungal

INDICATION: Treatment of esophageal candidiasis, invasive candidiasis, and invasive aspergillosis.

DOSAGE FORM: Solution for intravenous administration

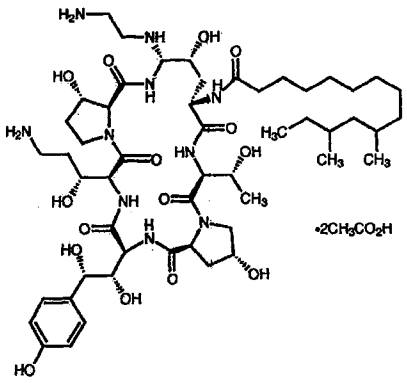
PRODUCT NAMES:

a. **PROPRIETARY:** Cancidas®

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

c. **CHEMICAL:** 1-[(4 *R* ,5 *S*)-5-[(2-amino-ethyl)amino]- N^2 -(10, 12-dimethyl-1-oxotetradecyl)-4- hydroxy-L-ornithine]-5-[(3 *R*)-3-hydroxy-L-ornithine] pneumocandin B_0 diacetate (salt).

STRUCTURAL FORMULA:



Molecular weight: 1213.42
Empirical formula: $C_{52}H_{88}N_{10}O_{15} \cdot 2C_2H_4O_2$

SUPPORTING DOCUMENTS: NDA# 21-227 (N-000, SE1-001, [redacted], and SE1-007).

TABLE OF CONTENT

1. EXECUTIVE SUMMARY:

Cancidas® (Caspofungin) is an approved drug for the treatment of invasive aspergillosis (in patients who are intolerant or refractory to amphotericin B treatment), esophageal candidiasis and invasive candidiasis. In this submission, the sponsor has provided studies in support of a post-marketing commitment. The objective of the post-marketing commitment was to evaluate resistance development by *Candida* and *Aspergillus* species. Resistance to caspofungin was measured by identification of mutations in the *FKS1* gene that encodes the catalytic subunit of β(1,3)-D-glucan synthase, inhibition of glucan synthase, and/or drug susceptibility in mice.

Mutants of *C. albicans* with reduced susceptibility were generated *in vitro* and in mice. The activity of caspofungin against these strains was decreased as measured by (i) inhibition of glucan synthase enzyme *in vitro*, and (ii) susceptibility of strains to the drug in mice. These effects appear to be correlated with changes at position 645 of the derived amino acid sequence of FKS1p for the mutants generated *in vitro* and position 641 of the derived amino acid sequence of FKS1p for strains selected in mice.

The clinical isolates (n = 111) of *Candida* species (*C. albicans*, n = 35; *C. glabrata*, n = 20; *C. tropicalis*, n = 8; *C. krusei*, n = 8; *C. guilliermondii*, n = 13; *C. parapsilosis*, n = 25; *C. lipolytica*, n = 1; *C. rugosa*, n =1) from patients with febrile neutropenia, invasive candidiasis or esophageal candidiasis, were characterized for resistance. Isolates associated with either an unfavorable clinical response and/or MIC values of ≥ 2 μg/ml in RPMI medium were characterized for changes in genotype and phenotype. Of the 35 *C. albicans* isolates, 16 had MIC values of ≥ 2 μg/ml in RPMI medium. Mutation in the *FKS1* gene was observed in 11 of 16 isolates of *C. albicans*. A higher concentration of caspofungin was required to inhibit glucan synthase enzyme and reduce fungal burdens in the kidneys of mice infected with the *C. albicans* isolate carrying amino acid substitutions at positions 641, 645, 647, 648 and 1361 of the FKS1 protein. Of all the *Candida* species tested, mutation in the *FKS1* gene was observed in only 1 *C. krusei* isolate with MIC value of ≥ 2 μg/ml in RPMI medium. Limited testing for all 3 parameters was performed for *Candida* species other than *C. albicans* due to technical problems with isolation of glucan synthase enzyme and due to isolates being avirulent in mice. Although it appears that mutations in the *C. albicans* FKS1 protein correlates with reduced caspofungin susceptibility, the number of isolates tested to correlate the specific point mutation with the susceptibility to caspofungin and clinical outcome was too small.

Attempts to generate mutants of *Aspergillus* with reduced susceptibility *in vitro* were stated to be unsuccessful. The clinical isolates (n = 26) of *Aspergillus* species (*A. fumigatus*, n = 23; *A. flavus*, n = 2; *A. terreus*, n = 1) from patients with febrile neutropenia or baseline aspergillus infection were characterized for resistance. None of the *Aspergillus* isolates had an MIC value of >2 μg/ml. Characterization of isolates from patients who failed therapy did not reveal any mutation in the *FKS1* gene or decreased inhibition of glucan synthase, where tested.

In summary, mutants with reduced susceptibility to caspofungin were obtained *in vitro*, in *C. albicans* infected mice treated with caspofungin, and from patients who failed caspofungin therapy. However, the breakpoints for caspofungin and methods for susceptibility testing of echinocandins have not been standardized.

The phase IV commitment to evaluate resistance development by *Candida* and *Aspergillus* species to caspofungin will continue until the year 2005.

1. INTRODUCTION AND BACKGROUND:

The subject of this NDA supplement is Cancidas® (Caspofungin), an approved drug for the treatment of invasive aspergillosis (in patients who are intolerant or refractory to amphotericin B treatment), esophageal candidiasis and invasive candidiasis. In this submission, the sponsor has provided an update for post-marketing study commitment #5. The objective of the phase IV commitment was to monitor resistance development by *Candida* and *Aspergillus* species to caspofungin and to characterize the resistance, where possible. These studies are to be done until the year 2005. Based on the findings, the sponsor has proposed changes to the microbiology section of the label.

3. PRECLINICAL MICROBIOLOGY:

Caspofungin belongs to the echinocandin class of antifungal agents that inhibit the synthesis of β (1, 3)-D-glucan, an essential component of fungal cell wall. Caspofungin is active *in vivo* and *in vitro* against *Candida* and *Aspergillus* species (for details see microbiology review dated 01-12-01).

3.1. DRUG RESISTANCE:

The development of resistance by *Candida* and *Aspergillus* species to caspofungin was evaluated by (1) generating mutants with reduced susceptibility to caspofungin *in vitro*, (2) selecting mutants with reduced susceptibility to caspofungin in infected mice treated with caspofungin, and (3) characterizing clinical isolates from patients with febrile neutropenia (clinical study 026) or with baseline *Candida* or *Aspergillus* infection (clinical studies 003, 004, 014, 019, 020, and 024). The mutant strains and clinical isolates were characterized by molecular, biochemical, and *in vivo* methods.

Molecular method: It has been reported that mutations in the β (1,3)-D-glucan synthase subunit (*FKS1*) gene leads to reduced *in vitro* and *in vivo* susceptibility to echinocandins including caspofungin (Kurtz *et al.,* 1996, Infect Immun, 64: 3244-3251; Douglas *et al.,* 1997, AAC, 41: 2471-2479). The amino acids 641-648 and 1357-1364 encoded by the 2.4 kb section of the *FKS1* gene have been identified to be critical for sensitivity of *C. albicans* to caspofungin. These amino acids were shown to be conserved among the various fungal species (*C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, *C. guilliermondii*, *C. rugosa*, *C. lipolytica*, *A. fumigatus* and *A. flavus*). Molecular characterization was performed for the isolates associated with either an unfavorable clinical response or MIC values of ≥ 2 μg/ml in RPMI medium by sequencing the 2.4 kb section of the *FKS1* gene. For this, the *FKS1* gene was amplified by polymerase chain reaction (PCR) using DNA from the laboratory strains or clinical isolates and *FKS1* primers. Sequencing was performed using a Dye-terminator cycle sequencing quick start kit and the sequencing reaction mix was run on a capillary electrophoresis DNA sequencer (Merck report 2003, Reference 6).

Biochemical method: It is known that caspofungin inhibits β (1,3)-D-glucan synthase. The glucan synthase enzyme was purified from the resistant *Candida* and *Aspergillus* strains and isolates and its sensitivity to the inhibitor, caspofungin, was measured. The 50% inhibitory concentration (IC_{50}) values were calculated (Merck report, April 2003, Reference 4, and Merck report, August 2003, Reference 1). The activity of glucan synthase from *C. parapsilosis* and most of the *Aspergillus* isolates was not measured due to technical difficulties encountered during enzyme purification.

***In vivo* method:** The activity of caspofungin against resistant *Candida* and *Aspergillus* strains and isolates was examined in infected mice (Merck report, August 2003, Reference 2). The activity of caspofungin against *C. albicans* (2.8×10^3 to 4.8×10^5 cfu) and *C. glabrata* (1.52×10^7cfu) was measured in immunocompetent C'5 deficient DBA/2J and immunocompromised CD-1 mice, respectively. The yeasts were inoculated by the intravenous route and treatment was initiated 15-30 minutes after infection with different doses of caspofungin administered intraperitoneally (qd for 4 days). The animals were sacrificed and the fungal burden in the kidneys measured 4 days post-infection. The effective dose for 90% reduction in fungal burden (ED_{90}) compared to control animals was calculated. The sponsor has stated that many *Candida* species other than *C. albicans* were not virulent in mice and therefore could not be tested.

The activity of caspofungin against *A. fumigatus* isolates from patients who failed caspofungin therapy was examined in DBA/2N mice. The mice were infected by intravenous inoculation of a 0.2 ml spore suspension (approximately 10^6 spores). Therapy was initiated with different doses of caspofungin within 15-30 minutes of challenge by the intraperitoneal route, for 5 days. Mice were followed for survival for up to day 28 post-challenge. The 50% effective dose (ED_{50}) values were estimated from survival rates calculated at day 28.

3.1.1. *Candida* species:

Candida strains with reduced susceptibility to caspofungin were generated *in vitro* and the resistance characterized by molecular, biochemical and *in vivo* methods. In addition, the resistance of *Candida* isolates characterized by similar methods. The criteria for selecting isolates for characterization included (a) isolates with caspofungin MIC values of ≥ 2 µg/ml in RPMI 1640 medium and ≥ 0.5 µg/ml in AM3 medium, (b) isolates with caspofungin MIC values of ≥ 2 µg/ml in RPMI 1640 medium but <0.5 µg/ml in AM3 medium, or (c) isolates obtained from patients who had unfavorable clinical response and/or persistent infection or had breakthrough infections after caspofungin therapy.

The susceptibility testing of the *Candida* strains and isolates was performed in RPMI 1640 and Antibiotic medium #3 (AM3) using the NCCLS M27-A method for yeast (Merck report, 2003, Reference 3). The identification of the yeast was confirmed at the Merck Research laboratory by morphological testing using CHROM agar and microscopic examination and biochemical testing using the Dade/MicroScan rapid yeast identification panel prior to susceptibility testing. The minimum inhibitory concentration (MIC) required for 100% inhibition compared to controls in RPMI medium was determined after 48 hours of incubation. The caspofungin MIC values in AM3 medium were determined after 24 hours of incubation. The term MIC_{90} indicates the MIC of the same isolates when tested 90% of the time.

3.1.1.1. Laboratory strains with reduced susceptibility to caspofungin generated *in vitro*:

Strains of *C. albicans* with reduced susceptibility to caspofungin were generated *in vitro* using the *C. albicans* strains CAI-4 and MY1055 by the fluctuation test method. For the fluctuation test, stationary phase *C. albicans* cultures were diluted in yeast extract peptone dextrose broth supplemented with 100 µg/ml adenine and 100 µg/ml uridine (YPDAU) to give a colony count of about 1 cfu/ml and an aliquot of 0.5 ml of this suspension was grown at 30°C for 2 days to the stationary phase. The cultures were plated on YPDAU agar containing 0.8 µg/ml of caspofungin. The sponsor has stated that 4 spontaneous resistant mutants were isolated based on >16 fold higher MIC values in RPMI medium

(caspofungin MIC_{90} >32 µg/ml) and in AM3 medium (caspofungin MIC_{90} 1 to >2 µg/ml) compared to wild type (caspofungin MIC_{90} values 0.12 to 0.5 µg/ml in RPMI medium and <0.06 µg/ml in AM3 medium). However, it is unclear if the 4 resistant strains were isolated from the same wild type strain or 2 different strains. These 4 mutant strains (NR-4, R-1, T-25, and NR-3) and the wild type strains were characterized by molecular, biochemical and *in vivo* methods (Table 1).

The strains, NR-4 and R-1, were heterozygous for *FKS1* gene with one wild type allele and one mutant allele. Both strains carried a mutant allele where the serine residue was mutated to a proline at position 645 of the derived amino acid sequence of FKS1 protein (FKS1p). In strain T-25, one of *FKS1* allele was knocked out and the second allele had a serine to proline mutation at position 645 of the amino acid sequence of FKS1p, while the strain NR-3 was homozygous for *FKS1* gene with both alleles carrying a serine to tyrosine mutation at amino acid position 645 of the FKS1p.

The caspofungin IC_{50} values representing inhibition of the glucan synthase enzyme activity from the 2 heterozygous strains showed a biphasic inhibition pattern (caspofungin IC_{50} values was 0.5 and 100 ng/ml). The caspofungin IC_{50} values against glucan synthase from the remaining strains, T-25 and NR-3, were >146-fold higher than wild type.

The susceptibility of the 2 heterozygous strains to caspofungin in mice (represented as ED_{90} values) was reduced (10 to 50 fold lower than the wild type). The susceptibility of the strain T-25 with the knock out mutation and the homozygous strain NR-3 was even further reduced (471 to 1600 fold lower than the wild type).

Table 1: Characteristics of wild-type caspofungin-susceptible *Candida albicans* isolates and laboratory generated *Candida albicans* isolates with reduced susceptibility to caspofungin.

Strain Number	*FKS1* Genotype	MIC_{90}† RPMI	MIC_{90} AM3	Glucan Synthesis IC_{50}‡	Mouse ED_{90}§
Wild-Type and Laboratory Derived Isolates					
MY1055	S645/S645	0.5	<0.06	0.29	0.007
CAI-4	S645/S645	0.12	<0.06	0.91	0.002
NR-4	S645P/wt 645	>32	1	0.5 & 100	0.07
R-1	645wt/S645P	>32	>2	0.5 & 100	0.1
T-25	Null/S645P	>32	>2	133	3.30
NR-3	S645Y/S645Y	>32	>2	2500	3.20
Gnotobiotic Mouse Derived Isolates					
2-1-#1	F641C/F641C	>32	2	77	2.42
2-1-#2	F641C/F641C	64	>2	50	1.05
2-2-#2	F641C/wt641	2	0.12	0.5 & 50	<0.25

† Minimum Inhibitory Concentration (MIC) in µg/mL of the isolate when tested 90% of the time (isolates were tested between 10 and 24 times).
‡ Glucan Synthase Inhibitory Concentration required for 50% inhibition of glucan synthesis ($IC_{50\%}$) in ng/mL.
§ Effective Dose required for 90% reduction of colony forming units of *Candida albicans* in kidneys of mice compared to nonmedicated controls ($ED_{90\%}$) in mg/kg/day.

3.1.1.2. Laboratory strains with reduced susceptibility to caspofungin generated *in vivo*:

Strains with reduced susceptibility to caspofungin were isolated at end of therapy from gnotobiotic mice. The mice were infected by swabbing the oral cavity and mildly abrading the mucosa with *C. albicans* and treated orally with 25 mg/kg/day of caspofungin for 43 days. Three strains (2-1#1, 2-1#2

and 2-2-#2) with increased MIC values (caspofungin MIC_{90} values in RPMI = 2 to 64 µg/ml and in AM3 medium = 0.12 to >2 µg/ml) were obtained (see Table 1 above). The details of the experimental design were not included. The caspofungin MIC_{90} values against the strains, 2-1#1 and 2-1#2, were similar to that of mutants generated by the fluctuation analysis method while that against strain 2-2#2 was lower. The sponsor has stated that the strains, 2-1#1 and 2-1#2, were obtained from the same mouse. The 2 strains were homozygous for *FKS1* gene with a phenylalanine to cysteine substitution (F641C) observed at amino acid position 641 of FKS1p. The strain 2-2#2 with caspofungin MIC_{90} of 2 and 0.12 µg/ml in RPMI and AM3 medium, respectively, was heterozygous for the *FKS1* gene. The strain had one allele with a phenylalanine to cysteine susbstitution at amino acid position 641 and one wild type allele.

The caspofungin IC_{50} values representing inhibition of glucan synthase activity against the 2 homozygous strains were high (50-77 ng/ml). Against the heterozygous strain, a biphasic inhibition pattern was observed (caspofungin IC_{50} values was 0.5 and 100 ng/ml). Please note that controls consisting of wild type strains or strains from mice with normal susceptibility to caspofungin *in vitro* and wild type *FKS1* gene sequence were not used as controls.

The activity of caspofungin in mice (represented as increased ED_{90} values) against the 2 homozygous mutant strains was lower (1.05 to 2.42 mg/kg/day) than the wild type strain. However, caspofungin was effective in reducing the fungal burden in the kidneys of mice infected with the heterozygous strain.

Please note that these studies were limited to *C. albicans*. Species other than *C. albicans* were not tested.

In summary, the activity of caspofungin was decreased as measured by (i) inhibition of glucan synthase enzyme, and (ii) activity in animals infected with strains of *C. albicans* with reduced susceptibility to caspofungin generated *in vitro*. These effects appear to be correlated with changes at position 645 of the derived amino acid sequence of FKS1p. Similarly, reduction in the activity of caspofungin against glucan synthase enzyme and in mice was observed against strains with reduced susceptibility selected from gnotobiotic mice infected with *C. albicans* and treated with the drug. These effects appear to be correlated with changes at position 641 of the derived amino acid sequence of FKS1p.

3.1.1.3. Clinical isolates of *Candida species* with reduced susceptibility to caspofungin:

Isolates from patients with febrile neutropenia:
Clinical isolates (n = 37) of *Candida* species from patients enrolled in study 026 were selected on the basis of (a) caspofungin MIC values of ≥2 µg/ml in RPMI 1640 medium and ≥0.5 µg/ml in AM3 medium or (b) caspofungin MIC values of ≥2 µg/ml in RPMI 1640 medium but <0.5 µg/ml in AM3 medium. These isolates were evaluated for changes in genotype and phenotype. Both *C. albicans* (n = 9) and *Candida* species other than *C. albicans* (*C. glabrata*, n = 3; *C. tropicalis*, n = 5; *C. krusei*, n = 3; *C. guilliermondii*, n = 2; *C. parapsilosis*, n = 15) were included for characterization (Table 2). Of the 9 *C. albicans* isolates (from 3 patients), 4 showed serine to phenylalanine substitution (S645F) at amino acid position 645 of FKS1p. These mutations were associated with high MIC values (≥ 4 µg/ml in RPMI medium; ≥ 2 µg/ml in AM3 medium), decreased inhibition of glucan synthase activity (IC_{50} >160 ng/ml) and >100-fold decreased susceptibility of the isolates to caspofungin in

mice (ED_{90} value of 0.7 to 10 mg/kg/day) compared to the wild type strains (see Tables 1 and 2). Although the remaining 5 isolates were from the same 3 patients with breakthrough infection, the caspofungin IC_{50} values for glucan synthase from these isolates and susceptibility of these isolates to the drug in mice were similar to wild type strains.

All the 3 *C. glabrata* isolates (2 from patients treated with caspofungin and 1 from patient treated with AmBisome) had a caspofungin MIC of 2 μg/ml in RPMI medium and ≤ 0.125 μg/ml in AM3 medium. All the isolates had the wild type *FKS1* gene. The caspofungin IC_{50} value for the glucan synthase enzyme ranged from 14 to 40 ng/ml. The sponsor has stated that these isolates were not related to infection in the patients based on the evaluation of an independent adjudicator. The criteria used to determine which isolates are related to infection are unclear. The activity of caspofungin against these isolates was not tested in mice. Also, a wild type *C. glabrata* strain with normal susceptibility to caspofungin was not used as a comparator.

All the 5 *C. tropicalis* isolates (4 were from patients treated with caspofungin and 1 from patient treated with AmBisome) did not show any mutation in the *FKS1* gene. Although 4 isolates were from patients who had breakthrough infection or had clinically failed caspofungin therapy, the caspofungin MIC values against these isolates were low (≤ 2 μg/ml in RPMI medium and ≤ 0.006 μg/ml in AM3 medium). The isolate from the patient with clinically favorable response but persistent infection after AmBisome therapy showed caspofungin MIC values similar to those obtained from patients treated with caspofungin. The caspofungin IC_{50} value for the glucan synthase enzyme from these isolates ranged between 0.7 and 4.4 ng/ml. A wild type *C. tropicalis* strain or an isolate from patient successfully treated with caspofungin was not used as a comparator. None of these isolates were tested in mice.

Of the 3 *C. krusei* isolates, only 1 obtained from a patient (in the caspofungin arm) with breakthrough infection showed an arginine to glycine substitution (R1361G) at amino acid position 1361 of FKS1p. This mutation was associated with a high MIC value (32 μg/ml in RPMI medium; 16 μg/ml in AM3 medium) and a high IC_{50} value against glucan synthase enzyme (795 ng/ml). The isolate was avirulent in immunocompetent mice and is currently being tested in immunocompromised mice. No mutation was observed in the remaining 2 isolates (1 in the caspofungin arm and 1 in the AmBisome arm). The caspofungin IC_{50} values for glucan synthase enzyme against the 2 isolates were 46 and 172 ng/ml. These IC_{50} values were associated with caspofungin MICs of 2 μg/ml in RPMI medium and ≤ 0.25 μg/ml in AM3 medium. However, the caspofungin IC_{50} values for wild type *C. krusei* were not included.

The 2 *C. guilliermondii* isolates (in the AmBisome arm) that were tested did not show a mutation in *FKS1* gene, although one of the isolates had a caspofungin MIC value of >8.0 μg/ml in RPMI medium and 0.5 μg/ml in AM3 medium. The caspofungin inhibitory concentrations against glucan synthase from this strain (IC_{50} value = 32 ng/ml) was 2.5 fold higher than that against the isolate (CLY11019) which was stated not to be associated with infection (caspofungin MIC value of 2 μg/ml in RPMI medium and 1 μg/ml in AM3 medium). These isolates were not tested in mice. Please note that these isolates were obtained from patients treated with AmBisome. Isolates from patients treated with caspofungin were not evaluated.

Table 2: Yeast isolates with potentially reduced susceptibility to caspofungin from patients treated with caspofungin in study protocol 026 and selected for further characterization.

Isolate	AN#	Treatment	Infection or clinical outcome	Caspofungin MIC(µg/ml)		Glucan Synthesis‡ IC_{50} (ng/ml)	FKS1p mutation	Mouse ED_{90} (mg/kg/day)
				RPMI 1640 100% inhibition	AM3 100% inhibition			
C. albicans								
CLY16996*	0815	Caspofungin	Breakthrough	>8.0	2.0	1997	S645F	1.09
CLY16998*	0815	Caspofungin	Breakthrough	0.5	0.06	0.56	None	<0.06
CLY18195*	0815	Caspofungin	Breakthrough	0.25	0.06	0.91	None	0.01
CLY16997*	0815	Caspofungin	Breakthrough	>8.0	4.0	162	S645F	9.98
CLY19228#	3342	Caspofungin	Breakthrough	0.5	0.06	0.40	None	0.005
CLY19229#	3342	Caspofungin	Breakthrough	0.5	0.06	0.40	None	0.002
CLY19230#	3342	Caspofungin	Breakthrough	4.0	2.0	420	S645F	0.76
CLY19231#	3342	Caspofungin	Breakthrough	4.0	2.0	456	S645F	0.73
CLY18603	3032	Caspofungin	Breakthrough	0.25	0.008	0.5	None	ND
C. glabrata								
CLY11012	0540	Caspofungin	None	2.0	0.125	14	None	ND
CLY10914	2082	Caspofungin	None	2.0	0.06	25	None	ND
CLY11018	2789	AmBisome	None	2.0	0.125	40	None	ND
C. tropicalis								
CLY10934	0362	Caspofungin	Breakthrough	1.0	0.06	4.4	None	ND
CLY16328	0742	Caspofungin	Breakthrough	0.5	0.015	3.1	None	ND
CLY18602	2455	Caspofungin	Failure	0.5	0.008	1.9	None	ND
CLY10910	0375	Caspofungin	Intermediate	2.0	0.03	0.7	None	ND
CLY16049	0468	AmBisome	Persistent	2.0	0.008	1.0	None	ND
C. krusei								
CLY16038	1040	Caspofungin	Breakthrough	32.0	16.0	795	R1361G	NC[&]
CLY10933	0361	Caspofungin	Failure	2.0	0.25	46	None	ND
CLY16245	0500	Ambisome	None	2.0	0.06	172	None	ND
C. guilliermondii								
CLY11020	2862	AmBisome	Breakthrough	>8.0	0.5	32	None	ND
CLY11019	2440	AmBisome	None	2.0	1.0	13	None	ND
C. parapsilosis								
CLY16036	2135	Caspofungin	Breakthrough	>8.0	1.0	ND	None	ND
CLY10939	2646	Caspofungin	Breakthrough	>8.0	0.5	ND	None	ND
CLY16237	2646	Caspofungin	Breakthrough	>8.0	0.5	ND	None	ND
CLY16016	3418	Caspofungin	Breakthrough	>8.0	0.06	ND	None	ND
CLY11013	0541	Caspofungin	None	2.0	2.0	ND	None	ND
CLY11014	0541	Caspofungin	None	2.0	0.5	ND	None	ND
CLY10921	0740	Caspofungin	None	>8.0	1.0	ND	None	ND
CLY18538	2489	Caspofungin	None	2.0	0.125	ND	None	ND
CLY18539	2489	Caspofungin	None	2.0	0.125	ND	None	ND
CLY18541	2489	Caspofungin	None	2.0	0.06	ND	None	ND
CLY18542	2489	Caspofungin	None	2.0	0.06	ND	None	ND
CLY10871	2183	AmBisome	Breakthrough	>8.0	0.25	ND	None	ND
CLY10872	2183	AmBisome	Breakthrough	2.0	0.5	ND	None	ND
CLY10873	2183	AmBisome	Breakthrough	2.0	2.0	ND	None	ND
CLY16819	3676	AmBisome	baseline	>8.0	1.0	ND	None	ND

*,# isolates were determined to be same based on random amplification of polymorphic DNA (RAPD) analysis and fingerprinting using CA3 probe for repetitive sequences.

NC = not calculable as the strain was avirulent in normal mice; [&]currently being tested in immunocompromised mice.

‡ Glucan Synthesis Inhibitory Concentration required for 50% inhibition of glucan synthesis (IC_{50}) in ng/ml.

ND = not done

In the case of the 15 *C. parapsilosis* isolates (11 from patients treated with caspofungin and 4 from patients treated with AmBisome), only sequencing of the *FKS1* gene was performed. The sponsor stated that technical problems were encountered in the extraction of enzyme from *C. parapsilosis* and therefore the inhibition of glucan synthase enzyme was not measured. The activity of caspofungin

against the *C. parapsilosis* isolates in mice was also not determined. All the *C. parapsilosis* isolates did not show any mutations in the *FKS1* gene, irrespective of their MIC values (2 to >8 μg/ml in RPMI and 0.06 to 2 μg/ml in AM3).

Overall, only limited characterization was performed for clinical isolates of *Candida* species other than *C. albicans*. Based on the limited testing in a small number of isolates of the *Candida* species, no conclusions can be drawn regarding correlation between clinical outcome, MIC values, mutations in the *FKS1* gene, inhibitory concentrations for glucan synthase, and activity *in vivo*.

Isolates from patients with baseline *Candida* infection:
In addition to the above isolates, clinical isolates (n = 48) of *Candida* species from patients enrolled in studies 003, 004, 014, 019, 020, and 024 selected on the basis of (a) caspofungin MIC values of ≥2 μg/ml in RPMI 1640 medium and ≥0.5 μg/ml in AM3 medium or (b) caspofungin MIC values of ≥2 μg/ml in RPMI 1640 medium but <0.5 μg/ml in AM3 medium were characterized for genotypic and phenotypic changes (Table 3). These included *C. albicans* (n = 9) and *Candida* species other than *C. albicans* (*C. glabrata*, n = 8; *C. guilliermondii*, n = 11; *C. krusei*, n = 5; *C. lipolytica*, n = 1; *C. parapsilosis*, n = 10; *C. rugosa*, n = 1; *C. tropicalis*, n = 3). Of the 9 *C. albicans* isolates, only 1 isolate with a high caspofungin MIC (4 μg/ml in RPMI medium and 1 μg/ml in AM3 medium) revealed a proline to histidine substitution (P648H) at amino acid position 648 of the FKS1p. The mutation was associated with decreased inhibition of glucan synthase enzyme (IC_{50} value = 209 ng/ml) and decreased susceptibility to the drug in mice (ED_{90} value = 0.867 mg/kg/day). However, the data on the clinical outcome of the patient from whom this isolate was obtained was not available. The analysis of 3 isolates from patients with an unfavorable clinical response and persistent yeast did not reveal any mutation in the *FKS1* gene even though the MIC value was high for one of the isolates in both medium (MIC >16 μg/ml in RPMI medium and >2 μg/ml in AM3 medium). The caspofungin IC_{50} and ED_{90} values against the 3 isolates were similar to the wild type strains (Tables 3 and 1). Four other isolates obtained from patients with a favorable clinical response (2 = persistence of yeast; 2 = eradication of yeast) did not show any mutations in the *FKS1* gene, although 2 of the isolates had high MIC values (>8 μg/ml in RPMI and 0.06 μg/ml in AM3). The inhibitory concentration for glucan synthase enzyme in 3 of the 4 isolates tested and the activity of caspofungin against 1 of the 4 isolates tested in mice were found to be similar to the wild type strains.

Of the 8 *C. glabrata* isolates, no mutation in the *FKS1* gene was observed in 6 isolates from patients with an unfavorable clinical response (MIC ≤ 4 μg/ml in RPMI medium and ≤ 0.5 μg/ml in AM3 medium) and 2 patients with a favorable clinical response (MIC ≤ 2 μg/ml in RPMI medium and ≤ 0.5 μg/ml in AM3 medium). The caspofungin IC_{50} values against glucan synthase enzyme appear to be similar for isolates obtained from patients with an unfavorable clinical response (0.3 to 2.3 ng/ml) and isolates obtained from patients with a favorable response (0.6 ng/ml). The activity of caspofungin against these isolates was not tested in mice.

All 11 *C. guilliermondii* isolates did not show any mutation in the *FKS1* gene. Two of the 11 isolates were from patients with an unfavorable clinical and mycological response and had high MIC values (>8 μg/ml in RPMI medium and 2 μg/ml in AM3 medium). The remaining 9 isolates from patients with a favorable clinical response also had high MIC values (>8 μg/ml in RPMI medium and 0.125 to >2 μg/ml in AM3 medium). The caspofungin IC_{50} values for glucan synthase from isolates obtained from patients with an unfavorable clinical response (20-27 ng/ml) was within the range observed for

isolates obtained from patients with a favorable clinical response (6.2 to 29 ng/ml). The activity of caspofungin against these isolates was not tested in mice.

All 5 *C. krusei* isolates did not show any mutation in the *FKS1* gene. One of the 5 isolates was from a patient with an unfavorable clinical response while the remaining 4 isolates were from patients with a favorable clinical response. The MIC values of isolates from both groups were similar. The caspofungin IC_{50} values for glucan synthase against the isolate obtained from the patient with an unfavorable clinical response (caspofungin IC_{50} = 69 ng/ml) was within the range observed for isolates from patients with a favorable clinical response (caspofungin IC_{50} = 11-206 ng/ml). These isolates were not tested in mice.

The single isolate of *C. lipolytica* obtained from a patient with an unfavorable clinical response did not show any mutation in the *FKS1* gene. The caspofungin IC_{50} for glucan synthase was 70 ng/ml. However, wild type strain of *C. lipolytica* or isolates from patients with a favorable response were not used for comparison. The clinical significance of such a finding is not known.

All 10 *C. parapsilosis* isolates did not show any mutation in the *FKS1* gene. Four of the 10 isolates were from patients with an unfavorable clinical response while the remaining 6 isolates were from patients with a favorable clinical response. The MIC values of isolates from patients showing an unfavorable clinical response overlapped with the MIC values of isolates from patients with a favorable clinical response. The glucan synthase activity was not measured for *C. parapsilosis* isolates due to technical difficulties in enzyme purification. The activity of caspofungin against these isolates was not tested in mice.

One isolate of *C. rugosa* with caspofungin MIC of >16 μg/ml in RPMI medium and 2 μg/ml in AM3 medium obtained from a patient with a favorable clinical response did not show any mutation in the *FKS1* gene. The caspofungin IC_{50} for glucan synthase was 1 ng/ml. No wild type strain of *C. rugosa* or isolates from patients with an unfavorable clinical response were used for comparison. The clinical significance of such a finding is not known.

Of the 3 isolates of *C. tropicalis*, none had any mutations in the *FKS1* gene. The caspofungin concentration required for inhibition of glucan synthase from an isolate obtained from a patient with an overall unfavorable response (caspofungin IC_{50} = 0.47 ng/ml) was similar to that of 2 isolates obtained from patients with a favorable response (caspofungin IC_{50} = 0.7 to 1.20 ng/ml). These isolates were not tested in mice.

A correlation between the MIC values, FKS1p mutation, inhibitory concentration for glucan synthase enzyme or susceptibility to the drug in mice and clinical outcome was not observed in the small number of isolates that were characterized.

Table 3: Yeast isolates with potentially reduced susceptibility to caspofungin from patients treated with caspofungin from clinical studies 003, 004, 014,020 and 024.

Isolate CLY#	Study	Outcome		Caspofungin MIC (µg/ml)		FKS1p mutation	Glucan synthesis‡ IC_{50} (ng/ml)	Mouse ED_{90}§ mg/kg/day
		Clinical	Micro	RPMI	AM3			
C. albicans								
10072	020	Unfav	Persis	>16	0.015	None	ND	0.004
10294	020	Unfav	Persis	0.25	0.03	None	ND	OT
9704B	020	Unfav	Persis	>16	>2	None	0.52	<0.125
10306	014	Fav	Persis	1	0.125	None	0.46	ND
9483	004	Fav	Persis	>8	0.06	None	ND	0.083
10274	020	Fav	Erad	0.25	0.03	None	0.40	ND
10753	014	Fav	Erad	>8	0.06	None	0.73	ND
9573	004	--	Persis	>8	0.125	None	ND	0.034
10885	024	--	--	4	1	P648H	209	0.867
C. glabrata								
10390	014	Unfav	Persis	4	0.06	None	0.30	ND
10391	014	Unfav	Persis	1	0.06	None	0.90	ND
9770	014	Unfav	--	2	0.5	None	ND	ND
9771	014	Unfav	--	2	0.5	None	ND	ND
9832	004	Unfav	--	2	0.5	None	ND	ND
9773	014	Unfav	Erad	2	0.125	None	2.3	ND
9394	014	Fav	Erad	1	0.015	None	0.60	ND
10737	014	Fav	--	2	0.5	None	ND	ND
C. guilliermondii								
9447	003	Unfav	--	>8	2	None	27	ND
9552A	004	Unfav	--	>8	2	None	20	ND
10357B	020	Fav	Erad	>8	0.5	None	14	ND
9612	004	Fav	--	>8	>2	None	12	ND
10337	020	Fav	--	>16	0.5	None	23	ND
10673	014	Fav	--	>8	>2 (0.125)	None	6.2	ND
9081B	003	Fav	--	>64	>2	None	22	ND
9173B	003	Fav	--	>8	>2	None	17	ND
10984	014	Fav	--	>8	>2	None	ND	ND
10892	014	Fav	--	>8	>2/0.5	None	ND	ND
9748B	004	Fav	--	>8	>2	None	29	ND
C. krusei								
9899	004	Unfav	Erad	2	0.5	None	69	ND
9535	014	Fav	Erad	2	1	None	206	ND
10954	014	Fav	Erad	2	0.5	None	11	ND
9796	014	Fav	--	2	1	None	ND	ND
10736	014	Fav	--	2	0.5	None	ND	ND
C. lipolytica								
9443	003	Unfav	--	2	1	None	70	ND
C. parapsilosis								
10398	014	Unfav	Persis	2	0.125	None	ND	ND
9767	014	Unfav	--	8	2	None	ND	ND
10966	014	Unfav	--	2	1	None	ND	ND
10958	014	Unfav	Erad	2	0.25	None	ND	ND
9834	020	Fav	Erad	4	2	None	ND	ND
9925	014	Fav	Erad	>8	1	None	ND	ND
9569B	004	Fav	Erad	>8	0.125	None	ND	ND
10719	014	Fav	--	2	0.5	None	ND	ND
9568B	004	Fav	--	>8	2	None	ND	ND
9080	003	Fav	--	2	1	None	ND	ND
C. rugosa								
10751	014	Fav	--	>16	2	None	1	ND
C. tropicalis								
9933	014	Unfav	Persis	0.5	0.03	None	0.47	ND
11023	014	Fav	Erad	0.5	0.03	None	1.20	ND
11028	014	Fav	-	>8	0.06	None	0.7	ND

‡ Glucan synthesis inhibitory concentration required for 50% inhibition of glucan synthesis (IC_{50}) in ng/ml.
§ Effective dose required for 90% reduction of colony forming units in kidneys of DBA/2 mice compared to controls (ED_{90}) in mg/kg/day.
ND = not done; OT = on test; -- = not available
Fav = Favorable; Unfav = Unfavorable; Erad = Eradicated; Persis = Persistent.

In addition to the isolates from clinical studies for caspofungin, 26 isolates from patients who failed caspofungin therapy were referred to Merck by outside investigators for genotypic and phenotypic characterization (Table 4). These include 17 *C. albicans* and 9 *C. glabrata* isolates. Of the 17 *C. albicans* isolates, mutation in the FKS1p was observed in 6 isolates. These mutations include a serine to tyrosine substitution at amino acid position 645 (S645Y), an arginine to histidine substitution at amino acid position 1361 (R1361H), phenylalanine to leucine substitution at amino acid position 641 (F641L), and an aspartic acid to tyrosine substitution at amino acid position 647 (D647Y). The isolate with the S645Y mutation was associated with high MIC values (64 µg/ml in RPMI medium and >16 µg/ml in AM3 medium). The isolate also showed a >3000-fold decrease in inhibition of glucan synthase activity and a >100-fold decreased susceptibility to the drug in mice. The caspofungin IC_{50} (0.66 ng/ml) for glucan synthase enzyme from the isolate with the heterozygous mutation (R1361H/wt) in *FKS1* gene was similar to wild type *C. albicans* strains (Tables 4 and 1). However, the susceptibility (ED_{90} = 0.67 mg/kg/day) against the isolate in mice was 95-335 fold lower than wild type (ED_{90} = 0.002-0.007 mg/kg/day). The caspofungin inhibitory concentrations for glucan synthase enzyme (IC_{50} = 39 ng/ml) against the isolate with the homozygous mutation (R1361H) in FKS1p was 42-134 fold higher than wild type *C. albicans* strains (Tables 4 and 1). The activity of caspofungin against this isolate is currently being tested in mice. Two isolate with caspofungin MIC values of 2 µg/ml in RPMI medium and ≤ 1µg/ml in AM3 medium showed homozygous mutation (F641L) in FKS1p. Only one of the 2 isolates was evaluated further. A 5 fold decrease in inhibition of glucan synthase activity (IC_{50} = 5 ng/ml) and 100 fold decrease in susceptibility (ED_{90} = 0.74 mg/kg/day) in mice was observed with this isolate compared to wild type strain. For the isolate with the D647Y mutation in the FKS1p, the caspofungin IC_{50} against this isolate was similar to the isolate with the homozygous R1361H mutation and 42-134 fold higher than wild type *C. albicans.* A 23 fold decreased susceptibility (ED_{90} = 0.164 mg/kg/day) in mice was observed with this isolate compared to wild type strain. These effects were associated with caspofungin MIC of 4.0 µg/ml in RPMI medium and 0.5 µg/ml in AM3 medium. The inhibition of glucan synthase and susceptibilities of 3 of the 11 isolates without any mutations in the *FKS1* gene in mice were same as wild type. The MIC values of these 3 isolates were low (≤1 µg/ml in RPMI medium and ≤ 0.25 µg/ml in AM3 medium). The 8 remaining isolates without mutations in *FKS1* gene were not characterized further.

Of the 9 *C. glabrata* isolates, none of the isolates showed any mutation in the *FKS1* gene. The caspofungin IC_{50} values against 3 isolates that were tested ranged between 12 and 24 ng/ml. One of these isolates was tested in immunocompromised mice and showed an ED_{90} value of 1.72 mg/kg/day. However, wild type *C. glabrata* strains were not used as controls in these experiments. The clinical significance of these IC_{50} and ED_{90} values is not known.

Table 4: Further characterization of additional *Candida* referred isolates from marketed use of caspofungin, including mouse efficacy.

Isolate CLY#	Patient ID	Caspofungin MIC (µg/ml)		FKS1p mutation	Glucan synthesis‡ IC_{50} (ng/ml)	Mouse ED_{90}§ (mg/kg/day)
		RPMI 1640 100% Inhibition	AM3 100% Inhibition			
C. albicans						
18559	1W	64	>16	S645Y	3335	1.29
18600@	2W	64	0.5	R1361H/wt	0.66	0.67
18560@	2W	0.5	0.125	None	1.3	0.007
18561$	3W	1.0	0.125	None	0.27	0.001
18563	4W	1.0	0.25	None	0.95	0.005
656	1G	1.0	0.015	None	ND	ND
10821	1G	1.0	0.015	None	ND	ND
10822	1G	1.0	0.015	None	ND	ND
10824	1G	1.0	0.06	None	ND	ND
10825	1G	0.5	0.03	None	ND	ND
10826	1G	1.0	0.06	None	ND	ND
719	1G	2.0	1.0	F641L	5	0.74
721	1G	2.0	0.125	F641L	ND	ND
18558	1Z	0.5	0.125	None	ND	ND
18550	1Z	0.25	0.06	None	ND	ND
16376	None[a]	>16.0	1.0	R1361H	39	OT
724	None[b]	4.0	0.5	D647Y	39	0.164
C. glabrata						
18601@	2W	1.0	1.0	None	12	1.72#
18562$	3W	1.0	0.5	None	24	NV¶
657	2G	2.0	0.125	None	ND	ND
10823	2G	2.0	0.125	None	ND	ND
10827	2G	2.0	0.125	None	ND	ND
10828	2G	2.0	0.125	None	ND	ND
10829	2G	2.0	0.125	None	ND	ND
10830	2G	2.0	0.125	None	ND	ND
718	2G	2.0	0.5	None	20	ND

‡ Glucan synthesis inhibitory concentration required for 50% inhibition of glucan synthesis (IC_{50}) in ng/ml.
§ Effective dose required for 90% reduction of colony forming units of *Candida albicans* in kidneys of DBA/2 mice compared to controls (ED_{90}) in mg/kg/day.
#Effective Dose required for 90% reduction of colony forming units of *Candida glabrata* in kidneys in cyclophosphamide treated CD-1 mice compared to controls (ED_{90}) in mg/kg/day.
¶ NV = Nonvirulent isolate.
ND = not applicable.
OT = on test
@, $ refer to isolates from the patients 2W, and 3W, respectively
[a] isolate referred by Dr. Brown;
[b] isolate obtained from Beth Israel Hospital
Note: The MIC values above were derived from the Clinical Microbiology Laboratory at Merck. Patient numbers followed by a "W" were referred by Dr. Waldrup, patient numbers followed by a "G" were referred by Dr. Graybill, patient numbers followed by a "Z" were referred by Dr. Zoller.

There were 2 abstracts reporting on caspofungin MICs for sequential isolates obtained from patients who either failed or relapsed after caspofungin therapy. These are discussed below:

In one report (Moudgal *et al.*, Abstract, 40th Meeting of the Infectious Disease Society of America, Chicago, 2002), a patient with aortic valve replacement developed fungemia with *C. parapsilosis*. The patient was treated sequentially with multiple antifungal agents (amphotericin B, flucytosine, fluconazole) including caspofungin. However, blood cultures were positive for *C. parapsilosis*. Five

isolates of *C. parapsilosis* collected at various time points of antifungal therapy were found to be similar by electrophoretic genotyping (the details of the genotyping method were not included). The MIC values for caspofungin, fluconazole and amphotericin B for 5 sequential isolates from the same patients were 2, 64, and 0.5 µg/ml, respectively (Table 5). However, an increase in micafungin MIC values was observed in sequential isolates from the same patient. The method used to determine *in vitro* susceptibility to the different antifungal agents was not specified. Please note that methods for *in vitro* susceptibility testing of echinocandins and breakpoints for caspofungin or micafungin have not been established. The clinical significance of this finding is not known.

Table 5 : MIC for different antifungals against 5 sequential isolates from a patient who failed caspofungin therapy.

Drug	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5
Fluconazole	64.0	64.0	64.0	64.0	64.0
Voriconazole	0.06	0.06	0.06	0.06	0.06
Caspofungin	2.0	2.0	2.0	2.0	2.0
Micafungin	4.0	8.0	16.0	16.0	16.0
Amphotericin B	0.5	0.5	0.5	0.5	0.5

In another report (Hernandez *et al.,* Abstract, Focus of fungal infections, Hawaii, 2003), isolates from a HIV positive patient with esophagitis due to *C. albicans* who relapsed after treatment with caspofungin (50 mg/day for 4 weeks) were tested for *in vitro* susceptibility by the NCCLS M27-A methods and in the mouse model for disseminated candidiasis. The patient had failed previous treatment with multiple antifungals, including azoles. The caspofungin MIC values for the baseline isolate, isolate at end of caspofungin therapy, and isolate at relapse were 0.25, 0.25, and <64 µg/ml, respectively. It was stated that mice infected with the isolate obtained at relapse required a higher dose (1 mg/kg) to reduce fungal cell counts in the kidneys of infected mice. The study suggests that isolates with reduced susceptibility to caspofungin can develop during caspofungin therapy.

The 3 characteristics measured to determine resistance did not show consistent correlation with *in vitro* susceptibility to caspofungin or clinical outcome. The sponsor has stated that baseline glucan synthesis inhibitory concentration values for caspofungin against *Candida* species other than *C. albicans* varied considerably and overlapped for isolates having high MIC values, known mutations in *FKS1* gene and reduced susceptibility in mouse model of disseminated candidiasis. However, data on the baseline glucan synthase activity of wild type strains of *Candida* species other than *C. albicans* were not provided. Based on the above data, it appears that mutation leading to amino acid substitution at positions 641, 645, 647, 648 and 1361 of the *C. albicans* FKS1 protein reduces *in vitro* and *in vivo* caspofungin susceptibility. However, conclusions based on amino acid substitution cannot be made at this time, as the number of isolates tested was too small. Also, the clinical relevance of these mutations needs to be further investigated. In addition, the role of altered expression of other proteins in reducing susceptibility to caspofungin was not measured. Studies have shown that overexpression of SBE2p and CDR2p in *Saccharomyces cerevisiae* or *C. albicans* strains was associated with reduced *in vitro* susceptibility to caspofungin (Osherov *et al.,* 2002, AAC 46: 2462-2469; Schuetzer-Muehlbauer *et al.,* 2003, Mol. Micro. 48: 225-235). The expression of these proteins was not examined in the clinical isolates.

Overall, the results suggest that mutants with reduced susceptibility to caspofungin can develop during caspofungin therapy as was observed previously in mice (see microbiology review dated 01-12-01).

3.1.2. *Aspergillus* species:

The resistance of some of the clinical *Aspergillus* isolates was characterized. These isolates with caspofungin MIC values of ≤ 2 µg/ml in RPMI 1640 medium or from invasive aspergillosis patients failing treatment with caspofungin from clinical studies 019 and 026 were seleted for further characterization. The susceptibility testing of the *Aspergillus* isolates was performed in RPMI 1640 using the NCCLS M38-A method for moulds (Merck report, 2003, Reference 3). The minimum inhibitory concentration required for 80% inhibition of growth (MIC_{80}) was measured.

3.1.2.1. Laboratory strains of *Aspergillus* species with reduced susceptibility to caspofungin generated *in vitro*:

The sponsor has stated that attempts to generate *Aspergillus* mutants with reduced susceptibility *in vitro* using methods similar to those described for *Candida* isolates above (see page 5) and by selective mutagenesis methods have been unsuccessful. However, the results were not included for review.

3.1.2.2. Laboratory strains of *Aspergillus* species with reduced susceptibility to caspofungin generated *in vivo*:

There were no studies conducted to select mutants with reduced susceptibility to caspofungin in animals infected with *Aspergillus* species.

3.1.2.3. Clinical isolates of *Aspergillus* species with reduced susceptibility to caspofungin:

No clinical *Aspergillus* isolate with reduced susceptibility to caspofungin (MIC >2 µg/ml in RPMI) has been identified to date. However, isolates obtained from patients who failed caspofungin therapy in clinical studies 026 and 019 were further characterized. Limited biochemical characterization using the glucan synthase enzyme was performed due to technical difficulties in purifying the enzyme from the *Aspergillus* isolates.

Isolates from patients with febrile neutropenia:
The 7 *Aspergillus* (4 *A. fumigatus*, 2 *A. flavus* and 1 *A. terreus*) isolates from 7 different patients who failed caspofungin therapy in study 026 had MIC values of 0.25 to 1 µg/ml (Table 6). No mutation was observed in the FKS1p in all the isolates that were tested. The caspofungin IC_{50} values for glucan synthase against these isolates ranged from 0.03 to 0.81 ng/ml. Wild type *Aspergillus* strains were not used as comparators. The activity of caspofungin against these isolates was not measured in mice.

Table 6: *Aspergillus* isolates with potentially reduced susceptibility to caspofungin from patients treated with caspofungin enrolled in study 026 selected for further characterization.

Isolate CLF#	AN#[§]	Outcome	Caspofungin MIC (µg/ml) RPMI 1640	[‡]Glucan Synthesis IC_{50} (ng/ml)	FKS1p mutation
A. fumigatus					
16234	3120	Failure	1	0.61	None
16999	0815	Breakthrough	0.25	0.77	None
9126	2460	PBreakthrough	0.25	0.81	None
18190	3081	Breakthrough	0.5	0.38	None
A. flavus					
16844	0528	Breakthrough	0.25	0.80	None
9156	0540	SBreakthrough	0.5	0.03	None
A. terreus					
9151	2002	Failure	1	0.31	On test

[‡] Glucan synthesis inhibitory concentration required for 50% inhibition of glucan synthesis (IC_{50}) in ng/ml.
[§] AN# refers to the patient number in Protocol 026;
Note: The outcomes were classified as Pbreakthrough = Possible Breakthrough or Sbreakthrough = Subsequent Breakthrough based on the evaluation of an independent adjudicator appointed by Merck. The basis for this classification was not included.

Isolates from patients with baseline *Aspergillus* infection:

Clinical isolates (n = 19) of *A. fumigatus* obtained from (a) patients who had an unfavorable clinical and mycological response to caspofungin, (b) patients who had a favorable clinical response but persistent infection, and (c) patients who had an overall favorable response in study 019 were characterized. All the isolates from the 3 patient groups had caspofungin MIC values of ≤ 2 µg/ml (Table 7). No mutation was observed in the FKS1p of these isolates. The glucan synthase activity against these isolates was not measured due to difficulties in enzyme purification. The caspofungin ED_{50} values (measured in mice) against the 3 isolates obtained from patients with an unfavorable response was <0.044 mg/kg/day. However, isolates from patients with a favorable response or wild type strains were not used as comparators. The clinical significance of the ED_{50} values is not known.

Table 7: *Aspergillus* isolates with potentially reduced susceptibility to caspofungin from patients treated with caspofungin from study 019.

Isolate CLF#	Outcome		Caspofungin MIC_{80}† (µg/ml) in RPMI 1640	FKS1p mutation	‡ Mouse ED_{50} (mg/kg/day)
	Clinical	Micro			
A. fumigatus					
9004	Unfav	Persis	0.06	None	ND
9009	Unfav	PPer	0.06	None	ND
9012	Unfav	Persis	≤0.03	None	ND
9014	Unfav	Persis	0.06	None	ND
9022	Unfav	Persis	0.125	None	ND
9032	Unfav	Persis	0.25	None	0.036
9049	Unfav	Persis	0.06	None	ND
9060	Unfav	Persis	0.25	None	0.044
9065	Unfav	Persis	0.25	None	ND
9095	Unfav	PPers	0.25	None	0.038
9023	Fav	Persis	0.125	None	ND
9024	Fav	Persis	0.125	None	ND
9043	Fav	Persis	0.125	None	ND
9044	Fav	Persis	≤0.03	None	ND
9083	Fav	Persis	0.5	None	ND
9011	Fav	Erad	2	None	ND
9099	Fav	Erad	0.25	None	ND
9019	Fav	Erad	0.125	None	ND
9021	Fav	PErad	0.125	None	ND

† Minimum Inhibitory Concentration for 80% inhibition of growth compared to control (MIC_{80}) in µg/ml of the isolate.

‡ Effective dose required for 50% survival of caspofungin treated DBA/2N mice infected with *A. fumigatus* compared to drug-free controls (ED_{50}) in mg/kg/day.

ND = not done; Fav = Favorable; Unfav = Unfavorable;
Erad = Eradicated; Pper = Presumed Persistent, Persis = Persistent.

4. CONCLUSIONS:

The sponsor has characterized resistance development to caspofungin by *Candida* using (1) mutants with reduced susceptibility to caspofungin generated *in vitro*, (2) mutants with reduced susceptibility to caspofungin *in vivo*, and (3) clinical isolates from patients with febrile neutropenia or baseline *Candida* infections with MICs of ≥ 2 µg/ml in RPMI medium and ≥ 0.5 µg/ml or ≤ 0.5 µg/ml in AM3 medium. The isolates were characterized by sequencing the *FKS1* gene, measuring inhibition of glucan synthase enzyme, and susceptibility to the drug in mice.

Four spontaneous *C. albicans* mutants with reduced susceptibility to caspofungin (MIC_{90} >32 µg/ml in RPMI medium and 1 to >2 µg/ml in AM3 medium) were isolated by the fluctuation analysis method. The isolates showed decreased inhibition of glucan synthase and decreased susceptibility to the drug in animals. These effects correlated with changes at position 645 of the derived amino acid sequence of FKS1p.

A reduction in the inhibition of glucan synthase and susceptibility to the drug in mice was also observed using isolates (caspofungin MIC_{90} values in RPMI = 2 to 64 μg/ml and in AM3 medium = 0.12 to >2 μg/ml) selected from gnotobiotic mice infected with *C. albicans* and treated with caspofungin. These effects correlated with changes at position 641 of the derived amino acid sequence of FKS1p.

The resistance of clinical isolates (n = 111) of *Candida* species (*C. albicans*, n = 35; *C. glabrata*, n = 20; *C. tropicalis*, n = 8; *C. krusei*, n = 8; *C. guilliermondii*, n = 13; *C. parapsilosis*, n = 25; *C. lipolytica*, n = 1; *C. rugosa*, n =1) from studies 003, 004, 014, 019, 020, 024, 026 and those referred to Merck by outside investigators were characterized. Please note that some of the isolates were from the same patient. Of the 35 *C. albicans* isolates tested, 16 had caspofungin MICs of ≥ 2 μg/ml in RPMI medium. Of these 16 isolates, 11 had mutations in the FKS1 gene. The mutations observed were serine to tyrosine substitution at position 645 (S645Y), proline to histidine substitution at position 648 (P648H), arginine to histidine substitution at position 1361 (R1361H), aspartic acid to tyrosine substitution at position 647 (D647Y) and phenylalanine to leucine substitution at position 641 (F641L) of the derived amino acid sequence of FKS1 protein. A 162 to >3000-fold increase in caspofungin IC_{50} for glucan synthase was observed in 5 isolates carrying the S645Y mutation. In addition, a >100-fold caspofungin concentration was required for reduction in fungal burden in the kidneys of mice infected with these isolates. These effects were associated with an MIC of ≥ 4 μg/ml in RPMI medium and ≥ 2 μg/ml in AM3 medium and the isolates were obtained from patients who failed caspofungin therapy. The isolate carrying the P648H mutation showed a 200-fold increase in caspofungin IC_{50} for glucan synthase and a >100-fold increase in the caspofungin ED_{90} in mice. These effects were associated with a MIC of 4 μg/ml in RPMI and 1 μg/ml in AM3. The clinical outcome of the patient was not available. The effect of the R1361H mutation in the FKS1p on glucan synthase inhibition and *in vivo* activity was 40-fold, and 100-fold higher than wild type strains, respectively. These effects were associated with an MIC of >16 μg/ml in RPMI medium and ≥ 0.5 μg/ml in AM3 medium and unfavorable clinical response in the patients. In the case of the isolate with the D647Y mutation in the FKS1p, the inhibition of glucan synthase was reduced by 40 fold and the susceptibility in mice was decreased by 23-fold compared to wild type strains. These effects were associated with a MIC of 4 μg/ml in RPMI and 0.5 μg/ml in AM3 and unfavorable clinical response in the patient. Only one isolate with the F641L mutation was characterized. The caspofungin IC_{50} against the isolate was 5 fold higher than the wild type strains. The caspofungin ED_{90} value was 100-fold higher than wild type strains in mice. These effects were associated with a MIC of 2 μg/ml in RPMI and 1 μg/ml in AM3 and unfavorable clinical response in the patient. Of the 5 isolates that did not have a mutation in the *FKS1* gene, inhibition of glucan synthase enzyme was measured in 2 isolates. The caspofungin IC_{50} against the 2 isolates were similar to wild type strains. The caspofungin ED_{90} value against 1 of the 2 isolates that was tested in mice was also similar to wild type. Four of the 5 isolates were obtained from patients with unfavorable clinical response and/ or mycological response and one was obtained from a patient with an overall favorable response. Mutations in the *FKS1* gene were absent in the 19 isolates with caspofungin MICs of <2 μg/ml in RPMI medium. Of these 19 isolates, 17 were obtained from patient with an unfavorable clinical response and 2 were from patients with a favorable clinical response to caspofungin. The inhibition of glucan synthase enzyme and susceptibility of 11 of the 19 isolates in mice was similar to wild type strains.

Of the 20 *C. glabrata* isolates (MIC = >1 μg/ml in RPMI medium and >0.015 μg/ml in AM3) tested, none had any mutations in the *FKS1* gene. The caspofungin IC_{50} for glucan synthase in these isolates

ranged from 0.3 to 40 ng/ml. Only 1 of the 20 *C. glabrata* isolates was tested in immunocompromised mice. This isolate was not virulent when tested in immunocompetent mice and a higher inoculum (10^7 cfu) was required to infect the immunocompromised mice. The caspofungin ED_{90} against this isolate was 1.72 mg/kg/day. However, a wild type strain of *C. glabrata* was not used as a control in these experiments. Please note that 15 of the 20 isolates were from patients with an unfavorable clinical response, 2 were from patients with a favorable clinical response and 3 were stated by sponsor as not associated with infection. The inhibition of glucan synthase enzyme from isolates obtained from patients with an unfavorable clinical response was similar to that of isolates from patients with a favorable clinical response.

Of the 8 *C. krusei* isolates tested, only 1 isolate obtained from a patient who failed caspofungin therapy (MIC of 32 µg/ml in RPMI medium and 16 µg/ml in AM3 medium) showed an arginine to glycine substitution at amino acid position 1361 of the FKS1 protein. The caspofungin IC_{50} for glucan synthase enzyme from this isolate was elevated (795 ng/ml). However, the isolate was avirulent in normal mice. [redacted] The MIC for the 7 isolates that did not have any mutations in the *FKS1* gene were 2 µg/ml in RPMI medium and ≥0.06 µg/ml in AM3 medium. The range of caspofungin IC_{50} values for glucan synthase from these isolates was 11 to 206 ng/ml and was similar for isolates from patients who had unfavorable and favorable clinical responses. These isolates were not tested in mice.

No mutations were found in the *FKS1* gene of *C. guilliermondii* (n = 13), *C. parapsilosis* (n = 25), *C. tropicalis* (n = 8), *C. lipolytica* (n = 1) and *C. rugosa* (n =1) isolates. The caspofungin IC_{50} values for glucan synthase from *C. guilliermondii*, *C. tropicalis*, *C. lipolytica* and *C. rugosa* were <32, <4.4, 70 and 1 ng/ml, respectively. The inhibition of glucan synthase enzyme was not measured in *C. parapsilosis* isolates as technical problems were encountered in enzyme extraction. The IC_{50} values against isolates from patients with an unfavorable clinical response were similar to isolates from patients with a favorable clinical response. None of these isolates were tested in mice. The sponsor stated that the baseline glucan synthesis inhibitory concentration values for caspofungin against *Candida* species other than *C. albicans* varied considerably and often overlapped with isolates having high MIC values, known mutations in *FKS1* gene and reduced susceptibility in mouse model of disseminated candidiasis. However, data on inhibition of glucan synthase and susceptibility to the drug in mice for wild type strains of *Candida* species were not included. Based on the limited testing performed on a small number of isolates of *Candida* species, no conclusions can be drawn regarding correlation between clinical outcome and the MIC values, FKS1p mutation, inhibitory concentrations for glucan synthase or susceptibility to the drug in mice.

In addition to the above isolates, two reports described the *in vitro* susceptibility of sequential isolates from 2 different patients who either relapsed or failed clinically after caspofungin therapy. An increase in caspofungin MIC values was observed for sequential isolates obtained from a patient who relapsed after caspofungin therapy. It was stated that a higher dose of caspofungin was also required for reduction of fungal burden in mice infected with the isolate obtained at relapse. In another report, an increase in MICs of micafungin (another echinocandin) was observed in sequential isolates obtained from a patient who failed caspofungin therapy. However, no increase in the caspofungin MICs was observed (caspofungin MICs for all isolates was 2 µg/ml). The details of the method used for susceptibility testing were not included. As standardized methods for susceptibility testing and breakpoints for echinocandins have not been established, the clinical significance of an increase in micafungin MIC while on caspofungin therapy is not known.

The sponsor has characterized resistance development to caspofungin by *Aspergillus* using clinical isolates from patients with febrile neutropenia or baseline *Aspergillus* infections, as no *Aspergillus* isolates with reduced susceptibility to caspofungin were isolated by *in vitro* mutagenesis methods. Additionally, no *Aspergillus* isolates with reduced susceptibility to caspofungin (MIC >2 μg/ml) have been identified in clinical studies. The testing of 26 *Aspergillus* isolates obtained from patients who had an unfavorable clinical outcome or had persistence of the mould showed that the caspofungin MICs against all these isolates were ≤ 2 μg/ml and none had mutations in the *FKS1* gene. The caspofungin IC_{50} for glucan synthase tested in 7 isolates was <1 ng/ml. The caspofungin ED_{90} values against 3 of the isolates tested in mice were ≤ 0.044 mg/kg/day.

[redacted]

Overall, the studies suggest that resistant isolates of *Candida* species can arise during treatment. However, breakpoints for caspofungin have not been established. Isolates with reduced susceptibility were also shown to arise in infected mice treated with caspofungin. The sponsor will continue to provide updates on the surveillance monitoring for resistance annually until year 2005 to fulfil post-marketing commitments.

5. LABEL:

5.1. Sponsor's proposed label:

The changes to the current approved label for caspofungin are underlined and deletions striked out.

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 susceptible *Aspergillus* species and *Candida* species. β(1,3)-D-glucan is not present in mammalian cells. Caspofungin has shown activity against *Candida* species and in regions of active cell growth of the hyphae of *Aspergillus fumigatus*.

Activity in vitro
Caspofungin exhibits *in vitro* activity against *Aspergillus* species (*Aspergillus fumigatus*, *Aspergillus flavus*, and *Aspergillus terreus*) and *Candida* species (*Candida albicans, Candida glabrata, Candida guilliermondii, Candida krusei, Candida parapsilosis,* and *Candida tropicalis*). Susceptibility testing was performed according to the National Committee for Clinical Laboratory Standards (NCCLS) method M38-A (for *Aspergillus* species) and M27-A (for *Candida* species). Standardized susceptibility testing methods for β (1,3)-D-glucan synthesis inhibitors have not been established for yeasts and filamentous fungi, and results of susceptibility studies do not correlate with clinical outcome.

Activity in vivo
Caspofungin was active when parenterally administered to immunocompetent and immunosuppressed mice as long as 24 hours after disseminated infections with *C. albicans,* in which the endpoints were prolonged survival of infected mice and reduction of *C. albicans* from target organs. Caspofungin, administered parenterally to immunocompetent and immunosuppressed rodents, as long as 24 hours after disseminated or pulmonary infection with *Aspergillus fumigatus*, has shown prolonged survival, which has not been consistently associated with a reduction in mycological burden.

Drug Resistance
Mutants of *Candida* with reduced susceptibility to caspofungin have been identified in some patients during treatment. MIC values for caspofungin should not be used to predict clinical outcome, since a correlation between MIC values and clinical outcome has not been established. ~~A study in mice infected with *C. albicans* and treated with orally administered doses of caspofungin suggests that there is a potential for resistance development to occur.~~ *In vitro* resistance development to caspofungin by *Aspergillus* species has not been studied. In limited clinical experience, drug resistance in patients with invasive aspergillosis has not been observed. The incidence of drug resistance by various clinical isolates of *Candida* and *Aspergillus* species is unknown.

5.2. Comments:

1. The sponsor has proposed to include the statement "Mutants of *Candida* with reduced susceptibility to caspofungin have been identified in some patients during treatment. MIC values for caspofungin should not be used to predict clinical outcome, since a correlation between MIC values and clinical outcome has not been established" in the Microbiology section of the label under the heading "Drug Resistance". This is acceptable based on the results of the phase IV studies evaluating development of resistance by *Candida* and *Aspergillus* species to caspofungin. However, the sponsor has deleted the statement "A study in mice infected with *C. albicans* and treated with orally administered doses of caspofungin suggests that there is a potential for resistance development to occur". As development of resistance was reported in mice infected with *C. albicans* and treated with caspofungin (for details see microbiology review dated 01-12-01), this statement should be included in the label. Please note that the inclusion of the statement will reinforce the observation made in clinical studies and during marketed use of caspofungin.

2. The statements "*In vitro* resistance development to caspofungin by *Aspergillus* species has not been studied. In limited clinical experience, drug resistance in patients with invasive aspergillosis has not been observed" should be deleted. Methods for evaluation of resistance in *Aspergillus* have not been standardized. The sponsor has stated that studies used to generate *Aspergillus* mutants with reduced susceptibility to caspofungin *in vitro* were unsuccessful. However, studies to select for mutants with reduced susceptibility to caspofungin in animals infected with *Aspergillus* species were not conducted. Also, limited testing of clinical isolates of *Aspergillus* species for resistance development was performed. The testing mainly consisted of an evaluation of mutational changes in the *FKS1* gene. Only few isolates of *A. fumigatus* obtained from patients with an unfavorable clinical and mycological response were tested in mice. The activity of caspofungin against these isolates was not compared to isolates obtained from patients with a favorable clinical and mycological response. Hence, it would be inappropriate to say that drug resistance has not been observed in patients with invasive aspergillosis.

5.3. FDA's version of the label:

Based on the above comments the label has been modified. Additions are double underlined and deletions striked out.

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 susceptible *Aspergillus* species and *Candida* species. β(1,3)-D-glucan is not present in mammalian cells. Caspofungin has shown activity against *Candida* species and in regions of active cell growth of the hyphae of *Aspergillus fumigatus*.

Activity in vitro
Caspofungin exhibits *in vitro* activity against *Aspergillus* species (*Aspergillus fumigatus*, *Aspergillus flavus*, and *Aspergillus terreus*) and *Candida* species (*Candida albicans, Candida glabrata, Candida guilliermondii, Candida krusei, Candida parapsilosis,* and *Candida tropicalis*). Susceptibility testing was performed according to the National Committee for Clinical Laboratory Standards (NCCLS) method M38-A (for *Aspergillus* species) and M27-A (for *Candida* species). Standardized susceptibility testing methods for β (1,3)-D-glucan synthesis inhibitors have not been established for yeasts and filamentous fungi, and results of susceptibility studies do not correlate with clinical outcome.

Activity in vivo
Caspofungin was active when parenterally administered to immunocompetent and immunosuppressed mice as long as 24 hours after disseminated infections with *C. albicans,* in which the endpoints were prolonged survival of infected mice and reduction of *C. albicans* from target organs. Caspofungin, administered parenterally to immunocompetent and immunosuppressed rodents, as long as 24 hours after disseminated or pulmonary infection with *Aspergillus fumigatus*, has shown prolonged survival, which has not been consistently associated with a reduction in mycological burden.

Drug Resistance

Mutants of *Candida* with reduced susceptibility to caspofungin have been identified in some patients during treatment. Similar observations were made in a study in mice infected with *C. albicans* and treated with orally administered doses of caspofungin. MIC values for caspofungin should not be used to predict clinical outcome, since a correlation between MIC values and clinical outcome has not been established. ~~*In vitro* resistance development to caspofungin by *Aspergillus* species has not been studied. In limited clinical experience, drug resistance in patients with invasive aspergillosis has not been observed.~~ The incidence of drug resistance by various clinical isolates of *Candida* and *Aspergillus* species is unknown.

6. RECOMMENDATIONS:

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

Kalavati Suvarna
Microbiologist, HFD-590

CONCURRENCES:

HFD-590/Deputy Dir. _____Signature _____Date
HFD-590/Micro TL _____Signature _____Date
CC:
HFD-590/Original IND
HFD-590/Division File
HFD-590/MO
HFD-590/Pharm
HFD-590/Chem
HFD-590/Review Micro
HFD-590/CSO/ChiC

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

/s/

Kalavati Suvarna
12/1/03 02:59:06 PM
MICROBIOLOGIST

Shukal Bala
12/1/03 03:03:19 PM
MICROBIOLOGIST

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-227 / S-014
21-227 / S-011

ADMINISTRATIVE and CORRESPONDENCE DOCUMENTS

NDA 21-227/S-011, S-014

Labeling Review of Supplemental Labeling Revisions (SLRs):

Executive Summary:
This review describes and responds to:

- Merck's response to the Division's approvable letter for S-011 dated December 4, 2003 for Microbiology labeling
- A new "changes being effected" (CBE) labeling supplement (S-014) deleting the word "isolated" from the **ADVERSE REACTIONS**/*General* subsection

This review recommends approval of those proposed labeling changes.

Sponsor: Merck and Co., Inc.

Product: Cancidas™ (caspofungin acetate) for Injection, 50 mg/vial, 70 mg/vial

Materials Reviewed:

Response to December 3, 2003 Approvable Letter:

SLR Amendments	Date submitted	Date received	Date completed
011	January 29, 2004	January 30, 2004	March 8 , 2004
011	March 2, 2004	March 3 , 2004	March 8 , 2004

New CBE/SLR:

SLR	Date submitted	Date received	Date completed
014	February 11, 2004	February 12, 2004	March 8, 2004

- Approved package insert for NDA 21-227 dated January 7, 2003
- Original Labeling and Microbiology Review of S-011 dated December 4, 2003
- Division's approvable letter for S-011 dated December 4, 2003

Background:
NDA 21-227 was originally approved on January 26, 2001. The last approved labeling change occurred on January 7, 2003 when SE1-007 was approved.

MICROBIOLOGY Labeling in S-011
Supplement 011 was submitted for prior approval on June 5, 2003. In the cover letter Merck stated that this submission provided for an update in the **MICROBIOLOGY** section of the package insert based on information described in the March 28, 2003 submission which responded to Postmarketing Study Commitment 5 for Cancidas. That submission contained:

1. preliminary and unaudited information related to four *Candida* isolates identified as having reduced susceptibility to caspofungin in Protocol 026

2. a description of one *Candida* isolate with reduced susceptibility to caspofungin which was referred to MRL from postmarketing use of caspofungin
3. a commitment by MRL to submit a prior approval labeling supplement in support of a labeling change to the **MICROBIOLOGY** section of the package insert

On December 4, 2003 an approvable letter for S-011 was sent to the company with the following FDA proposed labeling revisions for **MICROBIOLOGY**/*Drug Resistance*:

Double underline=added text
~~Strikethrough~~=deleted text

Drug Resistance
Mutants of *Candida* with reduced susceptibility to caspofungin have been identified in some patients during treatment. Similar observations were made in a study in mice infected with *C. albicans* and treated with orally administered doses of caspofungin. MIC values for caspofungin should not be used to predict clinical outcome, since a correlation between MIC values and clinical outcome has not been established. ~~*In vitro* resistance development to caspofungin by *Aspergillus* species has not been studied. In limited clinical experience, drug resistance in patients with invasive aspergillosis has not been observed.~~ The incidence of drug resistance by various clinical isolates of *Candida* and *Aspergillus* species is unknown.

On January 29, 2003 the company submitted a response to the December 3, 2003 approvable letter but the "mice findings" sentence in *Drug Resistance* noted in the approvable letter was not included. On February 23, 2003 the company was contacted via e-mail and advised as follows concerning the following FDA proposed labeling revisions for **MICROBIOLOGY**/*Drug Resistance* subsection:

> The reason we wanted to keep the statements about mice findings was to reinforce the occurrence of resistance. It is true that mutants were observed in some patients, but that's what they studied. If the company wants they can delete the word "some", and then we would be OK taking the statements about animals out."

A revised label that included the mice findings statement as noted above was received by the Division on March 3, 2004.

New CBE/S-014
Supplement 014 was submitted as "Changes Being Effected" and provides for the deletion of the word "isolated" from the **ADVERSE REACTIONS**/*General* subsection based on reports of possible histamine-related symptoms including rash, facial swelling, pruritis, sensation of warmth or bronchospasm. That sentence now reads:

> "Possible histamine-mediated symptoms have been reported including ~~isolated~~ reports of rash, facial swelling, pruritus, sensation of warmth, or bronchospasm. Anaphylaxis has been reported during administration of CANCIDAS."

In an e-mail message dated February 23, 2004, Dr. Eileen Navarro, Medical Officer stated the following:

> I have no objection to the proposed labeling that removes the word "isolated" from the hypersensitivity section (rash, facial edema, bronchospasm etc). The rate of rash was 6.2% in the ETFN study, and the aggregate number of patients with the other AES (bronchospasm,hypersensitivity) rise to numbers beyond what would be considered "isolated".

Electronic Labeling Comparison:
The approved Cancidas™ label dated January 7, 2003 was electronically compared to the proposed draft label dated March 2, 2004. The changes were as follows:

Double underline=added text
~~Strikethrough~~=deleted text

1. MICROBIOLOGY

- The *Drug Resistance* subsection was revised to read:

> *Drug Resistance*
> ~~A study in mice infected with *C. albicans* and treated with orally administered doses of caspofungin suggests that there is a potential for resistance development to occur. *In vitro* resistance development to caspofungin by *Aspergillus* species has not been studied. In limited clinical experience, drug resistance in patients with invasive aspergillosis has not been observed~~. The incidence of drug resistance by various clinical isolates of *Candida* and *Aspergillus* species is unknown.
>
> Mutants of *Candida* with reduced susceptibility to caspofungin have been identified in some patients during treatment. Similar observations were made in a study in mice infected with *C. albicans* and treated with orally administered doses of caspofungin. MIC values for caspofungin should not be used to predict clinical outcome, since a correlation between MIC values and clinical outcome has not been established. The incidence of drug resistance by various clinical isolates of *Candida* and *Aspergillus* species is unknown.

2. **ADVERSE REACTIONS**

- The *General* subsection was revised to read:

> *General*
> Possible histamine-mediated symptoms have been reported including ~~isolated~~ reports of rash, facial swelling, pruritus, sensation of warmth, or bronchospasm. Anaphylaxis has been reported during administration of CANCIDAS.

Conclusions/Recommendations:
These labeling changes are acceptable. A letter should be sent advising the applicant that these supplemental NDA submissions are approved.

Robin Anderson, R.N., M.B.A.
Labeling Reviewer

Kala Suvarna, Ph.D.
Microbiology Reviewer

Eileen Navarro, M.D.
Medical Officer

cc:
HFD-590/MO/E. Navarro
HFD-590/MedTL/L. Sacks
HFD-590/Micro/K. Suvarna
HFD-590/MicroTL/S. Bala
HFD-590/DivDir/R. Albrecht
HFD-590/PM/C. Chi

Concurrence:
HFD-590/MO/E. Navarro 3/8/04
HFD-590/MedTL/L. Sacks 3/15/04
HFD-590/Micro/K. Suvarna 3/8/04
HFD-590/MicroTL/S. Bala 3/8/04
HFD-590/DivDir/R. Albrecht 3/17/04

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/s/

Robin Anderson
3/17/04 03:12:18 PM
INTERDISCIPLINARY

Renata Albrecht concurred with this review on 3/17/04.

Renata Albrecht
3/17/04 10:43:20 PM
MEDICAL OFFICER

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20857

NDA 21-227/S-014

CBE-0 SUPPLEMENT

Merck & Co., Inc.
Attention: Tamra L. Goodrow, Ph.D.
Sumneytown Pike
P.O. Box , BLA-20
West Point, PA 19486

Dear Dr. Goodrow:

We have received your supplemental drug application submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for the following:

Name of Drug Product: CANCIDAS™ (caspofungin acetate) For Injection

NDA Number: 21-227

Supplement number: 014

Date of supplement: February 11, 2004

Date of receipt: February 12, 2004

This supplemental application, submitted as "Supplement - Changes Being Effected" proposes the following change:

- Deletion of the word "isolated" from the **ADVERSE REACTIONS**, *General* subsection of the package insert to read as follows:

  "Possible histamine-mediated symptoms have been reported including ~~isolated~~ reports of rash, facial swelling, pruritus, sensation of warmth, or bronchospasm. Anaphylaxis has been reported during administration of CANCIDAS."

Unless we notify you within 60 days of the receipt date that the application is not sufficiently complete to permit a substantive review, we will file the application on April 12, 2004 in accordance with 21 CFR 314.101(a).

All communications concerning this supplement should be addressed as follows:

U.S. Postal Service:
Center for Drug Evaluation and Research
Division of Special Pathogen and Immunologic Drug Products, HFD-590
Attention: Document Room
5600 Fishers Lane
Rockville, Maryland 20857

Courier/Overnight Mail:
Food and Drug Administration
Center for Drug Evaluation and research
Division of Special Pathogen and Immunologic Drug Products, HFD-590
Attention: Document Room N-115
9201 Corporate Blvd.
Rockville, Maryland 20850

If you have any question, call Christina H. Chi, Ph.D., Regulatory Project Manager, at (301) 827-2127.

Sincerely,

{See appended electronic signature page}

Ellen F. Molinaro, R.Ph.
Chief, Project Management Staff
Division of Special Pathogen and Immunologic
Drug Products
Office of Drug Evaluation IV
Center for Drug Evaluation and Research

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

/s/

Ellen Molinaro
2/25/04 08:10:53 AM
NDA 21-227/S-014

NDA 21-227/S-011

Labeling, Microbiology and Clinical Review of Supplemental Labeling Revision (SLR):

Sponsor: Merck and Co., Inc.

Product: Cancidas™ (caspofungin acetate) for Injection, 50 mg/vial, 70 mg/vial

Materials Reviewed:

SLR	Date submitted	Date received	Date completed
011	June 5, 2003	June 6, 2003	November 28, 2003
Amendment 011	September 30, 2003	October 1, 2003	November 28, 2003

Background:
NDA 21-227 was originally approved on January 26, 2001. The last approved labeling change occurred on January 7, 2003 when SE1-007 was approved.

Supplement 011 was submitted for prior approval. In the cover letter Merck stated that this submission provided for an update in the **MICROBIOLOGY** section of the package insert based on information described in the March 28, 2003 submission which responded to Postmarketing Study Commitment 5 for Cancidas. That submission contained:

1. preliminary and unaudited information related to four *Candida* isolates identified as having reduced susceptibility to caspofungin in Protocol 026
2. a description of one *Candida* isolate with reduced susceptibility to caspofungin which was referred to MRL from postmarketing use of caspofungin
3. a commitment by MRL to submit a prior approval labeling supplement in support of a labeling change to the **MICROBIOLOGY** section of the package insert

In S-011, the preliminary, unaudited Caspofungin Susceptibility Surveillance Monitoring summary previously provided in the March 28, 2003 submission was audited and finalized in support of this labeling change.

Electronic Labeling Comparison:
The approved Cancidas™ label dated January 7, 2003 was electronically compared to the proposed draft label dated June 5, 2003. The changes were as follows:

Double underline=added text
~~Strikethrough~~=deleted text

MICROBIOLOGY

The *Drug Resistance* subsection was revised to read:

Drug Resistance

[redacted]

In an e-mail message dated November 20, 2003, Dr.Shukal Bala, Microbiology Team Leader stated that the proposed labeling changes contained in S-011 were not acceptable and that specific comments would be forthcoming. In an e-mail message dated November 26, 2003, Dr. Kala Suvarna, Microbiology Reviewer stated the following:

- The sponsor has proposed to include the statement "Mutants of *Candida* with reduced susceptibility to caspofungin have been identified in some patients during treatment. [redacted] in the Microbiology section of the label under the heading "Drug Resistance". This is acceptable based on the results of the phase IV studies evaluating development of resistance by *Candida* and *Aspergillus* species to caspofungin. However, the sponsor has deleted the statement " [redacted] As development of resistance was reported in mice infected with *C. albicans* and treated with caspofungin (for details see microbiology review dated 01-12-01), this statement should be included in the label. Please note that the inclusion of the statement will reinforce the observation made in clinical studies and during marketed use of caspofungin.

- The statements "*In vitro* resistance development to caspofungin by *Aspergillus* species has not been studied. In limited clinical experience, drug resistance in patients with invasive aspergillosis has not been observed" should be deleted.

Dr. Suvarna stated that the *Drug Resistance* subsection should read as follows:

> Mutants of *Candida* with reduced susceptibility to caspofungin have been identified in some patients during treatment. Similar observations were made in a study in mice infected with *C. albicans* and treated with orally administered doses of caspofungin. MIC values for caspofungin should not be used to predict clinical outcome, since a correlation between MIC values and clinical outcome has not been established. ~~*In vitro* resistance development to caspofungin by *Aspergillus* species has not been studied. In limited clinical experience, drug resistance in patients with invasive aspergillosis has not been observed.~~ The incidence of drug resistance by various clinical isolates of *Candida* and *Aspergillus* species is unknown.

In an e-mail message dated December 2, 2003, Dr. Rigoberto Roca, Medical Team Leader stated that he concurred with Dr. Suvarna's proposed labeling revisions.

Conclusions/Recommendations:
These labeling changes are not acceptable. A letter should be sent advising the applicant that this supplemental NDA submission is approvable.

Robin Anderson, R.N., M.B.A.
Labeling Reviewer

Kala Suvarna, Ph.D.
Microbiology Reviewer

Rigoberto Roca, M.D.
Medical Team Leader

cc:
HFD-590/MedTL/R. Roca
HFD-590/Micro/K. Suvarna
HFD-590/MicroTL/S. Bala
HFD-590/DivDir/R. Albrecht
HFD-590/PM/C. Chi

Concurrence:
HFD-590/MedTL/R. Roca 12/2/03
HFD-590/Micro/K. Suvarna 12/1/03
HFD-590/MicroTL/S. Bala 12/1/03
HFD-590/DivDir/R. Albrecht 12/3/03

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

/s/

Robin Anderson
12/3/03 10:24:18 AM
INTERDISCIPLINARY

Renata Albrecht concurred with this review on 12/3/03.

Renata Albrecht
12/4/03 03:21:30 PM
MEDICAL OFFICER