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APPROVAL PACKAGE FOR:

APPLICATION NUMBER

NDA 21-632

NDA 21-948

Microbiology Review(s)

Product Quality Microbiology Review

Review for HFD-590

February 25 , 2004

NDA: 21-632

Drug Product Name

Proprietary:

Non-proprietary: Anidulafungin

Drug Product Classification: antifungal

Review Number: 1

Subject of this Review

Submission Date:, January 14, 2004

Receipt Date: April 25, 2003; January 14, 2004

Consult Date: May 28, 2003

Date Assigned for Review: June 3, 2003

Submission History (for amendments only)

Date(s) of Previous Submission(s): April 25, 2003

Date(s) of Previous Micro Review(s): Review #1 was held open for
January 2004 Amendment.

Applicant/Sponsor

Name: Vicuron Pharmaceuticals Inc.

Address: 455 South Gulph Road
King of Prussia, PA 19406

Representative: Timothy J. Henkel, MD, PhD, Ex. VP and CMO

All regulatory communications assigned to:

Harriette Nadler, Ph.D.

Director, regulatory Affairs

Telephone: (610) 491-2211

Name of Reviewer: James L. McVey

Conclusion: The application is recommended for approval from a product quality microbiology perspective.

Product Quality Microbiology Data Sheet

- A.
1. **TYPE OF SUPPLEMENT:** N.A.
 2. **SUPPLEMENT PROVIDES FOR:** N.A.
 3. **MANUFACTURING SITE:**
[
]
Registration Number []
 4. **DOSAGE FORM, ROUTE OF ADMINISTRATION AND STRENGTH/POTENCY:** A 50 mg dose is provided for Intravenous Infusion. The final package contains two 15 mL vials. The first has 50 mg of anidulafungin lyophilized for reconstitution with the 20% ethanol/water from the second vial. Subsequent dilution is required prior to infusion. The final use concentration is 0.5 mg/mL.
 5. **METHOD(S) OF STERILIZATION:** The active drug containing vial is [lyophilized. The diluent is []
 6. **PHARMACOLOGICAL CATEGORY:** Antifungal agent
- B. **SUPPORTING/RELATED DOCUMENTS:** DMF for manufacture at [] LOA provided is dated March 4, 2003 (see Section 3.2.R.3 link to letter in original CMC submission).
- C. **REMARKS:** The original submission contained no [] process validation information and did not identify the filling lines or equipment to be used at [] for this product. After advising Vicuron representative H. Nadler of this issue, a phone conference was conducted with [] (contract manufacturer), Vicuron (applicant) and myself. A summary of this teleconference was made by Vicuron and included in the amendment. The request made in the phone conversation was that the filling lines and equipment used in the manufacture of the product be identified along with the appropriate sections of the DMF.

filename: 21632r1

Executive Summary

I. Recommendations

- A. Recommendation on Approvability** – This application is recommended for approval.
- B. Recommendations on Phase 4 Commitments and/or Agreements, if Approvable** – N.A.

II. Summary of Microbiology Assessments

- A. Brief Description of the Manufacturing Processes that relate to Product Quality Microbiology** – Both the lyophilized drug product and the initial solvent are [] manufactured at []
 [] The lyophilized powder is []

Table 1. Quantitative Composition of Anidulafungin for Injection.

Ingredient	Theoretical Quantity per vial ^{a,b}	Function	Reference to standards
Anidulafungin	50 mg ^c	Active Ingredient	
Fructose	50 mg		USP
Mannitol	250 mg		USP
Polysorbate 80	125 mg		USP/NF
Tartaric Acid	5.6		USP/NF
Sodium hydroxide and/or hydrochloric acid solution	as needed	pH adjustment	USP/NF
^a 15 mL glass, round vial with a stopper, and sealed with an aluminium seal			

Table 2. Quantitative Composition of Diluent for Anidulafungin for Injection.

Ingredient	Theoretical Quantity per vial ^{a,b}	Function	Reference to standards
Dehydrated Alcohol			USP
Water for Injection			USP
^a ^b 15 mL — glass, round vial with a — stopper, and sealed with an aluminium seal			

- B. Brief Description of Microbiology Deficiencies -** No deficiencies are outstanding regarding the processes and equipment specified by [] for the manufacture of this drug product.
- C. Assessment of Risk Due to Microbiology Deficiencies –** Minimal risk is perceived.

III. Administrative

- A. Reviewer's Signature** _____
- B. Endorsement Block**
Microbiologist: James L. McVey
Microbiology Supervisor: P. H. Cooney
- C. CC Block**

DFS
HFD-805/Division File/NDA 21632r1

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 § 552(b)(5) Deliberative Process

 § 552(b)(4) Draft Labeling

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/s/

James McVey
2/25/04 02:57:11 PM
MICROBIOLOGIST

Peter Cooney
2/25/04 03:26:49 PM
MICROBIOLOGIST

3 Page(s) Withheld



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 § 552(b)(5) Deliberative Process

 § 552(b)(4) Draft Labeling

MICROBIOLOGY REVIEW
DIVISION OF SPECIAL PATHOGEN AND TRANSPLANT PRODUCTS

NDA#s # 21-632 and 21-948

Reviewer : Lynn Steele Moore

Sponsor:

Vicuron Pharmaceuticals Inc., a subsidiary of Pfizer
235 East 42nd Street
New York, NY 10017

Submissions Reviewed: NDA 21-632 (1/24/06 complete response); NDA 21-948

DRUG CATEGORY: Antifungal

INDICATION: Treatment of esophageal candidiasis, candidemia, and other forms of invasive candidiasis

DOSAGE FORM: Lyophilized powder for intravenous administration

PRODUCT NAMES:

a. PROPRIETARY: Eraxis

b. NONPROPRIETARY: Anidulafungin, VER002, V-echinocandin, LY303366

c. CHEMICAL:

1-[(4R,5R)-4,5-Dihydroxy-N(2)-[[4''-(pentyloxy)[1,1':4',1''-terphenyl]-4-yl]carbonyl]-L-ornithine]echinocandin B

Summary:

The subject of this NDA is Eraxis (anidulafungin), an echinocandin antifungal agent. In NDA 21-632 the sponsor is seeking approval for second line treatment of patients with esophageal candidiasis (EC). On November 25, 2005, the sponsor received a second approvable letter. On January 24, 2006, a complete response was submitted, containing revised labeling information (a combined label for esophageal candidiasis and invasive candidiasis/candidemia). No additional information was submitted as part of this complete response. Please refer to the DFSed reviews dated March 15, 2004 and November 21, 2005 for additional information regarding NDA 21-632.

In NDA 21-948, approval is being sought for candidemia, and invasive candidiasis (IC) indications. No new preclinical microbiology information was submitted. For details of the antifungal activity of anidulafungin *in vitro* and *in vivo* please see the microbiology review dated March 15, 2004 (NDA 21-632).

Four clinical trials were included in NDA 21-632 and reviewed previously (March 15, 2004). These were VER002-4 for EC, XBAF, a supportive study in EC, VER002-6 for IC, and VER002-11 for fluconazole refractory mucosal candidiasis. Additional patients were enrolled in study VER002- 11, and two studies for the treatment of IC (VER002-9,

VER002-9B) were reviewed.. Please refer to the November 21, 2005 review (NDA 21-632) for the full review.

Recommendations:

NDA 21-632 and 21-948 are recommended for approval with respect to microbiology.

Lynn Steele Moore
Microbiologist, DSPTP

Shukal Bala, Ph.D.
Microbiology Team Leader, DSPTP

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/s/

Kristen Miller
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CSO

Shukal Bala
2/16/2006 01:17:58 PM
MICROBIOLOGIST

MICROBIOLOGY REVIEW
DIVISION OF SPECIAL PATHOGEN AND TRANSPLANT PRODUCTS

NDA # 21-632, 21-948

REVIEWER : Lynn Steele Moore
CORRESPONDENCE DATE : 05-27-05
CDER RECEIPT DATE : 05-27-05
REVIEW ASSIGN DATE : 06-02-05
REVIEW COMPLETE DATE: 10-25-05

SPONSOR: Vicuron Pharmaceuticals
455 South Gulph Road
Suite 310
King of Prussia, PA 19406

SUBMISSION REVIEWED: NDA 21-632 (Complete response) and NDA 21-948 (N-000)

DRUG CATEGORY: Antifungal

INDICATION: Treatment of esophageal candidiasis, candidemia, and other forms of invasive candidiasis

DOSAGE FORM: Lyophilized powder for intravenous administration

PRODUCT NAMES:

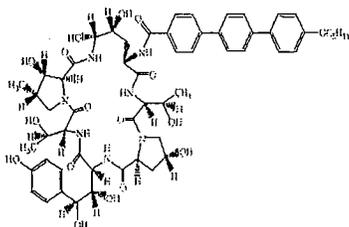
a. **PROPRIETARY:** *I*

b. **NONPROPRIETARY:** Anidulafungin, VER002, V-echinocandin, LY303366

c. **CHEMICAL:**

1-[(4R,5R)-4,5-Dihydroxy-N(2)-[[4''-(pentyloxy)[1,1':4',1''-terphenyl]-4-yl]carbonyl]-L-ornithine]echinocandin B

STRUCTURAL FORMULA:



Molecular weight: 1140.3
Empirical Formula: C₅₈H₇₃N₇O₁₇

SUPPORTING DOCUMENTS: IND# IND#54,597

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I. EXECUTIVE SUMMARY

The subject of this NDA is anidulafungin, an echinocandin antifungal agent. In NDA 21-632 the sponsor is seeking approval for second line treatment of patients with esophageal candidiasis (EC). In NDA 21-948 approval is being sought for candidemia, and invasive candidiasis (IC) indications.

Preclinical Microbiology:

No new information was submitted. For details of the antifungal activity of anidulafungin *in vitro* and *in vivo* please see the microbiology review dated 3/15/04 (NDA 21-632).

Clinical Microbiology

Four clinical trials were included in NDA 21-632 and reviewed previously (March, 2004). These were VER002-4 for EC, XBAF, a supportive study in EC, VER002-6 for IC, and VER002-11 for fluconazole refractory mucosal candidiasis. Additional patients were enrolled in study VER002-11 and is reviewed in its entirety in this review. In addition 2 studies for the treatment of IC (VER002-9, VER002-9B) were reviewed.

The phase 3 clinical trial, VER002-9 was designed to study the efficacy of anidulafungin vs. fluconazole in the treatment of patients with candidemia and other forms of IC and the prevention of complications. However, VER002-9B was an open label non comparative study. Two hundred sixty one patients were enrolled in VER002-9 and 33 patients were enrolled in VER002-9B.

In the VER002-9 study at the end of intravenous (IV) therapy there were 194 patients evaluable (103 patients in the anidulafungin treatment group and 91 in the fluconazole group). Of these, a majority were infected with *Candida albicans* (n=108), and the remaining with *Candida* species other than *C. albicans* (28 *C. glabrata*, 20 *C. parapsilosis*, 16 *C. tropicalis*, 2 *C. guilliermondii*, 2 *C. lusitaniae*, and 1 *C. famata*) in both treatment arms. Mixed infections were seen in both groups. Global success (combined clinical and microbiological) at end of treatment (EOT) in the MITT population was seen in 90 patients (87%) in the anidulafungin arm and 68 patients (75%) in the fluconazole arm. At the follow up (FU) visit, overall global success in the anidulafungin arm was seen in 71 out of 88 patients (80.7%) vs. 51/76 (67.1%) in the fluconazole arm. Baseline pathogen of a *Candida* species other than *C. albicans* was observed in 32 patients in the anidulafungin arm and 29 patients in the fluconazole arm. Anidulafungin treated patients had higher proportions of global success at EOT and at FU. Overall, there was no correlation between clinical outcome and anidulafungin or fluconazole MICs.

In the VER002-9B study (a noncomparative study) at the end of IV therapy there were 17 patients evaluable. *C. albicans* (n=8) and *C. glabrata* (n=7) were the most frequently isolated baseline pathogens. Global success at EOT in the MITT population was observed in 21 of 31 patients (67.7%). There was no correlation between clinical outcome and anidulafungin MICs. Microbiological success at the pathogen level was comparable to those in study VER002-9 at EOT (88% for all species). Global, clinical, and microbiological success was sustained at the same level (64.3%, 61.3%, 61.3% respectively) at the 2 and 6 week follow up visits.

VER002-11 was a study designed to study the safety and efficacy of IV anidulafungin for the treatment of azole refractory mucosal candidiasis (ARMC). Please note that the criteria used for patients to be considered azole refractory include the presence of active oropharyngeal and/or esophageal clinical disease at the conclusion of or within one month of receiving a 14 day course of fluconazole at a dose of at least 200 mg daily, voriconazole (dose not specified) and/or other

antifungal drugs. Nineteen severely immunocompromised patients were enrolled. *C. albicans* was the most common baseline pathogen identified from patients with both OPC and EC. Of these, 4 patients had more than one baseline pathogen. Clinical cure and microbiologic eradication at EOT in the MITT population was observed in 18 of 19 (95%) and 5 of 19 (27%) patients, respectively. Clinical cure and microbiological eradication was observed in 13 (93%) and 4 (29%) of 14 patients, respectively, with *C. albicans* as the baseline pathogen. There was one patient with *C. glabrata* which was clinically cured but not microbiologically eradicated at EOT. There was no correlation between clinical outcome and MICs. Clinical success was observed for approximately 47% of all the patients at FU. However, microbiologic eradication was observed in only 1 of the 19 patients (5.3%). There was a slight difference in clinical success rates between patients with OPC (43%) and EC (50%) at FU. Microbiologic eradication was observed for approximately 8% of patients with EC at FU.

Clinical and microbiologic success rates for all the clinical studies including those reviewed previously (with the exception of the azole refractory study; VER002-11) are shown in Table 1. Overall, anidulafungin is active against *C. albicans*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis* in patients with EC, candidemia and IC. The number of patients for the remaining *Candida* species, listed in Table 1, was too small to conclude the activity of anidulafungin.

Table 1: Clinical and microbiologic response in 11 clinical studies at EOT except VER002-11

Yeast	Anidulafungin		Fluconazole	
	Clinical Success	Mycologic Eradication	Clinical Success	Mycologic Eradication
<i>C. albicans</i>	258/310 (83.2%)	252/310 (81.3%)	186/218 (85.3%)	198/218 (90.8%)
<i>C. glabrata</i>	39/46 (84.8%)	41/46 (89.1%)	11/18 (61.1%)	13/18 (72.2%)
<i>C. tropicalis</i>	20/21 (95.2%)	20/21 (95.2%)	4/4 (100%)	4/4 (100%)
<i>C. parapsilosis</i>	14/17 (82.4%)	15/17 (88.2%)	11/11 (100%)	10/11 (90.9%)
<i>C. krusei</i>	3/6 (50%)	4/6 (66.7%)	0/0	0/0
<i>C. guilliermondii</i>	2/2 (100%)	2/2 (100%)	0/0	0/0
<i>C. lusitaniae</i>	1/1 (100%)	1/1 (100%)	1/1 (100%)	1/1 (100%)
<i>C. dubliniensis</i>	1/1 (100%)	1/1 (100%)	0/0	0/0
<i>C. famata</i>	1/1 (100%)	1/1 (100%)	1/1 (100%)	1/1 (100%)
<i>Candida</i> species	1/2 (50%)	1/1 (100%)	2/2 (100%)	0/0
<i>C. albicans</i> + <i>C. glabrata</i>	13/14 (92.9%)	11/14 (78.6%)	12/13 (92.3%)	12/13 (92.3%)
<i>C. albicans</i> + <i>C. krusei</i>	0/2 (0%)	0/1 (0%)	0/2 (0%)	2/2 (100%)
<i>C. albicans</i> + <i>C. parapsilosis</i>	1/2 (50%)	1/2 (50%)	2/3 (66.7%)	2/3 (66.7%)
<i>C. albicans</i> + <i>C. tropicalis</i>	1/1 (100%)	1/1 (100%)	2/2 (100%)	2/2 (100%)
<i>C. glabrata</i> + <i>C. tropicalis</i>	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>C. glabrata</i> + <i>C. parapsilosis</i>	0/0	0/0	0/1 (0%)	1/1 (100%)
<i>C. albicans</i> + <i>C. spp.</i>	1/1 (100%)	1/1 (100%)	0/0	0/0
TOTAL	356/428 (83.2%)	352/426 (82.6%)	232/277 (83.8%)	246/275 (89.5%)

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II. INTRODUCTION AND BACKGROUND

The subject of the NDAs 21-632 and 21-938 is the echinocandin anidulafungin. In NDA 21-632 the sponsor is seeking approval for the treatment of patients greater than 18 years of age with esophageal candidiasis (EC), an important cause of morbidity in patients with advanced HIV infection. A dose regimen of 100 mg IV as a loading dose on day one followed by 50 mg IV daily for 14-21 days is proposed. NDA 21-948 is for patients with invasive candidiasis (IC); 100 mg IV daily for a minimum of 14 days was used after a 200 mg loading dose on day 1. In the U.S., amphotericin B, fluconazole, itraconazole, caspofungin and voriconazole are currently approved for the treatment of EC and caspofungin and voriconazole are approved for the treatment of candidemia.

III. PRECLINICAL MICROBIOLOGY

The activity of anidulafungin *in vitro* and *in vivo* was reviewed earlier. For details please refer to the microbiology review for NDA 21-632 dated 3/15/04. No new information was included in this submission.

IV. CLINICAL MICROBIOLOGY

The sponsor measured the efficacy of anidulafungin for the treatment of EC (study VER002-4), candidemia/invasive candidiasis (studies VER002-9 and VER002-9B), and azole refractory mucosal candidiasis (study VER002-11).

1. Esophageal Candidiasis

Please refer to the microbiology review dated March 15, 2004 for a complete review of study VER002-4 for the treatment of EC.

2. Candidemia/Invasive Candidiasis

Study VER002-9:

This was a phase 3 clinical trial designed to study the efficacy of anidulafungin vs. fluconazole in the treatment of patients with candidemia and other forms of IC and the prevention of complications. The study was conducted in 33 sites in the United States, 8 in Canada and 6 in Europe. To be included in the study patients must have had at least one blood culture positive for yeast, or, a positive culture for yeast from a specimen from a normally sterile site with or without a positive blood culture, or a positive yeast culture from a newly placed drain in a normally sterile site. Exclusions included patients who previously received more than 48 hours of systemic antifungal therapy for the treatment of a *Candida* infection, had received prophylactic administration of fluconazole, itraconazole, or voriconazole, had received anidulafungin, or had a known *C. krusei* infection. The sponsor has stated that patients with *C. krusei* were excluded to maintain the blind. Please refer to the Medical Officer's review for more details. Higher dose (200 mg) of IV anidulafungin was administered on Day 1 followed by a lower dose (100 mg)

daily on Day 2 through end of treatment (EOT) for a minimum of 14 days from the last negative culture and improvement of clinical signs and symptoms of candidemia or IC. Total treatment duration was not to exceed 42 days. IV fluconazole was administered 800 mg on Day 1 and then 400 mg daily on Day 2 through EOT. Oral fluconazole (400 mg daily) was given to qualifying patients in either arm. Patients were followed for clinical and microbiologic response through the 6 week follow up visit (+/- 1 week after completion of study medication).

Efficacy was evaluated based on clinical and microbiological responses. The primary endpoint was the global response (combined clinical and microbiological) in the MITT population at EOT.

Clinical responses at EOT were defined as:

Success/Cure: Resolution of signs and symptoms; no additional therapy required

Success/Improvement: Significant improvement of signs and symptoms; no additional therapy required.

Failure: No significant improvement in signs and symptoms or death due to the *Candida* infection.

Indeterminate: Evaluation could not be made (lost to follow-up, death), patients receiving < 3 doses of study drug.

Microbiological responses at Patient Level:

Success: Eradication (documented or presumed). Culture was negative for all *Candida* species present at baseline (documented), or culture data not available for a patient with a successful clinical response (presumed).

Failure/Persistence (documented or presumed): Baseline *Candida* spp. present in repeat cultures, or culture data not available for a patient with a clinical response of presumed failure.

Failure/Recurrence (documented or presumed): Baseline *Candida* spp. isolated following documented eradication, or culture data not available for a patient with a clinical response of failure after a previous response of presumed success.

Superinfection: Emergence of a new *Candida* spp. at an original site of infection or at a distant, normally sterile site while on study medication.

New Infection: Emergence of a new *Candida* spp. at an original site of infection or at a distant, normally sterile site after study medication completion.

Indeterminate: Culture data not available for a patient with a clinical outcome of indeterminate.

Microbiological responses at the Baseline Pathogen Level:

Success: Eradication (documented or presumed). Culture was negative for all *Candida* species present at baseline (documented), or culture data not available for a patient with a successful clinical response (presumed).

Persistence (documented or presumed): Baseline *Candida* spp. present in repeat cultures (documented), or culture data not available for a patient with a clinical response of presumed failure.

Recurrence (documented or presumed): Baseline *Candida* spp. isolated following

documented eradication or culture data not available for a patient with a clinical response of failure after a previous response of presumed success (presumed).

Indeterminate: Culture data not available for a patient with a clinical outcome of indeterminate.

Global Outcome and Response:

Success: Both clinical and microbiological success.

Failure: Either a clinical or microbiological failure (excluded clinical and microbiological responses of indeterminate).

Indeterminate: A clinical and/or microbiological response of indeterminate and neither response was a failure.

Two hundred sixty one patients were enrolled. There were 256 patients in the Intent-to-Treat (ITT) population (131 received anidulafungin and 125 received fluconazole), 245 in Microbiological ITT (MITT; 127 patients in the anidulafungin arm and 118 patients in the fluconazole arm) population. At the end of intravenous (IV) therapy (the primary endpoint) there were 194 of the 245 MITT patients (103 in the anidulafungin arm and 91 in the fluconazole arm) evaluated. The MITT population included all subjects who received at least one dose of study medication and who had a positive *Candida* culture from a normally sterile site at baseline. The majority of the cultures were from blood (90.7% in the fluconazole arm, 94.5% in the anidulafungin arm). The remaining sterile sites from either of the treatment arms included peritoneal fluid (n=14), pleural fluid (n=2), IV catheter (n=42), eye (n=1), and others. For the purpose of this review the results of the baseline pathogen from different sites in the 2 treatment arms were combined due to a small number of isolates from organs other than blood. Multiple positive blood cultures were observed in 52.8% of patients in the anidulafungin treatment group and 60.2% of subjects in the fluconazole arm at baseline. Single baseline pathogen was seen in 93.7% of patients in the anidulafungin arm and 89.8% of patients in the fluconazole arm. Multiple baseline pathogens were seen in 6.3% of the anidulafungin patients and in 10.2% of the fluconazole subjects. *C. albicans* was the most common pathogen isolated in both treatment arms followed by *C. glabrata* and *C. parapsilosis*.

Tables 2 and 3 show the clinical and microbiological success by baseline pathogen in the efficacy evaluable population at EOT and at follow up (FU) visits, respectively. At EOT (Table 2) 36 patients in the anidulafungin arm and 33 patients in the fluconazole arm had a baseline pathogen of a *Candida* species other than *C. albicans*. In the anidulafungin treated group there were 12 patients with *C. glabrata*, 2 with *C. guilliermondii*, 1 with *C. lusitaniae*, 9 with *C. parapsilosis*, and 12 with *C. tropicalis*. In the fluconazole treated group there were 16 patients with *C. glabrata*, 11 with *C. parapsilosis*, 4 with *C. tropicalis*, 1 with *C. famata*, and 1 with *C. lusitaniae*. Global response for anidulafungin against *C. albicans* was 93.3% and 75% for fluconazole. Against *C. glabrata* the global success for anidulafungin was 66.7% at EOT and 50% for fluconazole. Of the 9 patients with *C. parapsilosis* in the anidulafungin group 7 were global success. There were 11 patients in the fluconazole arm with 100% activity against *C. parapsilosis*. Mixed infections due to two *Candida* spp. were seen in 7 patients in the anidulafungin arm and 10 in the fluconazole group. There were 8 patients in the anidulafungin arm (1 *C.*

tropicalis, 3 *C. glabrata*, 2 *C. albicans*, 2 *C. parapsilosis*) and 17 (9 *C. albicans*, 6 *C. glabrata*, 2 *C. parapsilosis*) in the fluconazole arm who had a persistent infection with the baseline pathogen at EOT. Overall global success (the primary endpoint of clinical and microbiological responses combined) in the anidulafungin arm was seen in 90 out of 103 patients (87.4%) vs. 68/91 (74.7%) in the fluconazole arm.

At the FU visit (Table 3), overall global success in the anidulafungin arm was seen in 71 out of 88 patients (80.7%) vs. 51/76 (67.1%) in the fluconazole arm. Baseline pathogen of a *Candida* species other than *C. albicans* was observed in 32 patients in the anidulafungin arm and 29 patients in the fluconazole arm. Mixed infections due to two *Candida* spp. were seen in 5 patients in the anidulafungin arm and 9 in the fluconazole group. The clinical and microbiological successes by baseline pathogen in patients with single or mixed infections is also shown in Table 3. In the anidulafungin treated group there were 12 patients with *C. glabrata*, 1 with *C. guilliermondii*, 1 with *C. lusitaniae*, 8 with *C. parapsilosis*, and 10 with *C. tropicalis*. In the fluconazole treated group there were 14 patients with *C. glabrata*, 11 with *C. parapsilosis*, 2 with *C. tropicalis*, 1 with *C. famata*, and 1 with *C. lusitaniae*. Global response for anidulafungin at FU against *C. albicans* was 88.2% and 68.4% for fluconazole. Against *C. glabrata* the global success for anidulafungin was 50% and 42.9% for fluconazole. Of the 8 patients with *C. parapsilosis* in the anidulafungin group, 6 were global success. There were 11 patients in the fluconazole arm with 90.9% activity against *C. parapsilosis*.

Table 2:
 Clinical and Per-Patient Microbiological Success by Baseline Pathogen(n)
 Efficacy Evaluable Population

Treatment Group	Baseline Species	Clinical Success	End of Therapy n/N (%)		Global Response
			Proven Eradication	Presumed Eradication	
ANIDULAFUNGIN	CANDIDA ALBICANS	56/60 (93.3%)	42/60 (70.0%)	15/60 (25.0%)	56/60 (93.3%)
	CANDIDA GLABRATA	8/12 (66.7%)	9/12 (75.0%)	1/12 (8.3%)	8/12 (66.7%)
	CANDIDA GUILLIERMONDII	2/2 (100.0%)	1/2 (50.0%)	1/2 (50.0%)	2/2 (100.0%)
	CANDIDA LUSITANIAE	1/1 (100.0%)	1/1 (100.0%)	0/1 (0%)	1/1 (100.0%)
	CANDIDA PARAPSILOSIS	7/9 (77.8%)	6/9 (66.7%)	2/9 (22.2%)	7/9 (77.8%)
	CANDIDA TROPICALIS	12/12 (100.0%)	10/12 (83.3%)	2/12 (16.7%)	12/12 (100.0%)
	CANDIDA ALBICANS + CANDIDA GLABRATA	3/3 (100.0%)	2/3 (66.7%)	0/3 (0%)	2/3 (66.7%)
	CANDIDA ALBICANS + CANDIDA PARAPSILOSIS	1/2 (50.0%)	1/2 (50.0%)	0/2 (0%)	1/2 (50.0%)
	CANDIDA ALBICANS + CANDIDA SPECIES	1/1 (100.0%)	0/1 (0%)	1/1 (100.0%)	1/1 (100.0%)
	CANDIDA GLABRATA + CANDIDA TROPICALIS	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
	TOTAL	91/103 (88.3%)	72/103 (69.9%)	22/103 (21.4%)	90/103 (87.4%)
FLUCONAZOLE	CANDIDA ALBICANS	37/48 (77.1%)	31/48 (64.6%)	9/48 (18.8%)	36/48 (75.0%)
	CANDIDA FAMATA	1/1 (100.0%)	0/1 (0%)	1/1 (100.0%)	1/1 (100.0%)
	CANDIDA GLABRATA	10/16 (62.5%)	8/16 (50.0%)	3/16 (18.8%)	10/16 (62.5%)
	CANDIDA LUSITANIAE	1/1 (100.0%)	1/1 (100.0%)	0/1 (0%)	1/1 (100.0%)
	CANDIDA PARAPSILOSIS	11/11 (100.0%)	8/11 (72.7%)	2/11 (18.2%)	10/11 (90.9%)
	CANDIDA TROPICALIS	4/4 (100.0%)	3/4 (75.0%)	1/4 (25.0%)	4/4 (100.0%)
	CANDIDA ALBICANS + CANDIDA GLABRATA	3/4 (75.0%)	2/4 (50.0%)	1/4 (25.0%)	3/4 (75.0%)
	CANDIDA ALBICANS + CANDIDA PARAPSILOSIS	2/3 (66.7%)	2/3 (66.7%)	0/3 (0%)	2/3 (66.7%)
	CANDIDA ALBICANS + CANDIDA TROPICALIS	1/1 (100.0%)	1/1 (100.0%)	0/1 (0%)	1/1 (100.0%)
	CANDIDA GLABRATA + CANDIDA PARAPSILOSIS	0/1 (0%)	1/1 (100.0%)	0/1 (0%)	0/1 (0%)
	CANDIDA GLABRATA + CANDIDA TROPICALIS	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
TOTAL	70/91 (76.9%)	57/91 (62.6%)	17/91 (18.7%)	68/91 (74.7%)	

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Table 3:
 Clinical and Per-Patient Microbiological Success by Baseline Pathogen(s)
 Efficacy Evaluable Population

Treatment Group	Baseline Species	Clinical Success	Proven Eradication	Follow-Up n/N (%)		Global Response	
				Presumed Eradication			
ANIDULAFUNGIN	CANDIDA ALBICANS	46/51 (90.2%)	34/51 (66.7%)	11/51 (21.6%)		46/51 (88.2%)	
	CANDIDA GLABRATA	6/12 (50.0%)	6/12 (50.0%)	3/12 (25.0%)		6/12 (50.0%)	
	CANDIDA GUILLIERMONDII	1/1 (100.0%)	1/1 (100.0%)	0/1 (0%)		1/1 (100.0%)	
	CANDIDA LUSITANIAE	1/1 (100.0%)	1/1 (100.0%)	0/1 (0%)		1/1 (100.0%)	
	CANDIDA PARAPSILOSIS	6/8 (75.0%)	4/8 (50.0%)	2/8 (25.0%)		6/8 (75.0%)	
	CANDIDA TROPICALIS	9/10 (90.0%)	5/10 (50.0%)	4/10 (40.0%)		9/10 (90.0%)	
	CANDIDA ALBICANS + CANDIDA GLABRATA	2/2 (100.0%)	2/2 (100.0%)	0/2 (0%)		2/2 (100.0%)	
	CANDIDA ALBICANS + CANDIDA PARAPSILOSIS	0/1 (0%)	0/1 (0%)	0/1 (0%)		0/1 (0%)	
	CANDIDA ALBICANS + CANDIDA SPECIES	1/1 (100.0%)	1/1 (100.0%)	0/1 (0%)		1/1 (100.0%)	
	CANDIDA GLABRATA + CANDIDA TROPICALIS	0/1 (0%)	0/1 (0%)	0/1 (0%)		0/1 (0%)	
	TOTAL	72/88 (81.8%)	54/88 (61.4%)	20/88 (22.7%)		71/88 (80.7%)	
	FLUCONAZOLE	CANDIDA ALBICANS	27/38 (71.1%)	22/38 (57.9%)	8/38 (21.1%)		26/38 (68.4%)
		CANDIDA PAPAIA	1/1 (100.0%)	1/1 (100.0%)	0/1 (0%)		1/1 (100.0%)
CANDIDA GLABRATA		7/14 (50.0%)	5/14 (35.7%)	1/14 (7.1%)		6/14 (42.9%)	
CANDIDA LUSITANIAE		1/1 (100.0%)	1/1 (100.0%)	0/1 (0%)		1/1 (100.0%)	
CANDIDA PARAPSILOSIS		11/11 (100.0%)	6/11 (54.5%)	4/11 (36.4%)		10/11 (90.9%)	
CANDIDA TROPICALIS		2/2 (100.0%)	0/2 (0%)	2/2 (100.0%)		2/2 (100.0%)	
CANDIDA ALBICANS + CANDIDA GLABRATA		2/3 (66.7%)	2/3 (66.7%)	0/3 (0%)		2/3 (66.7%)	
CANDIDA ALBICANS + CANDIDA PARAPSILOSIS		2/3 (66.7%)	2/3 (66.7%)	0/3 (0%)		2/3 (66.7%)	
CANDIDA ALBICANS + CANDIDA TROPICALIS		1/1 (100.0%)	1/1 (100.0%)	0/1 (0%)		1/1 (100.0%)	
CANDIDA GLABRATA + CANDIDA PARAPSILOSIS		0/1 (0%)	1/1 (100.0%)	0/1 (0%)		0/1 (0%)	
CANDIDA GLABRATA + CANDIDA TROPICALIS		0/1 (0%)	0/1 (0%)	0/1 (0%)		0/1 (0%)	
TOTAL		54/76 (71.1%)	41/76 (53.9%)	15/76 (19.7%)		51/76 (67.1%)	

Susceptibility testing of clinical isolates taken at baseline in the MITT population was performed at a central reference laboratory using Clinical and Laboratory Standards Institute (CLSI) method (M27A). Anidulafungin and fluconazole MICs were performed in RPMI 1640 broth medium, cultures incubated for 24 hours and 48 hours, and a prominent decrease in turbidity was used as the endpoint. For echinocandins, the endpoint at 24 hours incubation has been recommended by the CLSI (January, 2005). The sponsor, for reasons of uniformity, used the same parameters for fluconazole (24 hours incubation and prominent decrease in turbidity as the endpoint). Please note CLSI recommends that fluconazole endpoint be measured at 48 hours and not 24 hours and therefore the incidence of fluconazole non-susceptible isolates may be underestimated. Quality control strains were included each time a clinical trial isolate was tested and were within acceptable ranges. The MIC₅₀ and MIC₉₀ values for anidulafungin against all isolates of *Candida* species tested in both treatment groups were 0.008 µg/mL and 0.5 µg/mL, respectively; fluconazole MIC₅₀ and MIC₉₀ were 0.25 and 8 µg/mL, respectively (Table 4). Both anidulafungin and fluconazole MICs of isolates at EOT were within the same range as for baseline isolates.

Tables 5 and 6 show anidulafungin and fluconazole MICs respectively, for all *Candida* species in anidulafungin and fluconazole treated patients. There appears to be no correlation between clinical outcome and MIC. Table 7 shows the global success at EOT by pathogen. Overall global success was better in the anidulafungin arm (77.3%) than in the fluconazole arm (61.3%). Anidulafungin appears to be more active against *C. albicans* and *C. tropicalis*. However, fluconazole (83.3%) was more active than anidulafungin (63.6%) against the *C. parapsilosis* isolates. Activity of anidulafungin appears to be the same as fluconazole against *C. glabrata*. Further analysis of the data (MICs ~ baseline pathogen ~ clinical ~ microbiological response) will be done as an addendum to the review.

Table 4: MIC data for baseline isolates

Study VER002-9: Anidulafungin and Fluconazole MIC₅₀, MIC₉₀, and MIC Range (mg/L) against Baseline Isolates (Micro-ITT Population)

Species Drug/Incubation Time	Anidulafungin Arm			Fluconazole Arm			All Patients		
	MIC ₅₀	MIC ₉₀	MIC Range	MIC ₅₀	MIC ₉₀	MIC Range	MIC ₅₀	MIC ₉₀	MIC Range
All Species	n=122			n=120			n=242		
Anidulafungin 24 h	0.008*	0.25	≤ 0.002-2	0.008	0.5	≤ 0.002-2	0.008	0.5	≤ 0.002-2
Anidulafungin 48 h	0.008	0.5	≤ 0.002-4	0.015	0.5	≤ 0.002-4	0.008	0.5	≤ 0.002-4
Fluconazole 24 h	0.25	8	≤ 0.06-> 128	0.25	8	≤ 0.06-128	0.25	8	≤ 0.06-> 128
Fluconazole 48 h	0.5	16	≤ 0.06-> 128	1	16	≤ 0.06-> 128	0.5	16	≤ 0.06-> 128
Candida albicans	n=74			n=66			n=140		
Anidulafungin 24 h	0.004	0.015	≤ 0.002-0.03	0.004	0.015	≤ 0.002-0.06	0.004	0.015	≤ 0.002-0.06
Anidulafungin 48 h	0.004	0.015	≤ 0.002-0.12	0.004	0.015	≤ 0.002-0.06	0.004	0.015	≤ 0.002-0.12
Fluconazole 24 h	0.25	0.5	≤ 0.06-> 128	0.25	1	≤ 0.06-32	0.25	1	≤ 0.06-> 128
Fluconazole 48 h	0.5	4	≤ 0.06-> 128	0.5	8	≤ 0.06-> 128	0.5	4	≤ 0.06-> 128
Candida glabrata	n=17			n=27			n=44		
Anidulafungin 24 h	0.03	0.06	0.015-0.12	0.03	0.12	≤ 0.002-0.12	0.03	0.06	≤ 0.002-0.12
Anidulafungin 48 h	0.03	0.06	0.015-0.06	0.03	0.12	0.004-0.12	0.03	0.06	0.004-0.12
Fluconazole 24 h	8	16	1-32	4	32	0.12-128	4	16	0.12-128
Fluconazole 48 h	8	32	1-128	8	64	1-128	8	32	1-128
Candida parapsilosis	n=12			n=14			n=26		
Anidulafungin 24 h	1	2	0.03-2	1	2	0.25-2	1	2	0.03-2
Anidulafungin 48 h	2	4	0.25-4	2	2	0.5-4	2	4	0.25-4
Fluconazole 24 h	0.25	1	0.12-1	0.12	1	≤ 0.06-1	0.25	1	≤ 0.06-1
Fluconazole 48 h	0.5	2	0.12-64	0.25	1	0.12-2	0.25	2	0.12-64

Species Drug/Incubation Time	Anidulafungin Arm			Fluconazole Arm			All Patients		
	MIC ₅₀	MIC ₉₀	MIC Range	MIC ₅₀	MIC ₉₀	MIC Range	MIC ₅₀	MIC ₉₀	MIC Range
Candida tropicalis	n=14			n=10			n=24		
Anidulafungin 24 h	0.015	0.06	≤ 0.002-0.12	0.015	0.03	≤ 0.002-0.06	0.015	0.06	≤ 0.002-0.12
Anidulafungin 48 h	0.03	0.06	≤ 0.002-0.12	0.015	0.03	≤ 0.002-0.12	0.015	0.06	≤ 0.002-0.12
Fluconazole 24 h	0.25	2	≤ 0.06-2	0.25	0.25	0.12-1	0.25	1	≤ 0.06-2
Fluconazole 48 h	0.5	2	0.12-2	0.25	1	0.12-1	0.5	2	0.12-2
Candida krusei	n=2			n=0			n=2		
Anidulafungin 24 h	-	-	0.06-0.12	-	-	-	-	-	0.06-0.12
Anidulafungin 48 h	-	-	0.12	-	-	-	-	-	0.12
Fluconazole 24 h	-	-	8-64	-	-	-	-	-	8-64
Fluconazole 48 h	-	-	64	-	-	-	-	-	64
Candida lusitanae	n=1			n=2			n=3		
Anidulafungin 24 h	-	-	0.03	-	-	0.03-0.25	-	-	0.03-0.25
Anidulafungin 48 h	-	-	0.03	-	-	0.03-0.5	-	-	0.03-0.5
Fluconazole 24 h	-	-	0.5	-	-	0.25	-	-	0.25-0.5
Fluconazole 48 h	-	-	0.5	-	-	0.25-0.5	-	-	0.25-0.5
Candida guilliermondii	n=2			n=0			n=2		
Anidulafungin 24 h	-	-	0.25-0.5	-	-	-	-	-	0.25-0.5
Anidulafungin 48 h	-	-	0.5-1	-	-	-	-	-	0.5-1
Fluconazole 24 h	-	-	1-2	-	-	-	-	-	1-2
Fluconazole 48 h	-	-	2	-	-	-	-	-	2
Candida famata	n=0			n=1			n=1		
Anidulafungin 24 h	-	-	-	-	-	0.06	-	-	0.06
Anidulafungin 48 h	-	-	-	-	-	0.12	-	-	0.12
Fluconazole 24 h	-	-	-	-	-	1	-	-	1
Fluconazole 48 h	-	-	-	-	-	1	-	-	1

*MICs represent prominent inhibition in RPMI medium. Only strains sent to the reference laboratory are included.
 Source: VER002-9 CSR, Section 14.1, Table 1.7.1.

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Table 5: Anidulafungin global success by baseline pathogen at EOT (MITT)

Baseline Species Anidulafungin MIC (mg/L)	Anidulafungin n/N (%)
All Species	92/119 (77.3)
≤ 0.002	29/36 (80.6)
0.004	10/14 (71.4)
0.008	17/17 (100.0)
0.015	8/11 (72.7)
0.03	9/11 (81.8)
0.06	5/7 (71.4)
0.12	1/3 (33.3)
0.25	1/1 (100.0)
0.5	2/4 (50.0)
1	3/5 (60.0)
2	2/2 (100.0)
Unknown*	5/8 (62.5)

Only patients with a single baseline pathogen are included.

Table 6: Fluconazole global success EOT (MITT)

Baseline Species Fluconazole MIC Range (mg/L)	Fluconazole n/N (%)
All Species	65/106 (61.3)
≤ 8	59/97 (60.8)
16 to 32	1/3 (33.3)
≥ 64	1/1 (100.0)
Unknown*	4/5 (80.0)

Only patients with a single baseline pathogen are included.
 *Baseline pathogen not sent to central lab.

Table 7. GLOBAL SUCCESS AT END OF IV THERAPY BY PATHOGEN(MICRO-ITT POPULATION)

Baseline Species	Anidulafungin n/N (%)	Fluconazole n/N (%)
All species	92/119 (77.3)	65/106 (61.3)
<i>Candida albicans</i>	60/74 (81.1)	38/61 (62.3)
Non- <i>albicans</i> species	32/45 (71.1)	27/45 (60.0)
<i>Candida glabrata</i>	9/16 (56.3)	11/22 (50.0)
<i>Candida tropicalis</i>	13/14 (92.9)	4/8 (50.0)
<i>Candida parapsilosis</i>	7/11 (63.6)	10/12 (83.3)
<i>Candida guilliermondii</i>	2/2 (100.0)	--
<i>Candida krusei</i>	0/1 (0.0)	--
<i>Candida lusitanae</i>	1/1 (100.0)	1/2 (50.0)
<i>Candida famata</i>	--	1/1 (100.0)

Note: N=Number of patients with a single baseline pathogen. Source: Data from Section 14.2, Table 2.12 of the submission.

Study VER002-9B:

This was a phase 3 clinical trial designed to study the efficacy of anidulafungin in the treatment of patients with candidemia and other forms of invasive candidiasis (IC) and the prevention of complications. This was a non-comparative open label study conducted in conjunction with and following VER002-9 in 33 patients. The study was conducted in 11 sites in the United States and 2 in Canada. The protocol was similar to that described above (study VER002-9) except that it was non comparative.

All of the 33 patients enrolled were in the Intent-to Treat (ITT) population. Of these, 31 were in MITT. At the end of IV therapy there were 17 patients evaluable. The MITT population (31 patients) included all subjects who received at least one dose of study medication and who had a positive *Candida* culture from a normally sterile site at baseline. Multiple positive blood cultures were seen in >64% of patients at baseline. Candidemia was present in more than 90% of patients. At baseline 90.3% of the patients had a single pathogen.

The clinical success rates at EOT by pathogen for patients with a single baseline pathogen are shown in Table 8. Clinical success was seen in 8 of 11 patients (72.7%) with *C. albicans* and 8 of 9 (88.9%) patients with *C. glabrata*. The microbiological success rate for the two most common pathogens (*C. albicans* and *C. glabrata*) was over 90%.

Table 8: Clinical Success EOT single baseline pathogen (MITT)

Baseline Species	Anidulafungin n/N (%) success [1]
ALL SPECIES	21/28 (75.0)
CANDIDA ALBICANS	8/11 (72.7)
CANDIDA GLABRATA	8/9 (88.9)
CANDIDA PARAPSILOSIS	3/4 (75.0)
CANDIDA TROPICALIS	2/3 (66.7)
CANDIDA KRUSEI	0/1 (0.0)

n= Number of patients with a response of success. Success included cure and improvement
 N=Number of patients with a single baseline pathogen

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Table 9 shows the MIC values for the baseline isolates from patients in MITT. Susceptibility testing of clinical isolates was performed as described above (study VER002-9). Anidulafungin MICs against all *Candida* species ranged from $\leq 0.002 - 2 \mu\text{g/mL}$ with MIC₅₀ and MIC₉₀ of 0.004 and 0.5 $\mu\text{g/mL}$, respectively (24h readings). Fluconazole MICs against all *Candida* species ranged from $\leq 0.006 - 32 \mu\text{g/mL}$. MIC₅₀ and MIC₉₀ for all species was 0.25 and 8 $\mu\text{g/mL}$, respectively (24h readings). No correlation was observed comparing anidulafungin MICs to global success rate.

Table 9: MITT baseline isolate MICs

Species Drug/incubation Time	MIC ₅₀	MIC ₉₀	MIC Range
All Species			
n=34			
Anidulafungin 24 h	0.004	0.5	$\leq 0.002-2$
Anidulafungin 48 h	0.008	2	$\leq 0.002-2$
Fluconazole 24 h	0.25	8	$\leq 0.06-32$
Fluconazole 48 h	1	16	0.12-64
<i>Candida albicans</i>			
n=13			
Anidulafungin 24 h	≤ 0.002	≤ 0.002	$\leq 0.002-0.004$
Anidulafungin 48 h	≤ 0.002	≤ 0.002	$\leq 0.002-0.004$
Fluconazole 24 h	0.12	0.25	$\leq 0.06-0.25$
Fluconazole 48 h	0.25	16	0.25-64
<i>Candida glabrata</i>			
n=12			
Anidulafungin 24 h	0.004	0.008	0.004-0.008
Anidulafungin 48 h	0.008	0.008	0.004-0.008
Fluconazole 24 h	4	8	2-32
Fluconazole 48 h	8	16	4-64
<i>Candida parapsilosis</i>			
n=5			
Anidulafungin 24 h			0.25-2
Anidulafungin 48 h			0.5-2
Fluconazole 24 h			0.12-1
Fluconazole 48 h			0.25-1

Species with fewer than 5 isolates are not included.
 MICs are for prominent inhibition in RPMI medium.

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One can see a difference in the MIC₉₀ at the 24 vs. 48 hour readings for fluconazole in the *C. albicans* isolates. The MIC₉₀ at 48 hours for *C. albicans* and *C. glabrata* is in the S-DD range, while the 24 hour readings are in the susceptible range according to CLSI interpretive criteria/breakpoints thereby showing that changes in experimental conditions can alter the MICs. Therefore, it is important that testing and interpretation of *in vitro* methods be consistent with the standard used.

3. Azole refractory mucosal candidiasis

The phase 2 clinical trial VER002-11 was designed to study the safety and efficacy of IV anidulafungin as a treatment for azole refractory mucosal candidiasis (ARMC). ARMC was defined as active oropharyngeal and/or esophageal clinical disease at the conclusion of or within one month of receiving a 14 day course of fluconazole at a dose of at least 200 mg daily or any dosage of voriconazole. Please note that the sponsor has not stated what doses of voriconazole were used. On page 46 of the clinical study report the sponsor has defined ARMC patients as those that received fluconazole and/or voriconazole. However, it appears from page 46 that other antifungal drugs such as amphotericin B, caspofungin, itraconazole, and ketoconazole were also administered. Six patients received only fluconazole, two patients received fluconazole and voriconazole (at different time points), and 2 patients received voriconazole in combination with agents other than fluconazole. The remaining patients were treated with other antifungal agents as described above (one patient did not have antifungal therapy listed). The study was conducted in 4 sites in the United States, enrolling 19 patients that were severely immunocompromised and had multiple prior episodes of oropharyngeal candidiasis (OPC) and/or EC. To be included in the study patients must be 12 years of age or older

with a diagnosis of ARMC and microscopic/culture of yeast. Exclusions included patients requiring continued treatment with another systemic, topical, or orally administered antifungal agent. Please refer to the Medical Officer's review for details. IV anidulafungin was administered as an initial 100 mg dose on Day 1 followed by 50 mg daily on Day 2 through Day 14 up until Day 21 (maximum duration). Therapy was discontinued between Days 14 and 21 for patients who were deemed by the investigator to have achieved complete resolution of symptoms. Endoscopy was performed at screening and at EOT. EOT was considered the point at which drug was discontinued. Clinical and microbiological efficacy was also assessed at FU (10-14 days after EOT). Clinical success at EOT was observed in 18 of 19 patients (95%). Microbiological success was observed in 7 of 19 patients (37%) at EOT. Eighteen out of 19 patients with a positive culture had *C. albicans* (95%).

The sponsor has stated that there were 14 fluconazole non susceptible baseline isolates (as mentioned previously the methods used for susceptibility testing of fluconazole deviated from CLSI methods in that 24 hour readings were used). Of these isolates, 4 were proven eradicated at EOT (27%). The primary efficacy variable for patients with EC was endoscopic and clinical response at EOT in the MITT population. All patients who had a baseline *Candida* spp. were included in the MITT population. *C. albicans* was the most common baseline pathogen identified from patients with both OPC and EC. Four patients had more than one baseline pathogen.

Table 10 shows the clinical and microbiological success by baseline pathogen for all patients and for the EC patients separately at both EOT and FU visits.

Table 10: Clinical and per patient microbiological success by baseline pathogen

Study VER002-11
 Clinical and Per-Patient Microbiological Success by Baseline Pathogen(s)
 Micro ITT Population (All patients)

Baseline Species	End of Therapy n/N ^a (%)		Follow-Up n/N ^a (%)		
	Clinical Success	Proven Eradication	Clinical Success	Proven Eradication	Presumed Eradication
<i>Candida albicans</i>	13/14 (92.9)	4/14 (28.6)	7/14 (50.0)	1/14 (7.1)	4/14 (28.6)
<i>Candida glabrata</i>	1/1 (100.0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)
<i>C. albicans + C. glabrata</i>	3/3 (100.0)	1/3 (33.3)	1/3 (33.3)	0/3 (0)	1/3 (33.3)
<i>C. albicans + C. glabrata + C. tropicalis</i>	1/1 (100.0)	0/1 (0)	1/1 (100.0)	0/1 (0)	0/1 (0)
Total	18/19 (94.7)	5/19 (26.3)	9/19 (47.4)	1/19 (5.3)	5/19 (26.3)

^a n/N=number of patients with the specified baseline pathogen(s) and indicated outcome/total number of patients with the specified baseline pathogen(s).

Study VER002-11
 Clinical, Endoscopic and Per-Patient Microbiological Success by Baseline Pathogen(s)
 Micro ITT Population (Patients with Esophageal Candidiasis)

Baseline Species	End of Therapy n/N ^a (%)			Follow-Up n/N ^a (%)		
	Clinical Success	Proven Eradication	Endoscopic Success	Clinical Success	Proven Eradication	Presumed Eradication
<i>Candida albicans</i>	7/8 (87.5)	3/8 (37.5)	7/8 (87.5)	5/8 (62.5)	1/8 (12.5)	4/8 (50.0)
<i>Candida glabrata</i>	1/1 (100.0)	0/1 (0)	1/1 (100.0)	0/1 (0)	0/1 (0)	0/1 (0)
<i>C. albicans + C. glabrata</i>	3/3 (100.0)	1/3 (33.3)	3/3 (100.0)	1/3 (33.3)	0/3 (0)	1/3 (33.3)
Total	11/12 (91.7)	4/12 (33.3)	11/12 (91.7)	6/12 (50.0)	1/12 (8.3)	5/12 (41.7)

^a n/N=number of patients with the specified baseline pathogen(s) and indicated outcome/total number of patients with the specified baseline pathogen(s).

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Clinical success was observed for approximately 47% of all the patients at FU. There was a slight difference in clinical success rates between patients with OPC (43%) and EC (50%) at FU. Endoscopic data were not collected for all patients at FU. Microbiologic eradication was observed for approximately 8% of patients with EC at FU.

The sponsor states in the proposed label that 14 patients had fluconazole [] pathogens. The remaining 5 patients had *C. albicans* with MICs ranging from 0.25 – 4 µg/mL. Only 6 patients had a fluconazole MIC of >64 µg/mL (5 *C. albicans*, 1 *C. glabrata*) with the remaining 8 patients having fluconazole MICs of 16-32µg/mL. These data will be analyzed further and included in the addendum to the review.

Data from all clinical studies reviewed in NDA 21-632 (March, 2004) is shown in Table 11.-Anidulafungin has activity against *C. albicans*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis*.

Table 11: Clinical, all 4 clinical studies combined (EOT) for NDA 21-632 (March, 2004)

Yeast	Anidulafungin		Fluconazole	
	Clinical Success	Mycologic eradication	Clinical Success	Mycologic eradication
<i>C. albicans</i>	197/243*(81%)	191/243(79%)	149/170*(88%)	158/170(93%)
<i>C. glabrata</i>	26/28* (93%)	26/28(93%)	1/2*(50%)	2/2(100%)
<i>C. krusei</i>	3/5 (60%)	4/5(80%)	0/0	0/0
<i>C. tropicalis</i>	6/7 (86%)	6/7(86%)	0/0	0/0
<i>C. parapsilosis</i>	7/8 (88%)	7/8(88%)	0/0	0/0
<i>C. famata</i>	1/1 (100%)	1/1(100%)	0/0	0/0
<i>C. dubliniensis</i>	1/1 (100%)	1/1(100%)	0/0	0/0
<i>Candida</i> spp.(not speciated)	1/2 (50%)	1/1(100%)	2/2(100%)	0/0
<i>C. albicans</i> + <i>C. glabrata</i>	9/10 (90%)	8/10(80%)	9/9(100%)	9/9(100%)
<i>C. albicans</i> + <i>C. tropicalis</i>	1/1 (100%)	1/1(100%)	1/1(100%)	1/1(100%)
<i>C. albicans</i> + <i>C. krusei</i>	0/2 (0%)	0/1(0)	0/2 (0%)	2/2(100%)
Total	252/309 (82%)	247/307(80%)	162/186(87%)	172/184(93%)

*Does not include mixed infections

4. Interpretive criteria

As discussed previously no correlation between clinical and microbiological outcomes can be made based on the information reviewed. The sponsor has not requested interpretive criteria/breakpoints in the label. Rather the sponsor has proposed to add a statement that relationship between in vitro activity and clinical response remains to be elucidated.

1 Page(s) Withheld

 § 552(b)(4) Trade Secret / Confidential

 § 552(b)(5) Deliberative Process

 ✓ § 552(b)(4) Draft Labeling

2. Comments

The sponsor has proposed to describe the activity of anidulafungin against several *Candida* species in the microbiology section of the label. Although the sponsor has measured the activity of anidulafungin against other *Candida* spp., in the absence of standardized *in vitro* susceptibility methods the clinical significance of such *in vitro* testing is not known. At the present time the criteria for listing of species is limited to those against which the correlation of *in vitro* testing with activity *in vivo* has been established. Based on the review of clinical studies, anidulafungin is active against *C. albicans*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis*. It would be useful to cross reference the Microbiology section in the Indications and Usage as well as the Clinical Study sections of the package insert.

3. FDA's version of the label:

(Please note that additions are in blue and underlined, the deletions are striked out)

MICROBIOLOGY

Mechanism of action

Anidulafungin is a semi-synthetic echinocandin with antifungal activity. Anidulafungin inhibits glucan synthase, an enzyme present in fungal, but not mammalian cells. This results in inhibition of the formation of 1,3- β -D-glucan, an essential component of the fungal cell wall.

Activity in vitro

Anidulafungin is active *in vitro* against *Candida albicans*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis*. (see INDICATIONS AND USAGE, CLINICAL STUDIES)

MICs were determined according to the Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards) approved method M27-A.

However, there is no relationship between *in vitro* activity and clinical outcome.

Activity in vivo

Parenterally administered anidulafungin was effective against *Candida albicans* in immunocompetent mice and rabbits with disseminated infection.

as measured by prolonged survival and reduction in mycological burden. Anidulafungin also reduced the mycological burden of fluconazole-resistant *C. albicans* in an

oropharyngeal/esophageal infection model in immunosuppressed rabbits. [

]

Drug Resistance

Emergence of resistance to anidulafungin, [] has not
been [] studied. [

]

[] Anidulafungin was
active [] Candida species resistant to
fluconazole. [] Cross
resistance with other echinocandins has not been studied.

[]

VII. RECOMMENDATIONS:

The NDAs 21-632 and 21-948 are approvable with respect to microbiology pending an accepted version of the label.

Lynn Steele Moore
Microbiologist, DSPTP

CONCURRENCES:

Deputy Dir. _____ Signature _____ Date _____
MicroTL _____ Signature _____ Date _____

CC:
Original NDA
CSO / Duggan II, Donovan

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

/s/

Lynn S. Moore
11/18/2005 01:57:37 PM
MICROBIOLOGIST

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11/21/2005 09:37:58 AM
MICROBIOLOGIST

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

NDA # 21-632

REVIEWERS : Lynn Steele Moore and
Susan Peacock

CORRESPONDENCE DATE : 04-25-03

CDER RECEIPT DATE : 04-30-03

REVIEW ASSIGN DATE : 06-16-03

REVIEW COMPLETE DATE: 03-15-04

SPONSOR: Vicuron Pharmaceuticals
455 South Gulph Road
Suite 310
King of Prussia, PA 19406

SUBMISSION REVIEWED: N-000

DRUG CATEGORY: Antifungal

INDICATION: Treatment of esophageal candidiasis

DOSAGE FORM: Lyophilized powder for intravenous administration

PRODUCT NAMES:

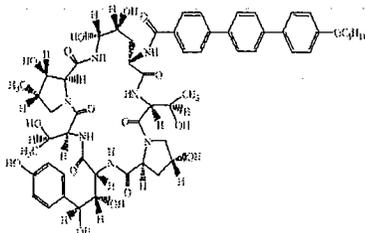
a. **PROPRIETARY:** ζ 3

b. **NONPROPRIETARY:** Anidulafungin, VER002, V-echinocandin, LY303366

c. **CHEMICAL:**

1-[(4R,5R)-4,5-Dihydroxy-N(2)-[[4''-(pentyloxy)[1,1':4',1''-terphenyl]-4-yl]carbonyl]-L-ornithine]echinocandin B

STRUCTURAL FORMULA:



Molecular weight: 1140.3
Empirical Formula: C₅₈H₇₃N₇O₁₇

SUPPORTING DOCUMENTS: IND#

IND#54,597

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I. EXECUTIVE SUMMARY:

The subject of this NDA is anidulafungin, an echinocandin antifungal agent. The sponsor is seeking approval for the treatment of patients with esophageal candidiasis (EC).

Preclinical Microbiology:

Mechanism of Action

Anidulafungin is a semisynthetic derivative of the natural product echinocandin B. Like other echinocandin antifungal agents, anidulafungin is a non-competitive inhibitor of (1,3)- β -D-glucan synthase. Glucan is the major component of the fungal cell wall and the proportion of this polysaccharide in the walls of different fungi varies. It is interesting to note that cilofungin, an analogue of echinocandin, was shown to decrease ergosterol and glucan contents of *C. albicans* with the drug for 18 hours, whereas chitin and mannan content were increased. It is not known at this time if a similar effect occurs with other echinocandins. To date, glucan synthase inhibition is the only documented mode of antifungal action of anidulafungin.

Activity *In vitro*

The methods for susceptibility testing of antifungal agents are evolving. Currently, the National Committee for Clinical Laboratory Standards (NCCLS) has published two approved documents, M27A2 for yeasts against the azoles, the new triazoles, 5-flucytosine (5-FC) and amphotericin B; M38A for the filamentous fungi against the azoles, the new triazoles, 5-FC, and amphotericin B. Neither of these documents address susceptibility testing of echinocandins nor are breakpoints established for this class of agents. Although a number of reference laboratories in the United States are following the NCCLS methods to perform susceptibility testing for the echinocandins, the usefulness of these methods for *in vitro* susceptibility testing of this class of drugs is not known. It is also unclear what the most relevant endpoint for inhibition of fungal growth is for echinocandins. Therefore, the usefulness of the NCCLS methods in predicting the activity *in vivo* for this new class of antifungal agents is not known.

Anidulafungin MICs for most *Candida* spp., with the exception of *C. parapsilosis*, were comparable to or lower than MICs of other antifungal agents tested, regardless of the test medium used. The clinical significance of this finding is not known. Anidulafungin has low MICs against fluconazole resistant (by NCCLS criteria) *Candida* spp. Anidulafungin shows no *in vitro* activity against *Cryptococcus neoformans*. The activity of anidulafungin was greater than that of itraconazole and amphotericin against *Aspergillus* spp. Usefulness of the MICs in predicting clinical outcome is not established.

Activity *in vivo*

Candida albicans

Anidulafungin was shown to improve the survival and reduce the mycological burden of immunocompetent and/or immunosuppressed mice and rabbits infected with *C. albicans*. With delay of treatment up to 24 hours after infection, intraperitoneal (i.p.) doses of <1 mg/kg of anidulafungin demonstrated 50% survival in immunosuppressed mice. Doses of 0.625 mg/kg intravenous (i.v.) or 1.25 mg/kg i.p. of anidulafungin 4-6 hours after infection demonstrated 100% survival.

Candida krusei

Orally administered anidulafungin was effective in reducing the fungal burden in the kidneys of immunocompetent mice infected with fluconazole-nonsusceptible strains of *C. krusei*.

Aspergillus fumigatus

Anidulafungin was shown to prolong the survival of immunosuppressed mice at doses of <0.625-1.25 mg/kg/day i.v. when treatment was initiated 24 hours after infection with *A. fumigatus*. When administered within 30 minutes after infection with *A. fumigatus*, a 5 mg/kg/dose of anidulafungin i.p. decreased the mycological burden in the kidneys of immunocompetent mice infected with *A. fumigatus*, and a 10 mg/kg/dose of anidulafungin i.p. cleared the kidneys of viable *A. fumigatus* organisms. In a pulmonary aspergillosis rabbit model, anidulafungin had no apparent effect on residual fungal burden despite improvement in survival and reduction in pulmonary tissue injury. In another pulmonary aspergillosis rabbit model study, anidulafungin at doses of 1 mg/kg/day and 10 mg/kg/day prolonged survival by 2-3 days whereas doses of 5 mg/kg/day and 20 mg/kg/day had no effect on survival. In this same model, anidulafungin was effective at reducing pulmonary injury as measured by pulmonary lesion scores and total lung weight; however, there was no improvement in the clearance of *A. fumigatus* from the lungs, in contrast to amphotericin B treated animals.

Pneumocystis carinii

Oral administration of anidulafungin (5 mg/kg/day for 4 days) reduced the number of cysts in the lungs of heavily infected, immunosuppressed rats by more than 99%. Prophylactic oral administration of 1 mg/kg twice daily for 4 weeks resulted in >90% reduction in all life-cycle forms of *P. carinii*. Intraperitoneal anidulafungin treatment (2 mg/kg/day) of immunosuppressed mice for 6 weeks reduced the number of detectable organisms in lung smears.

Drug resistance

A potential for development of resistance was examined *in vitro* by 13 serial passages of a single strain of *C. albicans*. The results showed no significant change in MICs. However, the clinical significance of such an observation is not known.

Cross-resistance

In vitro, strains of *C. albicans* with high fluconazole MICs were shown to have low anidulafungin MICs.

In vivo, anidulafungin was effective in reducing mycological burden in the tissue of immunosuppressed rabbits in a model of oropharyngeal and esophageal candidiasis infected with fluconazole-resistant *C. albicans*. Orally administered anidulafungin was effective in reducing the fungal burden in the kidneys of immunocompetent mice infected with fluconazole-nonsusceptible *C. krusei*. In addition, 5 fluconazole refractory patients with EC were clinically improved or cured at EOT after treatment with anidulafungin.

In immunosuppressed mice infected with amphotericin B-susceptible and –refractory *A. fumigatus* isolates, anidulafungin, at doses of ≥ 2.5 mg/kg/day administered within 18 hours after infection, increased the survival times and decreased the mycological burden (lungs and kidneys). Anidulafungin, at doses of 0.5-1.0 mg/kg/day, was effective in prolonging the survival of immunosuppressed mice infected with itraconazole-susceptible and –resistant *A. fumigatus* isolates but was not effective at reducing the mycological burden in the spleen and kidneys.

Drug Combinations

In vitro no antagonism between anidulafungin and other systemic antifungals was observed against *C. albicans*. Against test strains of *Aspergillus* spp. and *Fusarium* spp. the combination of anidulafungin or micafungin with amphotericin B exhibited synergistic to indifferent activity.

In vivo, the combination of anidulafungin and amphotericin B was effective in prolonging survival of immunosuppressed mice infected with itraconazole-resistant *A. fumigatus*, but the combination therapy was not effective in lowering the fungal burden in mice infected with either itraconazole susceptible or resistant strains. There was no additive or antagonistic effect of these two drugs on fungal burden.

Cidal vs Static

Anidulafungin is not cidal against *Candida* spp. or *Aspergillus* spp. Contrasting conclusions regarding the fungicidal activity of anidulafungin have been arrived at by different authors. The media used in the various *in vitro* tests could account for this, at least in part. For example, time kill studies conducted in RPMI 1640 media against *Candida* spp. did not show fungicidal activity for anidulafungin. However, when AM3 medium was used, MIC and Minimum Fungicidal Concentrations (MFC) values were comparable. Time kill Studies also showed some reduction in CFU. Against *Aspergillus* spp. there was a difference noted in MFC values between species when different media were used. MFC results were typically within 1-2 dilutions of the MIC in the studies reviewed. Methods for determining fungicidal activity of any antifungal agent are not standardized. Activity of anidulafungin should not be considered fungicidal.

Clinical Microbiology

Four clinical trials were included: Two Esophageal Candidiasis (EC) trials, one Invasive Candidiasis (IC) and one fluconazole refractory mucosal candidiasis trial (currently ongoing).

The pivotal phase 3 clinical trial, VER002-4 was designed to study the safety and efficacy of anidulafungin in the treatment of patients with EC. There were 601 patients enrolled, 180 patients were microbiologically evaluable (ME) at end of treatment (EOT) in the anidulafungin treatment group and 186 in the fluconazole group. Of these microbiologically evaluable patients 92.8% (359 isolates) of the 387 baseline *Candida* spp. were *C. albicans*, 19 *C. glabrata* (4.9%), 4 *C. parapsilosis* (1%), 3 *C. krusei* (0.8%) and 2 *C. tropicalis* (0.5%) in both treatment arms. At EOT the clinical and mycologic response was similar for both groups. At follow up (FU), however, failures were higher in the anidulafungin arm than in the fluconazole arm. There was no correlation between clinical outcome and anidulafungin or fluconazole MICs.

XBAF, a smaller supportive phase 2 dose ranging study to determine the efficacy and safety of anidulafungin in the treatment of patients with EC was included in the submission. This study enrolled 36 patients, 27 were ME at EOT. Thirty-five baseline isolates were recovered, 89% were *C. albicans* (n=31), 2 patients were infected with *C. albicans* and *C. glabrata*, and 1 patient had *T. beigeli*. The two treatment groups had overall similar clinical success rates. In the 50 mg loading dose followed by 25 mg once daily for 14-21 days regimen, the success rate was 36.8% while in the 70 mg loading dose followed by 35 mg schedule, success was 41.2%. Susceptibility data were not available for this study.

A supportive phase 2 clinical trial, VER002-6 was a dose ranging study of the safety and efficacy of anidulafungin in the treatment of patients with IC. There were 120 patients enrolled, 68 were ME at the primary evaluation endpoint of follow up (FU). Clinical success at FU was comparable at the 2 higher dosage regimens, and slightly lower at the lower dosage range (100 mg/50 mg = 72.2% success, 150 mg/75 mg = 84.6%, 200 mg/100mg = 83.3%). Seventy three baseline isolates of *Candida* spp. were recovered. Of these, 39 isolates (53%) were species other than *C. albicans*. Breakdown by species: 46.6% *C. albicans* (n=34), 28.8% *C. glabrata* (n=21), 9.6% *C. parapsilosis* (n=7), 8.2% *C. tropicalis* (n=6), 5.5% *C. krusei* (n=4), 1.4% *C. dubliniensis* (n=1). Non - albicans yeast were isolated from 57% of the evaluable patients at FU; 50% were either infected or co-infected with *C. albicans*. Cultures were required only if clinically indicated at EOT so most successes were presumed rather than proven.

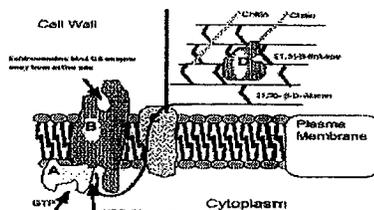
Clinical trial VER002-11 is an ongoing study of the safety and efficacy of anidulafungin in patients with fluconazole refractory mucosal candidiasis and has enrolled 5 patients to date (all 5 patients were considered clinical successes at EOT). All patients had advanced HIV infection and AIDS, OPC and 2 of the patients also had EC. Three patients had baseline isolates of *C. albicans*, 2 patients had both *C. albicans* and *C. glabrata*. Data is too limited to allow a definitive assessment of anidulafungin in patients with fluconazole refractory mucosal candidiasis.

There was no correlation between MICs and clinical or mycological outcome.

II. INTRODUCTION AND BACKGROUND:

The subject of this NDA is the echinocandin anidulafungin. The sponsor is seeking approval for the treatment of patients greater than 18 years of age with esophageal candidiasis (EC), an important cause of morbidity in patients with advanced HIV infection. In the U.S., amphotericin B, fluconazole, itraconazole, and caspofungin are currently approved for the treatment of EC. A dose regimen of 100 mg intravenously (IV) as a loading dose on day one followed by 50 mg IV daily for 14-21 days is proposed.

Anidulafungin is a semisynthetic derivative of the natural product echinocandin B. The echinocandins are a relatively new therapeutic class of antifungal agents that exert their effect at the level of the cell wall synthesis. Cell walls determine the shape of fungal cells and are essential for their integrity (Kollar, *et al.*, J Biol Chem **270** (3), 1170-1178, 1995). The cell wall of yeasts includes polymers of glucose, mannose, and N-acetylglucosamine, forming the polysaccharides, glucan, mannan and chitin (Cabib, E *et al.*, J Biol Chem **276** (23), 19679-19682, 2001). Glucan is the major component of the fungal cell wall. The cell wall composition can vary among different fungal species. For example, chitin concentration is higher in filamentous fungi as compared to yeast. Glucan synthase is a UDP-glucosyl-transferase enzyme located in the fungal cell membrane. This enzyme is important in the synthesis of β -(1,3)-D-glucan from glucose. Like other echinocandin antifungal agents, anidulafungin is a non-competitive inhibitor of (1,3)-beta-D-glucan synthase.



Diagrammatic representation of fungal glucan synthesis

At the proposed 100/50 mg regimen, maximum anidulafungin concentrations in human plasma are typically greater than 3 $\mu\text{g/mL}$ and are reached shortly after the end of the infusion. Concentrations are maintained above 1 $\mu\text{g/mL}$ through each dosing period, and are maintained above 2 $\mu\text{g/mL}$ for approximately 70% of each dosing period. Animal studies have shown that anidulafungin is quickly and extensively distributed throughout the body. Steady state is reached within the first few days of a daily dosing regimen. The elimination half-life ($t_{1/2}$) of anidulafungin in humans is approximately one day. Anidulafungin's protein binding is considered moderate, about 84% in human plasma. Its pharmacokinetics are linear in humans and animals; plasma C_{max} and area under the curve (AUC) are proportional to the dose administered.

III. PRECLINICAL MICROBIOLOGY:

1. Mechanism of Action:

1.1 Effect of anidulafungin on enzyme activity

Anidulafungin is a semi-synthetic echinocandin with activity against 1,3- β -D-glucan synthase derived from crude lysates of the yeast form of *C. albicans* and membrane fractions of the conidial forms of *A. fumigatus*.

The study by Tang *et al.* (ICAAC, 1993, abstract #367), measured *in vitro* glucan synthase reactions involving the polymerization of 14 C uridine diphosphate - glucose (UDPG) into TCA insoluble glucan. Details of the experimental design were not described in the abstract. The Dixon plot in Figure 1 shows the noncompetitive inhibition of the synthase using different concentrations of anidulafungin and UDPG substrate. The K_{iapp} of anidulafungin against the enzyme from *C. albicans* was $0.7 \pm 0.1 \mu\text{M}$ ($0.77 \mu\text{g/mL}$). Against *A. fumigatus*, the K_{iapp} was $0.11 \pm 0.01 \mu\text{M}$ ($0.12 \mu\text{g/mL}$).

Figure 1: Comparative activity of anidulafungin against glucan synthase from *C. albicans* and *A. fumigatus*.

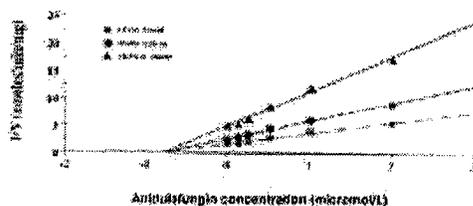


Figure 2 shows the comparative inhibitory activity of anidulafungin against the enzymes from *C. albicans* and *A. fumigatus*. The IC_{50} (50% inhibitory concentration) for anidulafungin was found to be $0.72 \pm 0.26 \mu\text{g/mL}$ against *C. albicans* and $0.17 \pm 0.08 \mu\text{g/mL}$ against *A. fumigatus*. Anidulafungin is more active against the *A. fumigatus* glucan synthase than against the *C. albicans* enzyme in a cell-free assay. To date, glucan synthase inhibition is the only documented mode of antifungal action of anidulafungin.

Figure 2: Comparative activity of anidulafungin against glucan synthase from *C. albicans* and *A. fumigatus*



2. Activity *in vitro*

Antifungal susceptibility testing of yeasts and filamentous fungi is standardized by the National Committee for Clinical Laboratory Standards (NCCLS) and published in the form of documents M27A2 and M38A, respectively for the azoles, the new triazoles, 5-flucytosine (5-FC), and amphotericin B. Echinocandins are not addressed in these documents. Although a number of reference laboratories in the United States are following the NCCLS documents M27A2 and M38A to perform susceptibility testing for the echinocandins, the usefulness of these methods for *in vitro* susceptibility testing of this class of drugs is not known. It is also unclear what the most relevant endpoint for inhibition of fungal growth is for echinocandins. For the purpose of this review the term MIC refers to minimum inhibitory concentration. MIC-0 and MIC-100 refer to MICs determined at a clear well (95-100% inhibition) endpoint. MIC-2 and MIC-50 refer to MICs determined at approximately 50% inhibition. MIC-1 and MIC-80 refer to MICs determined at approximately 80% inhibition. The terms MIC₅₀ and MIC₉₀ indicate the concentration of drug required for inhibiting 50% and 90% of the isolates tested, respectively. MFC is the minimum fungicidal concentration, usually the lowest concentration that kills 99.9% of the starting inoculum. The MFC₉₀ represents the concentration of drug required to kill 90% of the isolates tested. Breakpoints do not exist for the echinocandins against yeasts or moulds.

The development of anidulafungin was initiated before there was an NCCLS reference method. A variety of media and conditions of testing were used in various studies included in the submission. The MICs of anidulafungin and other antifungal agents are affected by several factors, including test media and endpoint determination. *In vitro* susceptibility testing was performed by the NCCLS microdilution method unless specified otherwise.

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2.1 Activity against *Candida* spp.

The *in vitro* activity of anidulafungin against 2,000 isolates of *Candida* spp. from patients with blood stream infections was determined in a surveillance study [Rex, JH; NIAID Mycoses Study Group (MSG) 33-34 surveillance studies; NIAID MSG 33-34 Candidemia Susc. Surv. Database; May 2001 Summary; also subsequently published by Ostrosky-Zeichner *et al.* AAC 47 (10), 3149-3154, 2003]. Isolates were collected between 1995-1999 from 39 US centers and evaluated against anidulafungin as well as 8 other antifungal agents. *In vitro* susceptibility testing was performed to determine MICs using the NCCLS M27A microdilution method in RPMI 1640 medium with MOPS buffer (with the exception of amphotericin B in which AM-3 medium was used). Inoculum was 1 to 5 x 10³ CFU/mL. Microtiter trays were incubated at 35°C and all MICs were determined visually and spectrophotometrically (570 nm after agitation at both 24 and 48 hours) using an endpoint of 50%, 80% and 95%. The report focused on spectrophotometric readings at 24 hours. Results were not shown for visual readings and 48 hour MICs. The authors of the MSG report state that 24 hour MIC 50% inhibition reading is most likely the appropriate reading for echinocandins; however, this is not addressed in the published article by Ostrosky-Zeichner.

A comparison of the *in vitro* activity of anidulafungin with currently approved antifungal agents is shown in Tables 1 to 3. Please note that Table 2 includes results of clinical isolates in addition to the isolates collected in the surveillance study. Data show anidulafungin to have lower MICs than caspofungin, fluconazole, itraconazole, amphotericin B and 5-FC against the 733 isolates of *C. albicans* and the 307 isolates of *C. tropicalis*. Activity of anidulafungin was comparable to voriconazole against these two groups of isolates. Against the 50 isolates of *C. krusei*, anidulafungin had lower MICs than all the other agents tested, however, the anidulafungin MIC₉₀ was only one dilution lower than the voriconazole MIC₉₀. *C. glabrata* MIC₉₀ for anidulafungin was comparable to 5-FC but was lower than all other currently approved agents tested. Overall, against most isolates the anidulafungin MICs were low (0.03-0.06 µg/mL) with the exception of *C. parapsilosis*, which had an MIC₉₀ of 2.0 µg/mL (range 1-4 µg/mL). Caspofungin also had an MIC₉₀ of 2.0 µg/mL against *C. parapsilosis*. While these MIC values are higher than those seen against other *Candida* spp. it has not been established that this represents resistance.

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Table 1: Comparative data from MSG 33-34 studies

Species	N of isolates	Inhibitory concentration (µg/L)		
		Antifungal agent	MIC ₅₀	MIC ₉₀
<i>C. albicans</i>	735	Anidulafungin	≤ 0.03	≤ 0.03
		Caspofungin	0.3	0.5
		Micafungin	≤ 0.03	≤ 0.03
		Fluconazole	≤ 0.12	0.25
		Itraconazole	≤ 0.03	0.06
		Voriconazole	≤ 0.03	≤ 0.03
		Posaconazole	≤ 0.03	≤ 0.03
		Amphotericin B	0.06	0.25
<i>C. glabrata</i>	458	Anidulafungin	≤ 0.03	0.13
		Caspofungin	0.5	1
		Micafungin	≤ 0.03	≤ 0.03
		Fluconazole	4	8
		Itraconazole	0.25	1
		Voriconazole	0.06	0.25
		Posaconazole	0.12	0.5
		Amphotericin B	0.12	0.5
<i>C. krusei</i>	50	Anidulafungin	0.06	0.12
		Caspofungin	1	1
		Micafungin	0.12	0.25
		Fluconazole	16	32
		Itraconazole	0.25	0.5
		Voriconazole	0.12	0.25
		Posaconazole	0.12	0.5
		Amphotericin B	0.25	0.5
<i>C. parapsilosis</i>	391	Anidulafungin	1	2
		Caspofungin	1	2
		Micafungin	0.5	1
		Fluconazole	0.25	1
		Itraconazole	≤ 0.03	0.13
		Voriconazole	≤ 0.03	≤ 0.03
		Posaconazole	≤ 0.03	0.06
		Amphotericin B	0.12	0.5
<i>C. tropicalis</i>	367	Anidulafungin	≤ 0.03	0.06
		Caspofungin	0.5	0.5
		Micafungin	≤ 0.03	≤ 0.03
		Fluconazole	0.25	0.5
		Itraconazole	0.06	0.12
		Voriconazole	≤ 0.03	0.06
		Posaconazole	≤ 0.03	0.06
		Amphotericin B	0.12	0.5
Total	2000	Anidulafungin	≤ 0.03	1
		Caspofungin	0.5	1
		Micafungin	≤ 0.03	0.5
		Fluconazole	0.25	4
		Itraconazole	0.03	0.25
		Voriconazole	≤ 0.03	0.12
		Posaconazole	≤ 0.03	0.25
		Amphotericin B	0.12	0.5

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Table 2: Anidulafungin against *Candida* spp. Comparative data from non-clinical isolates, MSG survey, and anidulafungin clinical trial isolates.

Organism	N	Minimum Inhibitory Concentration (mg/L)		
		MIC ₅₀	MIC ₉₀	Range
All isolates combined				
<i>Candida albicans</i>	1833	0.12	0.25	0.004 - 4
<i>Candida dubliniensis</i>	20	≤ 0.05	0.12	≤ 0.05 - 0.5
<i>Candida famata</i>	1	0.12	0.12	0.12 - 0.12
<i>Candida glabrata</i>	689	0.12	0.5	≤ 0.05 - >8
<i>Candida guilliermondii</i>	9	2	4	0.12 - 4
<i>Candida kefyr</i>	7	0.25	2	≤ 0.05 - 2
<i>Candida krusei</i>	65	0.25	0.5	≤ 0.05 - 1
<i>Candida lipolytica</i>	2	≤ 0.05	1	≤ 0.05 - 1
<i>Candida lusitanae</i>	35	0.5	2	≤ 0.05 - 2
<i>Candida parapsilosis</i>	438	2	4	≤ 0.05 - >8
<i>Candida pelliculosa</i>	1	0.015	0.015	0.015 - 0.015
<i>Candida rugosa</i>	7	1	4	0.05 - 4
<i>Candida sphaerica</i>	1	0.25	0.25	0.25 - 0.25
<i>Candida tropicalis</i>	547	0.06	0.5	≤ 0.05 - >8
Total of all <i>Candida</i> spp	5455	0.12	2	0.004 - >8

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Table 3: Activity of anidulafungin against *Candida* spp. from MSG 33-34 studies. Values are in µg/mL.

RPMI, 24h incubation, 50% inhibition endpoint. * Data from Rex 2001

(*C.dubliniensis*-18 isolates, *C.lusitanae*-20, *guilliermondii*- 9, *C.kefyr*-4, *C.rugosa*-7, *C.sphaerica*-1, *C.lipolytica*-

2).

Species	N of isolates	MIC ₅₀	MIC ₉₀	MIC Range
<i>C. albicans</i>	733	≤ 0.05	≤ 0.05	≤ 0.05-2
<i>C. glabrata</i>	438	≤ 0.05	0.15	≤ 0.05-4
<i>C. krusei</i>	30	0.06	0.15	≤ 0.05-0.25
<i>C. parapsilosis</i>	391	1	2	≤ 0.05-4
<i>C. tropicalis</i>	307	≤ 0.05	0.06	≤ 0.05-2
Other <i>Candida</i> spp.*	61	≤ 0.05	0.5	≤ 0.05-1
All species	3000	≤ 0.05	2	≤ 0.05-4

The activity of anidulafungin against fluconazole resistant strains of yeast that were included in the MSG studies is shown in Table 4.

Table 4: Activity of anidulafungin against fluconazole non-susceptible *Candida* isolates-MSG 33-34 study

Species (n)	No. of isolates	Anidulafungin (mg/L)		
		MIC ₅₀	MIC ₉₀	MIC Range
<i>C. albicans</i>	33	≤ 0.05	0.15	≤ 0.05 - 1
<i>C. glabrata</i>	81	0.06	0.15	≤ 0.05 - 2
<i>C. Krusei</i>	49	0.06	0.15	≤ 0.05 - 0.25
<i>C. parapsilosis</i>	15	0.06	2	≤ 0.05 - 2
<i>C. tropicalis</i>	35	≤ 0.05	0.15	≤ 0.05 - > 4

RPMI, 48-hour incubation, 50% inhibition endpoint. Species having less than 10 fluconazole-resistant isolates are not included. Data from Rex 2001.

In another study by Marco *et al.* (Diag Micro Inf Dis, 46 (4), 259-264, 2003), the activity of anidulafungin was compared to that of five other antifungal agents against 218 isolates of *Candida* spp. from blood collected between 1996 and 2001. *In vitro* susceptibility testing was done following the NCCLS M27A broth microdilution methods using RPMI 1640 with MOPS broth medium for all drugs and the inoculum size was 0.5 - 2.5 x 10³ CFU/mL. MICs were determined visually with the aid of a mirror reader after 48 hours of incubation in air at 35°C. The endpoint for anidulafungin was the lowest concentration of drug that completely inhibited growth. The results from this study are shown in Table 5. MIC results for anidulafungin are comparable to the MSG 33-34 study data [Rex, JH; NIAID Mycoses Study Group (MSG) 33-34 surveillance studies; NIAID MSG 33-34 Candidemia Susc. Surv. Database; May 2001 Summary].

Table 5: Susceptibilities of 218 *Candida* spp. from blood cultures

Organisms (No. of isolates)	Antifungal agent	Range	MIC (µg/ml)	
			50%	90%
<i>C. albicans</i> (91)	voriconazole	≤0.03-8	≤0.03	≤0.03
	fluconazole	≤0.12-7-128	0.25	0.5
	itraconazole	≤0.03-8	≤0.03	0.12
	amphotericin B	0.06-1	0.25	0.5
	5-fluorocytosine	≤0.12-1	≤0.12	0.5
<i>C. parapsilosis</i> (48)	anidulafungin	≤0.03-0.25	0.06	0.12
	voriconazole	≤0.03-0.06	≤0.03	≤0.03
	fluconazole	0.12-8	0.5	F
	itraconazole	≤0.03-1	0.06	0.25
	amphotericin B	0.25-1	0.5	F
<i>C. tropicalis</i> (35)	5-fluorocytosine	≤0.12-7-128	≤0.12	0.25
	anidulafungin	2-8	2	4
	voriconazole	≤0.03-8	≤0.03	0.25
	fluconazole	0.25-7-128	1	8
	itraconazole	≤0.03-1	0.06	F
<i>C. glabrata</i> (26)	amphotericin B	0.25-1	0.5	F
	5-fluorocytosine	≤0.12-2-128	≤0.12	0.5
	anidulafungin	≤0.03-0.5	0.12	0.25
	voriconazole	≤0.03-1	0.12	0.5
	fluconazole	0.5-32	4	32
<i>C. lusitana</i> (13)	itraconazole	0.06-4	0.5	F
	amphotericin B	0.12-1	0.5	F
	5-fluorocytosine	≤0.12-4	≤0.12	0.25
	anidulafungin	0.12-0.25	0.12	0.12
	voriconazole	0.03-0.5	0.5	0.5
<i>Candida</i> spp. (51)*	fluconazole	32-64	64	64
	itraconazole	0.12-1	0.5	F
	amphotericin B	0.5-1	1	F
	5-fluorocytosine	8-32	16	32
	anidulafungin	0.12-0.5	0.25	0.5
All strains (248)	voriconazole	0.03-0.06	0.06	
	fluconazole	1-4	2	
	itraconazole	0.12-1	0.5	
	amphotericin B	0.06-0.25	0.12	
	5-fluorocytosine	≤0.12-1	≤0.12	
All strains (248)	anidulafungin	≤0.03-32	0.12	
	voriconazole	≤0.03-8	≤0.03	0.25
	fluconazole	≤0.12-7-128	0.5	8
	itraconazole	≤0.03-8	0.06	0.5
	amphotericin B	0.06-1	0.5	F
All strains (248)	5-fluorocytosine	≤0.12-7-128	≤0.12	F
	anidulafungin	≤0.03-32	0.12	4

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In a study by Espinel-Ingroff (J Clin Micro, 36 (10), 2950-2956, 1998), 104 isolates of yeast were tested using NCCLS M27 microdilution methods. Inoculum size varied from 0.8 to 4.2 x 10³ CFU/mL as determined by plate counts. MICs were read visually and spectrophotometrically (490 nm after agitation at 48 hours). The endpoint for anidulafungin was read as the OD that was 90% lower than the OD of the growth control well. Anidulafungin MIC results (Table 5a) were comparable to those in the studies described above. The results of the 89 *Candida* isolates, 10 of which were fluconazole resistant isolates (as defined by NCCLS criteria) of *C. albicans*, are shown in Table 5b. Anidulafungin MICs were generally lower than caspofungin MICs; against the azole resistant isolates anidulafungin MICs

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were two 2 fold dilutions lower than caspofungin MICs (0.25 µg/mL for anidulafungin and 1µg/mL for caspofungin). The clinical significance of these data is unclear.

No difference was observed in the anidulafungin MIC results between the ten isolates of fluconazole susceptible and 10 isolates of fluconazole non-susceptible (MIC >16 µg/mL) *C. albicans* (Table 5a and 5b) included in this study. MFCs were measured by subculturing 10 µl from each well that showed complete inhibition onto Sabouraud Dextrose agar plates which were incubated at 28-30° C until growth was seen in the broth control subculture. The MFC was the concentration at which there was no growth or <3 colonies. MFC₉₀ results were typically within 1-2 dilutions of the MIC₉₀ with the exception of the fluconazole susceptible strains which were 3 dilutions higher than the MIC₉₀ (Table 5a).

Table 5a: MICs of 104 yeast

Fungus (no. tested)	Antifungal agent	MIC range (µg/ml)	MIC ₅₀ (µg/ml)	MIC ₉₀ (µg/ml)	MFC range (µg/ml)	MFC ₉₀ (µg/ml)
<i>C. albicans</i> (10) ^a	SEI156592	0.06-1.0 (>16) ^b	0.5	1.0	NID ^c	NID ^c
	MK-9991	0.25-2	0.5	1.0	0.5->16	2
	LY303366	<0.03-0.25	0.06	0.25	0.25-4	1.0
<i>C. albicans</i> (10) ^d	SEI156592	<0.03-0.06 (>16)	<0.03	<0.03	NID	NID
	MK-9991	0.25-2	0.5	1.0	0.25-1.0	1.0
	LY303366	<0.03-0.25 (<0.03)	0.06	0.06	0.25-1.0	0.5
<i>C. glabrata</i> (12)	SEI156592	<0.03-4	1.0	4	>16	>16
	MK-9991	0.5-2	0.5	1.0	1-4	2
	LY303366	0.06-0.25	0.12	0.25	0.12-1.0	0.5
<i>C. guilliermondii</i> (8)	SEI156592	<0.03-0.25 (0.06-4)	0.25	NID ^e	4->16	NID
	MK-9991	>16 (0.5-8)	2	NID	NID	NID
	LY303366	0.25-4	2	NID	4-8	NID
<i>C. lusitana</i> (13)	SEI156592	0.5-1.0	1.0	1.0	1.0-2	1.0
	MK-9991	0.5-4	1.0	2	1.0-2	2
	LY303366	0.12-1.0 (<0.03-0.5)	0.5	1.0	0.25-1.0	1.0
<i>C. lusitana</i> (12)	SEI156592	<0.03-0.25 (<0.03-1.0)	0.06	0.06	0.06-4	0.25
	MK-9991	1.0-4	1.0	2	1.0-4	1.0
	LY303366	0.25-2 (0.06-2)	1.0	2	1.0-2	2
<i>C. parapsilosis</i> (12)	SEI156592	0.06-0.5 (0.06->1.0)	0.25	0.5	1.0->16	8
	MK-9991	0.5-2 (2-4)	1.0	2	1.0->16	2
	LY303366	0.5-2	2	2	1.0-4	4
<i>C. tropicalis</i> (12)	SEI156592	0.06-8 (>16)	0.25	0.25	NID	NID
	MK-9991	0.5-2 (<0.03-2)	1.0	1.0	0.5-1.0	1.0
	LY303366	0.12-0.5 (<0.03-0.2)	0.25	0.5	0.12-1.0	1.0
<i>C. zeylanoides</i> (10)	SEI156592	0.25-0.5	0.25	0.25	0.25-0.5	0.5
	MK-9991	1.0->16	>16	>16	NID	NID
	LY303366	>16	>16	>16	NID	NID
<i>T. longibrachii</i> (5)	SEI156592	0.12-1.0	1.0	NID	0.5->16	NID
	MK-9991	1.0->16	>16	NID	>16	NID
	LY303366	>16	>16	NID	NID	NID
Total (104)						

^a Fluconazole MIC: >16 µg/ml; itraconazole MIC: 0.06 to >16 µg/ml.
^b Values in parentheses are MICs for SEI156592 and MK-9991 (for agar) and MICs for LY303366 (for broth).
^c For MFC column, NID indicates not done.
^d Fluconazole MIC: >16 µg/ml; itraconazole MIC: <0.03 to 0.06 µg/ml.
^e For the MIC₅₀ column, NID indicates not determined.

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Table 5b: MICs against *Candida* spp., including fluconazole resistant isolates

Species (no.)	Agent	MIC-B (mg/L)		
		MIC ₅₀	MIC ₉₀	MIC range
<i>C. albicans</i> azole-resistant (10) ^a	Anidulafungin	0.06	0.25	<0.03-0.25
	Caspofungin	0.5	1	0.25-2
<i>C. albicans</i> azole-susceptible (10)	Anidulafungin	0.06	0.06	<0.03-0.25
	Caspofungin	0.5	1	0.25-2
<i>C. glabrata</i> (12)	Anidulafungin	0.12	0.25	0.03-0.25
	Caspofungin	0.5	1	0.5-2
<i>C. lusitana</i> (13)	Anidulafungin	0.5	1	0.12-1
	Caspofungin	1	2	0.5-4
<i>C. tropicalis</i> (12)	Anidulafungin	0.25	0.5	0.12-0.5
	Caspofungin	1	1	0.5-2
<i>C. parapsilosis</i> (12)	Anidulafungin	2	2	0.5-2
	Caspofungin	1	2	0.5-2
<i>C. lusitana</i> (12)	Anidulafungin	1	2	0.25-2
	Caspofungin	1	2	1-4
<i>C. guilliermondii</i> (8)	Anidulafungin	2	ND	0.25-4
	Caspofungin	2	ND	>16

^a Fluconazole MIC, ≥16 mg/L. Incubation was in RPMI medium for 48 h. Complete inhibition (MIC=0) was measured spectrophotometrically (Espinel-Ingroff, 1998).

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In a study by Pfaller *et al.* [AAC 41(4), 763-766, 1997], *in vitro* activity was determined for anidulafungin and other antifungal agents against 435 clinical yeast isolates using NCCLS M27 methods as described above. Anidulafungin MICs were comparable to those in the above mentioned studies (Table 6). This study also looked at the effect of medium on the activity of anidulafungin. Anidulafungin MICs when tested in AM-3 media were substantially lower than those obtained using RPMI 1640. Although anidulafungin appears more active *in vitro* when AM-3 is used as the test medium, most investigators have used RPMI 1640 since studies aimed at standardizing MIC methodologies have been in RPMI 1640.

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Table 6: 435 yeasts, media comparison for anidulafungin

Organism(s)	Antifungal agent	Test medium	MIC (µg/mL)		
			Range	50%	90%
<i>C. albicans</i> (186)	LY303366	Antibiotic medium 3	0.001-0.250	0.001	0.003
	LY303366	RPMI 1640	0.015-0.250	0.12	0.5
	Fluconazole	RPMI 1640	0.015-0.800	0.03	0.25
	Fluconazole	RPMI 1640	0.12-0.128	0.25	2.0
	Amphotericin B 5FC	RPMI 1640	0.25-1.0	0.5	4.0
<i>C. glabrata</i> (67)	LY303366	Antibiotic medium 3	0.001-0.5	0.007	0.007
	LY303366	RPMI 1640	0.12-0.250	0.25	0.5
	Itraconazole	RPMI 1640	0.015-0.60	0.5	2.0
	Fluconazole	RPMI 1640	0.25-0.128	0.1	1.0
	Amphotericin B 5FC	RPMI 1640	0.06-1.0	0.06	0.12
<i>C. tropicalis</i> (58)	LY303366	Antibiotic medium 3	0.001-0.25	0.003	0.003
	LY303366	RPMI 1640	0.06-2.0	0.25	0.5
	Itraconazole	RPMI 1640	0.015-0.800	0.06	0.12
	Fluconazole	RPMI 1640	0.12-0.128	0.5	1.0
	Amphotericin B 5FC	RPMI 1640	0.25-1.0	1.0	4.0
<i>C. parapsilosis</i> (28)	LY303366	Antibiotic medium 3	0.001-2.0	0.25	2.0
	LY303366	RPMI 1640	0.12-0.250	2.0	4.0
	Itraconazole	RPMI 1640	0.015-0.25	0.12	0.12
	Fluconazole	RPMI 1640	0.25-2.0	0.5	4.0
	Amphotericin B 5FC	RPMI 1640	0.5-1.0	1.0	4.0
<i>C. krusei</i> (36)	LY303366	Antibiotic medium 3	0.001-0.015	0.007	0.015
	LY303366	RPMI 1640	0.12-1.0	0.25	0.5
	Itraconazole	RPMI 1640	0.015-1.0	0.5	0.5
	Fluconazole	RPMI 1640	0.25-128	32	64
	Amphotericin B 5FC	RPMI 1640	0.5-1.0	1.0	4.0
<i>C. melanosidea</i> (10)	LY303366	Antibiotic medium 3	0.001-0.014	0.001	0.003
	LY303366	RPMI 1640	0.12-0.5	0.12	0.5
	Itraconazole	RPMI 1640	0.015-0.12	0.015	0.03
	Fluconazole	RPMI 1640	0.25-0.5	0.25	0.5
	Amphotericin B 5FC	RPMI 1640	0.25-1.0	0.5	0.5
<i>C. lusitanae</i> (12)	LY303366	Antibiotic medium 3	0.001-0.007	0.004	0.007
	LY303366	RPMI 1640	0.03-2.0	0.5	2.0
	Itraconazole	RPMI 1640	0.007-0.25	0.12	0.25
	Fluconazole	RPMI 1640	0.12-4.0	1.0	4.0
	Amphotericin B 5FC	RPMI 1640	0.5-2.0	1.0	4.0
<i>C. guilliermondii</i> (9)	LY303366	Antibiotic medium 3	0.06-0.25	0.06	1.0
	LY303366	RPMI 1640	0.12-4.0	0.5	4.0
	Itraconazole	RPMI 1640	0.12-1.0	0.5	1.0
	Fluconazole	RPMI 1640	2.0-64	4.0	16
	Amphotericin B 5FC	RPMI 1640	0.25-1.0	0.5	0.12
<i>C. rugosa</i> (7)	LY303366	Antibiotic medium 3	0.007-0.15	0.007	0.007
	LY303366	RPMI 1640	1.0-4.0	4.0	4.0
	Itraconazole	RPMI 1640	0.015-0.12	0.03	0.03
	Fluconazole	RPMI 1640	1.0-8.0	1.0	1.0
	Amphotericin B 5FC	RPMI 1640	0.5-1.0	1.0	1.0
<i>S. cerevisiae</i> (22)	LY303366	Antibiotic medium 3	0.007-0.06	0.03	0.03
	LY303366	RPMI 1640	0.25-1.0	0.5	1.0
	Itraconazole	RPMI 1640	0.03-0.5	0.5	0.5
	Fluconazole	RPMI 1640	0.5-1.0	1.0	1.0
	Amphotericin B 5FC	RPMI 1640	0.12-1.0	0.5	1.0
All organisms (435)	LY303366	Antibiotic medium 3	0.001-0.12	0.006	0.012
	LY303366	RPMI 1640	0.001-0.250	0.003	0.03
	Itraconazole	RPMI 1640	0.015-0.250	0.25	1.0
	Fluconazole	RPMI 1640	0.007-0.800	0.12	0.5
	Amphotericin B 5FC	RPMI 1640	0.12-0.128	0.5	3.0

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In a Spanish study (Cuenca-Estrella, *et al.*, J Antimicrob Chemo, 46: 475-477, 2000), the activities of anidulafungin, itraconazole and amphotericin B, were compared against 156 fluconazole nonsusceptible (MIC ≥16 µg/mL) clinical isolates collected at 51 different hospitals between 1995 and 2000. A broth microdilution test was performed according to NCCLS M27A methods with the exceptions of the RPMI 1640 media supplemented with 2% glucose and a higher concentration of the final inoculum (0.5-2.5x10⁵ CFU/mL) was used. The exact time for reading of MICs (24/48 hours) was not specified in the article. The authors state that all isolates produced detectable growth after 24 - 48 hours of incubation. Spectrophotometric readings were performed at 540 nm and MICs were read as 80% inhibition of growth compared to the growth control well. Table 7 below shows that anidulafungin had low MICs against the test isolates of *C. albicans* (0.0002-0.015 µg/mL), *C. glabrata* (<0.0002-0.25 µg/mL), *C. krusei* (<0.0002-0.5 µg/mL) and *C. tropicalis* (<0.0002-0.12 µg/mL). There were too few isolates of *C. parapsilosis* (n=5) and *C. guilliermondii* (n=3) to comment.

Table 7: Fluconazole resistant *Candida* spp.

Isolate	Antifungal agent	MICs (mg/L)		
		MIC ₅₀	MIC ₉₀	range
<i>Candida albicans</i> (n = 63)	amphotericin B	0.25	1	0.12-1
	itraconazole	0.5	1	0.12-1
	LY303366	≤0.0002	0.015	≤0.0002-0.015
<i>Candida glabrata</i> (n = 42)	itraconazole	1	4	0.5-≥8
	amphotericin B	0.5	1	0.25-2
	LY303366	≤0.0002	0.12	≤0.0002-0.25
<i>Candida krusei</i> (n = 28)	itraconazole	0.25	1	0.12-1
	amphotericin B	0.5	1	0.5-2
	LY303366	≤0.0002	0.03	≤0.0002-0.5
<i>Candida tropicalis</i> (n = 15)	itraconazole	0.25	≥8	0.25-≥8
	amphotericin B	0.5	1	0.25-1
	LY303366	≤0.0002	0.06	≤0.0002-0.12
<i>Candida parapsilosis</i> (n = 5)	itraconazole			0.015-0.25
	amphotericin B			0.25-1
	LY303366			0.015-0.5
<i>Candida guilliermondii</i> (n = 3)	itraconazole			0.5-2
	amphotericin B			0.5-1
	LY303366			1-2

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In another Spanish study (Chavez *et al.*, J Antimicrob Chemo, 44: 697-700, 1999), 219 oral isolates of *Candida* spp. from HIV infected patients were examined. The authors state that NCCLS M27A methods were used with the exception that RPMI 1640 was supplemented with 2% glucose. Results in Tables 8 and 9 show the anidulafungin MICs to be similar to those seen in the other studies reviewed above that included fluconazole resistant *Candida* spp. Criteria used for defining fluconazole resistance was not described. However, it was stated that 43 isolates had a fluconazole MIC of ≥64 µg/mL. There were too few isolates of *C. parapsilosis* (n=3) included.

Table 8: MICs of 219 clinical isolates of *Candida* spp. (µg/mL) Fluconazole

Organism (n)	Antifungal agent	range	MIC ₅₀	MIC ₉₀
<i>C. albicans</i> (183)	voriconazole	≤0.03-≥16	≤0.03	0.125
	LY303366	≤0.03-≥16	0.06	0.25
	fluconazole	0.125-≥64	1	≥64
	itraconazole	≤0.03-≥16	0.125	1
	SPC	≤0.06-≥32	0.125	0.5
<i>C. glabrata</i> (12)	amphotericin B	≤0.03-1	0.125	0.5
	voriconazole	≤0.03-2	0.12	0.25
	LY303366	≤0.03-0.5	≤0.03	0.5
	fluconazole	≤0.125-≥64	8	≥64
	itraconazole	0.12-8	1	2
<i>C. krusei</i> (31)	SPC	≤0.06-10	0.125	0.25
	amphotericin B	≤0.03-0.5	0.125	0.25
	voriconazole	≤0.03-0.25	0.12	0.25
	LY303366	≤0.03-0.5	0.06	0.25
	fluconazole	16-≥64	32	≥64
<i>C. tropicalis</i> (10)	itraconazole	0.06-1	0.5	1
	SPC	0.5-16	2	≥16
	amphotericin B	0.06-1	0.25	0.5
	voriconazole	≤0.03-0.12	≤0.03	0.12
	LY303366	≤0.03-0.12	0.06	0.125
<i>C. parapsilosis</i> (3)	fluconazole	≤0.125-≥64	2	≥64
	itraconazole	≤0.03-8	0.125	1
	SPC	≤0.06-2	0.125	1
	amphotericin B	≤0.03-0.25	0.125	0.25
	voriconazole	≤0.06-0.5	≤0.06	-
All organisms (219)	LY303366	≤0.06-2	1	-
	fluconazole	0.12-8	2	-
	itraconazole	0.12-2	0.5	-
	SPC	0.3-1	0.12	-
	amphotericin B	0.12-0.25	0.12	-

Table 9: MICs of 216 susceptible and resistant *Candida* spp.

Species (n of isolates)	MIC of fluconazole (mg/L)	MIC of LY303366 (mg/L)		MIC of voriconazole (mg/L)	
		range	MIC ₅₀ MIC ₉₀	range	MIC ₅₀ MIC ₉₀
<i>C. albicans</i> (183)	≤8	≤0.03-2	0.06 0.125	≤0.03-≥16	≤0.03 0.06
	16-32	≤0.03-≥16	0.06 0.25	≤0.03-≥16	≤0.03 0.25
	≥64	≤0.03-8	0.06 0.25	≤0.03-2	≤0.03 0.25
<i>C. glabrata</i> (12)	≤8	≤0.03-4	≤0.03 0.5	≤0.03-2	0.125 0.25
	16-32	≤0.03-0.25	0.125 0.25	0.125	0.125 0.125
	≥64	≤0.03-0.12	≤0.03 0.125	0.25	0.25 0.25
<i>C. krusei</i> (11)	≤8				
	16-32	≤0.03-0.5	0.06 0.25	≤0.03-0.25	0.125 0.25
	≥64	≤0.03-0.5	≤0.03 0.25	≤0.03-0.5	≤0.03 0.5
<i>C. tropicalis</i> (10)	≤8	≤0.03-0.125	≤0.03 0.125	≤0.03-0.125	0.125 0.125
	16-32	0.125	0.125 0.125	0.125	0.125 0.125
	≥64	≤0.03-1	≤0.03 1	≤0.03	≤0.03 ≤0.03
All organisms (216)	≤8	≤0.03-≥16	0.06 0.125	≤0.03-≥16	≤0.03 0.125
	16-32	≤0.03-≥16	0.125 0.25	≤0.03-≥16	0.06 0.25
	≥64	≤0.03-8	0.06 0.25	≤0.03-2	≤0.03 0.25

A study from the UK (Moore *et al.*, European Soc Clin Micro and Inf Dis, 7:11-16, 2001) tested the activity of anidulafungin against *Candida* spp. (99 recent clinical isolates plus 6 ATCC control strains). Overall 55.2% of the isolates were fluconazole resistant (defined as fluconazole MIC of >16 µg/mL). A fluconazole MIC of 16 is considered susceptible dose dependent by the NCCLS definition. MIC testing was performed using a modified NCCLS microdilution method. AM3 medium with 2% glucose was used as the broth medium. Final inoculum was 1x10³ organisms/mL and microtiter trays were incubated at 37°C for 48 hours. Following incubation, plates were agitated for 5 minutes and growth was read spectrophotometrically (490 nm). The MIC was defined as 80% reduction in OD₄₉₀ compared to the growth control well. There was little to no trailing seen when interpreting the MICs. When RPMI 1640 is used it has been reported that trailing endpoints are seen. MFCs were determined by removing 100 µl from the MIC well and all wells above it that had no visible growth. Plates were incubated at 37°C for 48 hours. The MFC was defined as the lowest concentration of drug with <2 colonies (99% killing). The MFC was the same as or one dilution higher than the MIC for 69.5% of the isolates for anidulafungin (Figure 3 and Table 10). This study included 12 isolates of *C. parapsilosis* with an MIC₉₀ of 1.0 µg/mL which is one dilution lower than the studies mentioned above that used RPMI 1640 as the test medium. All other MICs in this study were lower than those reported in the studies above that used RPMI 1640 as the broth medium.

Figure 3: MIC/MFC for anidulafungin against 105 *Candida* spp.

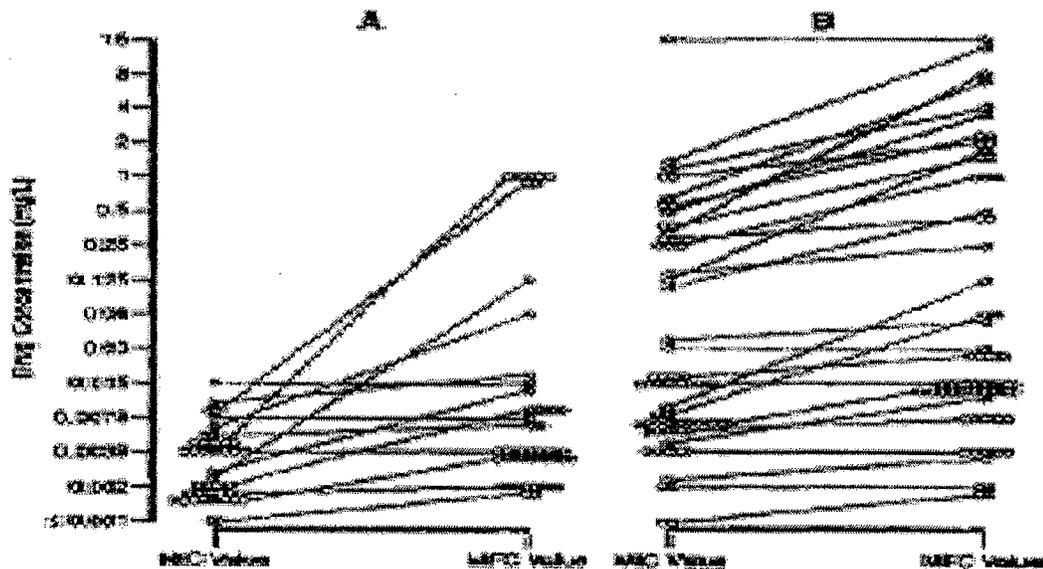


Table 10: MICs against *Candida* spp.

Species (No. of isolates)	Antifungal agent	MIC (mg/L)			
		Geometric mean	Range	50%	90%
<i>C. albicans</i> (42)	LY	0.0029	≤ 0.001-0.075	0.002	0.0078
	AMB	0.08	0.015-0.06	0.06	0.06
	FLU	0.24	≤ 0.125 -> 128	8	128
<i>C. glabrata</i> (13)	SFC	0.28	≤ 0.03 -> 32	0.25	1
	LY	0.01	0.0039-0.03	0.0078	0.03
	AMB	0.075	0.06-0.125	0.06	0.125
<i>C. parapsilosis</i> (32)	FLU	80.88	16 -> 128	32	> 128
	SFC	0.084	0.06-0.125	0.06	0.06
	LY	0.4	0.125-16	0.25	1
<i>C. parapsilosis</i> (32)	AMB	0.025	0.015-0.03	0.03	0.03
	FLU	0.79	0.5-2	1	1
	SFC	0.31	0.08-0.25	0.125	0.25
<i>C. lusitana</i> (1)	LY	0.011	0.0078-0.015	0.0078	0.075
	AMB	0.084	0.06-0.125	0.06	0.125
	FLU	58.42	32-128	64	128
<i>C. tropicalis</i> (10)	SFC	9.66	4-16	8	16
	LY	0.008	0.002-0.015	0.0078	0.0078
	AMB	0.037	0.015-0.06	0.03	0.06
<i>C. tropicalis</i> (10)	FLU	3158	0.5 -> 128	8	> 128
	SFC	0.61	0.06 -> 32	0.125	> 32
	LY	0.46	0.25-1	0.5	1
<i>C. guilliermondii</i> (8)	AMB	0.025	0.0078-0.06	0.03	0.03
	FLU	734	4-16	4	16
	SFC	0.072	≤ 0.03-0.125	0.06	0.125
<i>C. lusitana</i> (3)	LY	0.015	0.015	0.015	0.075
	AMB	0.048	0.03-0.06	0.03	0.06
	FLU	0.2	≤ 0.125-0.5	≤ 0.125	0.5
<i>C. inconspicua</i> (3)	SFC	6.26	0.06 -> 32	0.06	> 32
	LY	0.0015	≤ 0.001-0.032	≤ 0.001	0.002
	AMB	0.024	0.0078-0.06	0.0078	0.06
<i>C. inconspicua</i> (3)	FLU	32	16-64	16	64
	SFC	4	4	4	4
	LY	0.0024	≤ 0.001-0.0039	≤ 0.001	0.0039
<i>C. norvegensis</i> (3)	AMB	0.038	0.015-0.06	0.015	0.06
	FLU	32	32	32	32
	SFC	6.35	4-8	4	8
All isolates (105)	LY	0.011	≤ 0.001-16	0.0078	0.25
	AMB	0.046	0.0078-0.125	0.06	0.06
	FLU	6.72	≤ 0.125 -> 128	16	128
All isolates (105)	SFC	0.393	≤ 0.03 -> 32	0.125	8

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Zhanel *et al.* (AAC, 41 (4), 863-864,1997), reported the activity of anidulafungin against 137 isolates of *Candida* spp. from patients with systemic infections. MICs were performed according to NCCLS M27 macrodilution methods. MICs were read after 48 hours incubation at 35°C and MIC endpoints for anidulafungin were defined as the lowest concentration of drug that inhibited 100% of the visible growth. The MIC₉₀ for *C. albicans* was 0.08 µg/mL, *C. glabrata* 0.3 µg/mL and *C. tropicalis* 0.3 µg/mL. *C. parapsilosis* MICs ranged from 1.28 - 5.12 µg/mL (Table 11).

In a study by Klepser *et al.* (AAC, 42 (6), 1387-1391, 1998), an evaluation for determination of endpoints for anidulafungin was done using NCCLS microdilution methods as previously described. In addition to RPMI 1640 as the test broth, AM3 was also used for MIC and Time kill testing. MFCs were performed after the 48 hour MIC reading. Plates were incubated at 35°C for 48 hours before

Anidulafungin/LY303366/V-echinocandin/VER-002
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reading MFCs. The MFC was the lowest concentration of drug that resulted in <4 CFU. MFCs were consistently greater than the MIC₈₀ determinations (Table 12).

Table 11: 137 systemic isolate MICs

<i>Candida</i> species (no. of isolates)	Antimicrobial agent	MIC (µg/ml)		
		Range	50%	90%
<i>C. albicans</i> (99)	LY-303366	≤0.005-0.16	0.02	0.08
	Amphotericin B	≤0.0313-1	0.50	1
	SFC	≤0.0156-4	0.125	0.5
	Fluconazole	≤0.0625-2	0.25	1
	Ketoconazole	≤0.0039-2	≤0.0039	0.0625
<i>C. glabrata</i> (18)	LY-303366	0.04-0.32	0.16	0.32
	Amphotericin B	0.0625-2	0.5	2
	SFC	≤0.0156-0.5	0.0625	0.125
	Fluconazole	2-32	4	32
	Ketoconazole	≤0.0039-2	0.0625	1
<i>C. tropicalis</i> (10)	LY-303366	0.08-0.32	0.16	0.32
	Amphotericin B	0.25-2	1	1
	SFC	0.125-8	0.5	0.5
	Fluconazole	0.125->128	1	128
	Ketoconazole	≤0.0039-4	≤0.0039	4
<i>C. parapsilosis</i> (10)	LY-303366	1.28-5.12	2.56	5.12
	Amphotericin B	0.25-1	0.5	1
	SFC	0.0313-0.125	0.125	0.125
	Fluconazole	0.25-4	0.5	2
	Ketoconazole	≤0.0039-0.0078	≤0.0039	≤0.0039

* 50% and 90% MIC₅₀ and MIC₉₀, respectively.

Table 12: Klepser anid. MIC/MFC results

Isolate	MIC ₅₀ (µg/ml) (n = 6)	MIC ₁₀₀ (µg/ml) (n = 3)	MFC (range) (µg/ml) (n = 4)
<i>C. albicans</i> 90028	0.015	0.5	0.5 (0.12-0.5)
<i>C. albicans</i> OY315	0.015	0.12	0.185 (0.12-0.25)
<i>C. glabrata</i> 350	0.06	0.25	0.12 (0.12-0.25)
<i>C. glabrata</i> 582	0.045	0.25	0.06 (0.06-0.12)
<i>C. tropicalis</i> 2697	0.03	0.25	0.185 (0.12-0.25)
<i>C. tropicalis</i> 3829	0.03	0.5	0.09 (0.06-0.12)

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The MIC of anidulafungin is affected by test medium and endpoint determination. The most relevant endpoint for echinocandins is not established. In the reports reviewed, anidulafungin MICs for most *Candida* spp., with the exception of *C. parapsilosis*, were comparable to or lower than MICs of other antifungal agents tested, regardless of the test medium used. The clinical significance of this finding is not known.

2.2 Activity against *Aspergillus* spp.

The activity of anidulafungin was measured by the NCCLS M38A method unless specified otherwise. A variety of media were used in the studies included in the submission.

In a study by Zhanel *et al.* (AAC 41 (4), 863-865, 1997), anidulafungin MIC results against 20 isolates of *A. fumigatus* were reported. *In vitro* susceptibility testing was performed using M27 methods as at the time, there was no NCCLS M38 document for susceptibility testing of moulds. *Aspergillus* suspensions were prepared from mature cultures grown on Sabouraud dextrose agar (SAB) at 30°C. Suspensions containing *Aspergillus* conidia were adjusted spectrophotometrically to 82-85% transmission at 530 nm. A final inoculum of 0.5x10³ to 2.5x10³ CFU/mL was used to inoculate tubes. MIC endpoints were defined as the lowest concentration of drug that inhibited 100% of the visible growth. The MIC and Minimum Effective Concentration (MEC) were read after 48 hours incubation at 30°C. The MEC endpoint was described as an abrupt transition from test tubes containing a hyphal mass to test tubes containing small, distinct spherical colonies. Table 13 shows the individual MIC/MECs by species. The MEC₉₀ for *Aspergillus* spp. is 0.02 µg/mL and the MIC₉₀ is 10.24

µg/mL. MFCs were not measured.

Table 13: MIC/MEC

Fungus species (no. of isolates)	Antifungal agent	MIC (µg/ml) ^a		
		Range	50% ^b	90% ^c
<i>C. neoformans</i> (15)	LY-303366	>10.24	>10.24	>10.24
	Amphotericin B	±0.03125-0.25	0.125	0.25
	5FC	±0.0156-16	2	4
	Fluconazole	1-4	4	4
<i>B. dermatitidis</i> (29)	LY-303366	4-64	8	16
	Amphotericin B	±0.03125-0.25	0.0625	0.25
	5FC	64	>64	>64
	Fluconazole	2-32	8	16
<i>Aspergillus</i> species (20) ^d	LY-303366	0.00125-10.24	0.005/5.12 ^e	0.02/10.24 ^f
	Amphotericin B	0.5-4	1	2
	5FC	1-128	4	16
	Fluconazole	16-128	>128	>128
	Ketoconazole	±0.0156-4	1	2

^a 50% and 90% MIC₅₀ and MIC₉₀, respectively.
^b Five *A. fumigatus*, six *A. fumigatus*, three *A. glaucus* group, four *A. niger*, and *A. versicolor* isolates.
^c N/A=NTIC.

<i>Aspergillus</i> species (no. of isolates)	MIC/MEC (µg/ml) of LY-303366	MIC (µg/ml) range of:			
		Ampho- tericin B	5FC	Fluconazole	Ketoconazole
<i>A. fumigatus</i> (6)	0.00125/10.24	1-2	2-128	64-128	1-4
<i>A. flavus</i> (5)	0.005/10.24	0.5-4	4-128	16-128	0.125-2
<i>A. niger</i> (4)	NP ^g /0.01	1-2	1-32	16-128	±0.0156-2
<i>A. glaucus</i> group (3)	0.0025/10.24	1-2	1-8	64-128	0.5-2
<i>A. versicolor</i> (2)	NP/0.005	1	8-16	>128	1

^g NP, not present.

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Pfaller *et al.* (Diag Micro Inf Dis, 30, 251-255, 1998), compared the *in vitro* activity of anidulafungin and caspofungin against 51 clinical isolates of filamentous fungi, including *A. fumigatus* (n=12) and *A. flavus* (n=10) using NCCLS methods. A spectrophotometric method was used for inoculum preparation. Turbidity of conidial suspensions was measured at 530 nm and adjusted to a final inoculum of 0.4x10⁴ to 5x10⁴ CFU/mL. Microdilution trays were incubated at 35°C and read at 24, 48, and 72 hours. MIC endpoints were interpreted with the aid of a reading mirror. The anidulafungin endpoint was read as 75% reduction in growth compared to the growth control well. Results are shown in Table 14. Anidulafungin was 2-4 fold more active than caspofungin against the *Aspergillus* spp. Both echinocandins were more active than itraconazole, amphotericin B, and 5-FC against all the isolates. MFCs were not measured.

Table 14: MICs of 51 clinical isolates of filamentous fungi

Organism (no. of isolates)	Antifungal Class	MIC (µg/ml)			
		Amphotericin B	5-FC	Fluconazole	ITRACONAZOLE
<i>Aspergillus</i> spp. (3)	LY-303366	0.03
	Amphotericin B	2
	5-FC
	Fluconazole
<i>Aspergillus flavus</i> (10)	LY-303366	0.0125-0.03	0.03	0.03	0.03
	Amphotericin B	0.03125-0.5	0.03125	0.03125	0.03125
	5-FC	0.03125-16	0.03125	0.03125	0.03125
	Fluconazole	1-4	1	1	1
<i>Aspergillus fumigatus</i> (12)	LY-303366	0.00125-10.24	0.005-0.128	0.02	0.02
	Amphotericin B	0.03125-0.25	0.03125	0.03125	0.03125
	5-FC	1-128	1-128	1-128	1-128
	Fluconazole	16-128	>128	>128	>128
<i>Aspergillus</i> spp. (27)	LY-303366	0.00125-10.24	0.005-0.128	0.02	0.02
	Amphotericin B	0.03125-0.25	0.03125	0.03125	0.03125
	5-FC	1-128	1-128	1-128	1-128
	Fluconazole	16-128	>128	>128	>128
<i>Fusarium</i> spp. (17) ^h	LY-303366	0.03125-0.25	0.03125	0.03125	0.03125
	Amphotericin B	0.03125-0.25	0.03125	0.03125	0.03125
	5-FC	0.03125-16	0.03125	0.03125	0.03125
	Fluconazole	1-4	1	1	1
<i>Penicillium</i> sp. (3)	LY-303366	0.03125	0.03125	0.03125	0.03125
	Amphotericin B	0.03125	0.03125	0.03125	0.03125
	5-FC	0.03125	0.03125	0.03125	0.03125
	Fluconazole	1-4	1	1	1
<i>Pseudallescheria boydii</i> (5)	LY-303366	0.03125-0.25	0.03125	0.03125	0.03125
	Amphotericin B	0.03125-0.25	0.03125	0.03125	0.03125
	5-FC	0.03125-16	0.03125	0.03125	0.03125
	Fluconazole	1-4	1	1	1
<i>Rhizoglyphus</i> spp. (5)	LY-303366	0.03125-0.25	0.03125	0.03125	0.03125
	Amphotericin B	0.03125-0.25	0.03125	0.03125	0.03125
	5-FC	0.03125-16	0.03125	0.03125	0.03125
	Fluconazole	1-4	1	1	1
<i>Trichoderma</i> sp. (1)	LY-303366	0.03125	0.03125	0.03125	0.03125
	Amphotericin B	0.03125	0.03125	0.03125	0.03125
	5-FC	0.03125	0.03125	0.03125	0.03125
	Fluconazole	1-4	1	1	1
All <i>Aspergillus</i> spp. (31)	LY-303366	0.00125-10.24	0.005-0.128	0.02	0.02
	Amphotericin B	0.03125-0.25	0.03125	0.03125	0.03125
	5-FC	1-128	1-128	1-128	1-128
	Fluconazole	16-128	>128	>128	>128

^h Includes one isolate each of *Aspergillus* spp. and *Aspergillus* spp.

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In another study by Oakley *et al.* (AAC, 42 (10), 2726-2730, 1998), 60 isolates of *Aspergillus* spp. including 35 *A. fumigatus*, 8 *A. terreus*, 8 *A. flavus*, 8 *A. niger* and 1 *A. nidulans* were evaluated against anidulafungin, itraconazole and amphotericin B. Broth microdilution methods were employed using AM3 and casitone agar supplemented with 2% glucose (CAS) for susceptibility testing of anidulafungin; RPMI 1640 was used for itraconazole and amphotericin B. Trays were incubated at 37°C for 48 hours. The inoculum used was 2x10³ conidia/mL for anidulafungin and 10⁶ conidia/mL were used for determination of the anidulafungin MEC, which was defined as the first well to show small subspherical colonies and no hyphal growth. Itraconazole and amphotericin B endpoints were read as the first well with no visible growth. Table 15 shows the MEC/MICs of all the drugs against the *Aspergillus* spp. tested. Anidulafungin was more active than itraconazole and amphotericin regardless of test medium.

MFCs were also determined against the 60 isolates of *Aspergillus* spp. For all drugs, 100 µl was removed from wells showing no growth and plated to horse blood agar plates which were then incubated at 37°C for 48 hours. The MFC for anidulafungin was defined as the lowest concentration of drug allowing 2 or fewer colonies (98% killing). Itraconazole and amphotericin B MFCs were defined as the lowest concentration of drug allowing 5 or fewer colonies (99.99% killing). MFC data are shown in Table 16. There was a difference noted in anidulafungin MFCs between species, ranging from 0.0018->0.5 µg/mL. Itraconazole and amphotericin B MFCs were higher, ranging from 2 - >16 µg/mL. The authors state in the article that anidulafungin is fungicidal for 86.7% of the isolates when AM3 is used as the test medium, and 68% of the isolates when casitone is used as the test medium. Itraconazole was stated to be fungicidal in 35% of the isolates while amphotericin B was fungicidal in 70% of the isolates tested.

Table 15: MEC/MIC

Species (no. of isolates)	Antifungal agent	MEC or MIC (µg/ml)		
		Range	99%	99.9%
<i>A. fumigatus</i> (35)	LY in AM3	0.0018-0.035	0.0018	0.0075
	LY in CAS	0.0018-0.025	0.0075	0.0075
	ITZ	0.25->16	0.5	2
	AMB	0.5-2	2	2
<i>A. terreus</i> (8)	LY in AM3	0.0018-0.020	0.0018	0.005
	LY in CAS	0.0075-0.025	0.005	0.0075
	ITZ	0.125-0.25	0.25	0.25
	AMB	2-8	4	8
<i>A. flavus</i> (8)	LY in AM3	0.015->0.5	0.015	1
	LY in CAS	0.0075->0.5	0.01	1
	ITZ	0.5-8	0.5	4
	AMB	2-16	4	4
<i>A. niger</i> (8)	LY in AM3	0.0018-0.030	0.003	0.005
	LY in CAS	0.0075-0.025	0.0075	0.0075
	ITZ	0.5->16	1	8
	AMB	0.25-1	1	1
<i>A. nidulans</i> (1)	LY in AM3	0.003	NC*	0.005
	LY in CAS	0.0075	NC	0.0075
	ITZ	0.125	NC	0.125
	AMB	2	NC	2
All isolates (60)	LY in AM3	0.0018-0.035	0.003	0.005
	LY in CAS	0.0018-0.025	0.0075	0.0075
	ITZ	0.125->16	0.5	8
	AMB	0.25-16	2	2

* MECs and MFCs for LY only; MICs and MFCs for ITZ and AMB.

** NC, not calculable.

Table 16: MFCs

Species (no. of isolates)	Antifungal agent	MFC (µg/ml)		
		Range	99%	99.9%
<i>A. fumigatus</i> (35)	LY in AM3	0.0018->0.5	0.015	1
	LY in CAS	0.0018->0.5	0.25	1
	ITZ	4->16	16	16
	AMB	4->16	8	16
<i>A. terreus</i> (8)	LY in AM3	0.0018-0.0075	0.003	<0.002
	LY in CAS	0.003-0.0075	0.003	<0.002
	ITZ	2->16	8	16
	AMB	>16	16	16
<i>A. flavus</i> (8)	LY in AM3	0.003->0.5	0.015	<0.5
	LY in CAS	0.003->0.5	0.5	<0.5
	ITZ	2->16	16	16
	AMB	16->16	16	16
<i>A. niger</i> (8)	LY in AM3	0.0018-0.0075	0.003	<0.002
	LY in CAS	0.0075->0.5	0.06	0.5
	ITZ	0.5->16	16	16
	AMB	2->16	16	16
<i>A. nidulans</i> (1)	LY in AM3	<0.003	NC*	<0.002
	LY in CAS	<0.003	NC	<0.002
	ITZ	4	NC	8
	AMB	16	NC	16
All isolates (60)	LY in AM3	0.0018->0.5	0.003	1
	LY in CAS	0.0018->0.5	0.125	1
	ITZ	2->16	16	16
	AMB	4->16	16	16

* NC, not calculable.

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2.3 Activity against fungal species other than *Candida* and *Aspergillus*

The *in vitro* activity of anidulafungin was measured against non-clinical trial isolates of *Cryptococcus neoformans* (n=38), *Saccharomyces cerevisiae* (n=26), and *Rhodotorula rubra* (n=4). These data are from the Vicuron MIC database ([] 1998 Eli Lilly blood culture study report and Zhanel, 1998 study report). MICs for anidulafungin were determined using NCCLS M27 microbroth dilution methods using RPMI 1640 after 48 hour incubation. The results in Table 17 show that anidulafungin demonstrated poor *in vitro* activity against the 38 isolates of *C. neoformans* (MIC₉₀ >16 µg/mL). Against the 26 isolates of *S. cerevisiae*, the anidulafungin MIC₉₀ of 2 µg/mL, makes it comparable to the comparator drugs, fluconazole, itraconazole and amphotericin B. Four isolates of *R. rubra* were included, however, this number is too small to be evaluated.

In another study, the activity of anidulafungin was measured against 10 isolates of *C. neoformans* (Espinel-Ingroff, AAC, 36, 2950-2956, 1998). The results in Table 5a shows the MIC₉₀ for *C. neoformans* to be >16 µg/mL (printing error in published article table, >6). This is in line with the Vicuron database MICs mentioned above.

Krishnarao and Galgiani (AAC, 41, 1957-1960, 1997) evaluated the activity of anidulafungin against 15 isolates of *C. neoformans*. The activity was compared with caspofungin and fluconazole by the NCCLS M27A macrobroth dilution method. The results in Table 18 show that anidulafungin MICs are similar to the above reports demonstrating an MIC₉₀ against the 15 isolates of *C. neoformans* of >8 µg/mL. The other echinocandin included in the study, caspofungin, also had an MIC₉₀ of >8 µg/mL.

Table 17: MICs of non-*Candida* spp.

Organism	Drug	N	MIC ₅₀	(µg/L)	
				MIC ₉₀	MIC range
<i>Saccharomyces cerevisiae</i>	Anidulafungin	26	1	2	0.5-2
	Fluconazole	26	0.5	2	0.05-4
	Itraconazole	26	0.5	2	0.5-16.5
<i>Cryptococcus neoformans</i>	Amphotericin B	26	1	1	0.12-2
	Anidulafungin	38	>16	>16	0.5- >16
	Fluconazole	38	2	8	0.12-8
	Itraconazole	38	0.12	0.5	0.008-0.5
<i>Rhodotorula rubra</i>	Amphotericin B	38	1	1	0.5-1
	Anidulafungin	4			32
	Fluconazole	4			0.12-0.64
	Itraconazole	4			0.25-0.5

Table 18: Macrobroth MIC data

Species	No. of strains	MIC (µg/L)								
		MIC ₅₀			MIC ₉₀			Resistance		
		µg	mg	µg	µg	mg	µg	mg	µg	
<i>C. albicans</i>	15	0.25	0.25	0.008-0.25	0.25	0.5	0.25-10	1	1	0.5-50
<i>C. lusitana</i>	15	0.25	1	0.125-10	0.5	1	0.25-10	15	31	0.03-31
<i>C. tropicalis</i>	15	0.25	0.5	0.125-10	1	2	0.125-10	1	2	0.5-10
<i>C. guilliermondii</i>	15	0.25	0.5	0.125-10	4	8	10-100	1	1	10-100
<i>C. parapsilosis</i>	15	0.5	2	0.25-20	>10	>10	>10-200	1	1	0.25-10
<i>C. lusitana</i>	15	1	2	0.125-20	8	>10	>10-100	1	2	0.5-10
<i>C. guilliermondii</i>	15	>10	>10	>10-200	>10	>10	>10-200	1	1	0.25-10

The activity of anidulafungin was also evaluated against small numbers of other filamentous and dimorphic fungi as listed in Tables 14 and 19. The report by Espinel-Ingroff (AAC, 36, 2950-2956, 1998) shows that *in vitro*, anidulafungin appears less active than posaconazole against the strains of dimorphic fungi tested (*Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Sporothrix schenckii*) and comparable to caspofungin. There were only 5 isolates each of these 3 organisms included in that study so the numbers are too small to comment further. The filamentous fungi (*Acremonium strictum*, *A. flavus*, *A. fumigatus*, *A. terreus*, *Bipolaris* spp., *Cladophialophora bantiana*, *Fusarium oxysporum*, *F. solani*, *Phialophora* spp., *Pseudallescheria boydii*, *Rhizopus arrhizus*, *Scedosporium prolificans*), included in the same study tested small numbers of isolates per species.

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However, the anidulafungin MICs for most species were comparable to caspofungin (with the exception of the 1 isolate of *A. strictum*).

The Pfaller study (Diag Micro Inf Dis, 30, 251-255, 1998) measured activity of caspofungin and anidulafungin against 51 filamentous fungi (*Acremonium* spp., *A. flavus*, *A. fumigatus*, *Aspergillus* spp., *Fusarium* spp., *Paecilomyces* spp., *Pseudallescheria boydii*, *Rhizopus* spp., *Trichoderma* spp.). The MIC results were similar to those reported by Espinel-Ingroff in the study described above.

Table 19: MICs for filamentous and dimorphic fungi

Fungus (no. isolates)	Antifungal agent	MIC range (µg/ml)	Minimum inhibitory concentration MIC (µg/ml)	MIC range (µg/ml)
Filamentous Filamentous fungi <i>Acremonium strictum</i> (1)	SCHE56992	0.06	ND ^a	≥16
	MEK-0991	0.5	ND	≥16
	LY303366	≥16	ND	ND ^b
<i>Aspergillus flavus</i> (11)	SCHE56992	0.02-0.12	0.16	0.25-2
	MEK-0991	0.5	0.5	ND
	LY303366	<0.005-0.12	0.004	ND
<i>Aspergillus fumigatus</i> (25) ^d	SCHE56992	<0.005-1.0	0.12	0.25-≥16
	MEK-0991	0.5-≥16	2.12	ND
	LY303366	0.06	0.06	ND
<i>Aspergillus terreus</i> (2)	SCHE56992	<0.005-0.5	ND	1.0
	MEK-0991	0.5	ND	ND
	LY303366	<0.02	ND	ND
<i>Biziania</i> spp. ^e (6)	SCHE56992	0.02-0.25	0.14	0.3-1
	MEK-0991	1.0-2	1.7	ND
	LY303366	1.0-4	2.7	ND
<i>Candida lusitana</i> (5)	SCHE56992	<0.005-0.06	0.05	0.06-0.5
	MEK-0991	2-6	3.6	6-≥16
	LY303366	1.0-4	2	6-≥16
<i>Fusarium oxysporum</i> (6)	SCHE56992	1-6-16	4.16	2-≥16
	MEK-0991	≥16	≥16	ND
	LY303366	2-≥16	≥16	ND
<i>Fusarium solani</i> (6)	SCHE56992	≥16	≥16	ND
	MEK-0991	2-≥16	≥16	ND
	LY303366	≥16	≥16	ND
<i>Paecilomyces</i> spp. ^f (5)	SCHE56992	0.06-1.0	0.4	2-≥16
	MEK-0991	1.0-16	2.6	≥16
	LY303366	1.0-≥16	9	≥16
<i>Pseudallescheria boydii</i> (6)	SCHE56992	0.5-2	1.0	≥16
	MEK-0991	0.5-4	1.3	≥16
	LY303366	2-4	2.8	≥16
<i>Rhizopus oryzae</i> (5)	SCHE56992	2	2	2-16
	MEK-0991	≥16	≥16	ND
	LY303366	≥16	≥16	ND
<i>Trichoderma reesei</i> (2)	SCHE56992	≥16	ND	ND
	MEK-0991	4-8	ND	≥16
	LY303366	4	ND	ND
Dimorphic fungi <i>Blastomyces dermatitidis</i> (5)	SCHE56992	<0.005-0.06	0.05	2-16
	MEK-0991	0.5-8	2	ND
	LY303366	2-6	4	ND
<i>Blastomyces capsulatus</i> (5)	SCHE56992	<0.005-0.06	0.04	0.5-2
	MEK-0991	0.5-4	1.3	ND
	LY303366	2-4	3.6	ND
<i>Histoplasma capsulatum</i> (5)	SCHE56992	0.12-1.0	0.7	1-4
	MEK-0991	1.0-≥16	5.4	ND
	LY303366	0.25-≥16	3.2	ND

Total (82)

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2.4 Time-kill studies with *Candida* spp.

The effect of anidulafungin against 2 clinical isolates of *C. albicans* [one fluconazole susceptible (MIC 0.5 µg/mL) and one fluconazole resistant (MIC 128 µg/mL)] was measured over 24 hours using RPMI 1640 broth and NCCLS macrodilution methods (Karlowisky *et al.*, AAC, **41** (11), 2576-2578, 1997). Table 20 presents the MICs for the antifungal agents tested against the 2 isolates. Concentrations tested for anidulafungin were 0.001, 0.01, 0.1, 1, 10, 50, 100 and 1000 times the MIC. Aliquots of 100 µl were removed at 1, 2, 3, 4, 5, 6, 12 and 24 hours. Log₁₀ killing for anidulafungin was calculated after 24 hour incubation at 35°C. Kill curves for the 2 isolates of *C. albicans* grown in cultures supplemented with antimicrobial agent concentrations ranging from 0.001 to 1,000 times the MIC are shown in Figure 4. Anidulafungin displayed concentration independent killing of 1 to 2 log₁₀ CFU/mL for all concentrations between 0.1 and 1,000 times the MIC against both isolates. Anidulafungin at a concentration of 0.001 times the MIC did not appear to significantly alter the growth of either isolate. Regrowth was not seen at 24 hours in cultures of either isolate at anidulafungin concentrations ≥0.1 times the MIC. The activity of anidulafungin against the 2 isolates of *C. albicans* was similar to amphotericin B and greater than that of fluconazole. For all the strains tested, both anidulafungin and amphotericin B resulted in approximately 1.5 log₁₀ killing.

Figure 4: 24h anidulafungin kill curves of fluconazole susceptible/resistant *C.albicans*

A: fluconazole susceptible; B: fluconazole resistant *C.albicans*
 Symbols: ●growth control, ◆fluconazole, ○anidulafungin, ▲ amphotericin control
 ◆+MICx0.001, *MICx0.01, MICx0.1, ■MICx1, MICx10, ▲MICx50, ○MICx100, ○MICx1000

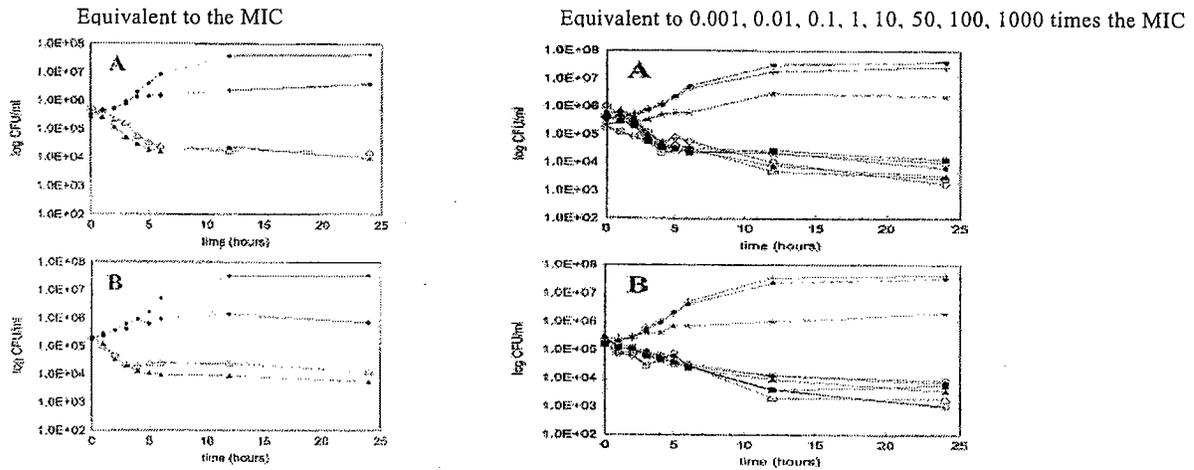


Table 20: MICs for 4 clinical isolates of *Candida* spp.

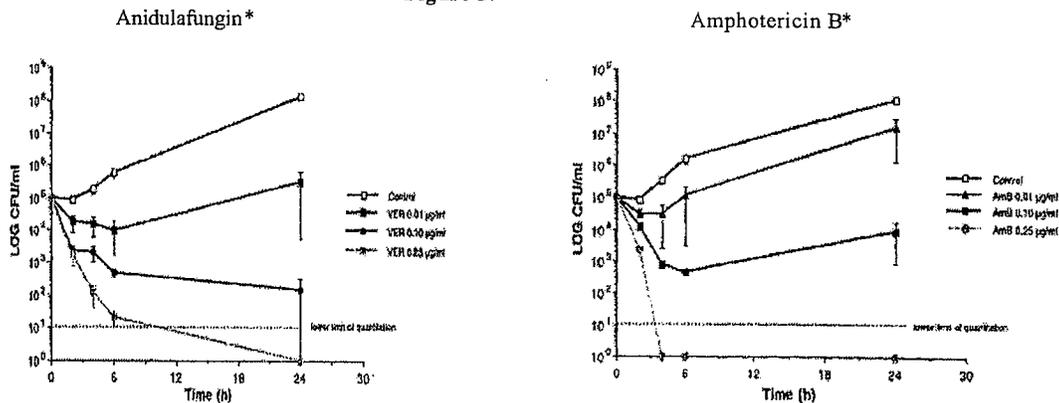
<i>Candida</i> isolate	MIC (µg/ml) of:		
	LY303366	Amphotericin B	Fluconazole
<i>C. albicans</i> Y88	0.04	0.5	0.5
<i>C. albicans</i> Y180	0.04	0.5	128
<i>C. glabrata</i> Y7	0.08	1	32
<i>C. krusei</i> Y171	0.16	1	32

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In another study by Petraitis *et al.* (AAC, **45** (2), 471-479, 2001), two isolates of fluconazole resistant *C. albicans* (MIC >64 µg/mL) were used in time kill studies performed in AM3 medium with anidulafungin or amphotericin B. Test isolates were obtained from two HIV patients who had EC. MICs were done according to NCCLS M27 methods. Concentrations tested were 0.01, 0.1, and 0.25 µg/mL for both anidulafungin and amphotericin B. After a logarithmic phase growth was achieved the suspension was centrifuged and washed 3 times. Time zero inoculum concentration was approximately 3×10^5 CFU/mL and sampling occurred at time 0, 2, 4, 6, and 24 hours. Aliquots were sampled from each time and colonies counted after 48 hours incubation at 37° C. Figure 5 shows the kill curves obtained from these experiments. Killing was achieved at 4-6 hours (>99.9% killing) for both drugs at 0.1 and 0.25 µg/mL and was sustained. Regrowth was not seen at 24 hours for anidulafungin at concentrations of 0.1 and 0.25 µg/mL. The concentration of 0.25 µg/mL of amphotericin B caused a more rapid decline in the number of organisms in the initial part of incubation. No significant differences in killing between anidulafungin and amphotericin B were detected at 24 hours. Concentration dependent fungicidal activities of both drugs are shown in Figure 5. This is not consistent with the above reported Karlowsky experiments which showed concentration independent killing for anidulafungin. It is unclear if the different media used (RPMI 1640/AM 3) could account for this difference.

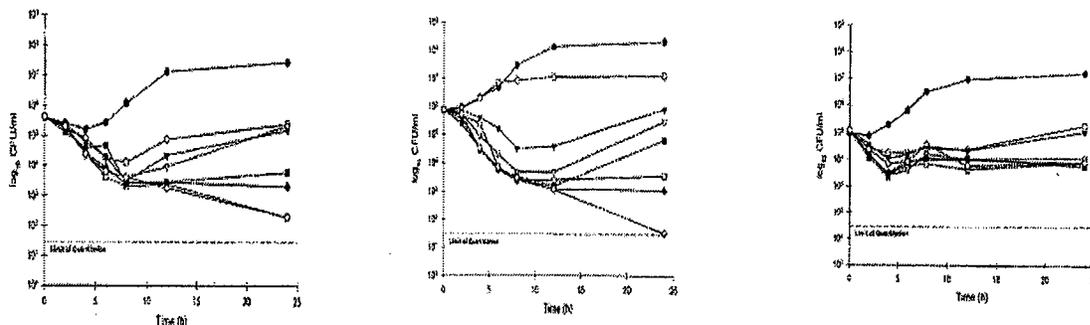
Figure 5:



*Data plotted are mean +/- SEM

In the study by Klepser *et al.* (AAC, **42** (6), 1387-1391, 1998), time kill studies were conducted against 2 isolates each of *C. albicans*, *C. glabrata* and *C. tropicalis*. Time kill studies were done in both RPMI 1640 and AM3 broth and dilutions ranged from 0.25-16x MIC₈₀. Predetermined time points were assayed. Figure 6 demonstrates representative plots for one isolate of each species tested. In AM3 medium, anidulafungin was fungicidal against all 6 test isolates. Fungicidal activity was defined as > a 3-log₁₀ reduction of CFU/mL compared with starting inoculum. Using 80% reduction of growth compared to the control well for the MIC endpoint, correlation was better between the MIC and the MFC/time kill data. When tested in RPMI, anidulafungin did not always exhibit cidal activity. When experiments were repeated in AM3 correlation between MIC₁₀₀ and MFC/time kill results was improved. In AM3, anidulafungin was fungicidal against all six test isolates and trailing in the MIC wells was greatly reduced or absent. The authors concluded that anidulafungin is not a uniformly fungicidal agent and recommend an endpoint of 80% reduction in growth.

Figure 6 : Time kill of 1 *C. albicans* isolate Time kill of 1 *C. glabrata* isolate Time kill of 1 *C. tropicalis* isolate



Key: ●=control, ○=0.25xMIC₈₀, ▼=0.5xMIC₈₀, ▽=MIC₈₀, ■=2xMIC₈₀, □=4xMIC₈₀, ◆=8xMIC₈₀, ◇=16xMIC₈₀

Variable conclusions from the above reports regarding the concentration dependence of the fungicidal activity of anidulafungin appears to be the result of testing methods/conditions. In addition, the clinical significance of such observations is not known.

2.5 Post antifungal effect

The post antifungal effect (PAFE) of anidulafungin was assessed against two isolates each of *C. albicans* and *C. neoformans*. MICs were determined using NCCLS M27 microdilution methods (Ernst *et al.* AAC, **44** (4), 1108-1111, 2000). A standard inoculum (1.5×10^5 CFU/mL) was used. *Candida* isolates were incubated with concentrations ranging from 0.125 to 4 times the MIC for 0.25 hours to 1 hour and the yeast washed 3 times with normal saline. Samples were removed at predetermined time points and inoculated to potato dextrose agar plates. The procedures for determining PAFE against *Cryptococcus* were similar to those above with the exception that only one anidulafungin concentration (80 µg/mL) was tested and incubation was at 30°C. The PAFE was stated to be the difference in time for control and test isolates to grow 1 log₁₀ above that observed following the final wash of drug removal. Figure 7 shows the PAFE for the *C. neoformans* isolate. Table 21 shows the MIC values for the test isolates and Table 22 the PAFEs after 1 hour of exposure to drug for the 2 isolates of *C. albicans*. Anidulafungin had a prolonged PAFE of >12 hours after 1 hour exposure at concentrations greater than or equal to the MIC against both isolates of *C. albicans*. At concentrations below the MIC, anidulafungin displayed a PAFE of >12 hours after 1 hour exposure for one of the *C. albicans* isolates (the other isolate of *C. albicans* at a concentration of 0.125 times the MIC produced no measurable PAFE). PAFEs observed were prolonged and dose dependent. Clinical relevance of such an effect is unknown.

Table 21: MICs for PAFEs

Isolate	MIC (µg/ml)		
	FLC	MIK991	LY303366
<i>C. albicans</i> 90028	0.25	0.03	0.015
<i>C. albicans</i> OY31.5	0.25	0.03	0.015
<i>C. neoformans</i> 015.012	4	>2	>2
<i>C. neoformans</i> 1435.019	4	>2	>2

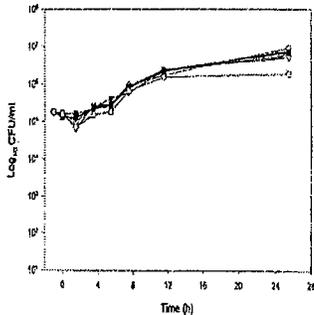


FIG. 7. PAPE of FLC, MIK991, and LY303366 after 1h of exposure to *C. albicans* 90028, *C. albicans* OY31.5, *C. neoformans* 015.012, and *C. neoformans* 1435.019.

Figure 7

Table 22: PAPE after 1h of drug exposure

Drug concn (multiple of MIC)	PAPE (h)			
	FLC	MIK991	LY303366	AMB
<i>C. albicans</i> OY31.5				
0.125	NT ^a	0	>12	2
0.25	NT	0	>12	4
0.5	NT	0	>12	>12
1.0	0	>12	>12	>12
2.0	0	>12	>12	>12
4.0	0	>12	>12	>12
<i>C. albicans</i> 90028				
0.125	NT	0	0	2
0.25	NT	0	>12	>12
0.5	NT	0	>12	>12
1.0	0	>12	>12	>12
2.0	0	>12	>12	>12
4.0	0	>12	>12	>12

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2.6 Effect of Protein Binding

Approximately 781 yeast isolates from the blood were evaluated for their susceptibility to anidulafungin in a study report by [] in 1998 (Eli Lilly report CM21). In this report the authors utilized NCCLS M27 methodologies as previously described. In addition, they repeated anidulafungin MICs for all isolates in RPMI 1640 (50%) plus 50% pooled human serum. MICs for anidulafungin were read as complete growth inhibition. The activity of anidulafungin is reduced in the presence of serum on an average of 2-4 fold for *C. albicans*, *C. tropicalis*, *C. glabrata* and *C. parapsilosis*. However, activity against *C. neoformans* was not changed in the presence of serum. See Tables 23-27. Clinical relevance of these findings is not known.

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Table 23

C. ALBICANS					
Final Report					
Antibiotic	n	MIC 80	MIC 90	Range	
FLU	313	0.25	0.5	<=0.03	-12
SFC	313	0.12	1	<=0.03	>=64
IFRA	313	0.06	0.12	<=0.008	-0.5
LY	313	0.03	0.12	<=0.015	-0.5
KETO	313	0.04	0.12	<=0.015	-0.5
AMB	313	1	1	0.05	-2
LY(SERA)	313	0.31	0.5	0.25	-4

Table 24

C. NEOFORMANS					
Final Report					
Antibiotic	n	MIC 80	MIC 90	Range	
FLU	38	2	2	0.12	-8
SFC	38	2	2	<=0.03	-8
IFRA	38	0.12	0.5	<=0.008	-0.5
LY	38	0.12	0.5	0.5	-32
KETO	38	0.12	0.5	<=0.008	-0.5
AMB	38	1	1	0.5	-1
LY(SERA)	38	0.32	0.5	<=0.015	>=16

Table 25

C. GLABRATA					
Final Report					
Antibiotic	n	MIC 80	MIC 90	Range	
FLU	66	8	16	1	-32
SFC	66	0.06	0.12	<=0.03	<=0.25
IFRA	66	1	4	0.08	-4
LY	66	0.04	0.12	<=0.015	-2
KETO	66	1	2	0.25	-4
AMB	66	1	1	0.5	-2
LY(SERA)	66	1	2	0.25	-2

C. PARAPSILOSIS					
Final Report					
Antibiotic	n	MIC 80	MIC 90	Range	
FLU	78	0.5	1	0.25	-12
SFC	78	0.12	0.25	<=0.03	<=0.3
IFRA	78	0.12	0.5	0.03	<=0.3
LY	78	2	2	<=0.015	-14
KETO	78	0.12	0.5	0.08	<=0.3
AMB	78	1	2	0.1	-2
LY(SERA)	20	0.5	>16	<=0.015	>=16

Table 26

C. TROPICALIS					
Final Report					
Antibiotic	n	MIC 80	MIC 90	Range	
FLU	62	1	2	0.25	>=16
SFC	62	0.25	1	<=0.03	>=64
IFRA	62	0.25	0.5	0.03	-1
LY	62	0.12	0.5	<=0.015	<=0.5
KETO	62	0.25	0.5	0.03	-1
AMB	62	1	2	0.1	-2
LY(SERA)	58	1	2	0.25	-2

Table 27

Tables 23-27: MIC data with and without serum

2.7 Activity of Metabolite

The sponsor has measured the antifungal activity of the metabolite by a bioassay (report ANI02M-001). In brief, solutions of anidulafungin were incubated at room temperature or 37°C for up to 10 days. At different time intervals a small aliquot of the stock solution was diluted and tested for antifungal activity by a bioassay. Bioassay was performed using an overnight culture of *C. albicans*, strain ATCC 90027. Freshly prepared stock of anidulafungin was used to prepare the standard curve. Each concentration of drug was dispensed (10µl) into pre-cut wells in the agar and plates were incubated overnight at 30°C. MIC testing was performed following NCCLS guidelines (M7-A5) using AM3 media. Results in Table 28 show MIC data at time zero through 240 hours. MICs were not significantly different. The usefulness of these MICs in determining the antifungal activity of the metabolite is not known.

Table 28: Biological activity of anidulafungin samples over 10 days

Time (hr)	5 µg/ml; PBS, pH 7.4				20 µg/ml; PBS, pH 7.4			
	37°C		Room Temp		37°C		Room Temp	
	Conc. (µg/ml)	MIC	Conc. (µg/ml)	MIC	Conc. (µg/ml)	MIC	Conc. (µg/ml)	MIC
0	5.66	<=0.001	6.97	0.002	29.17	<=0.001	43.50	<=0.001
24	5.60	0.004	5.60	0.002	29.17	0.004	29.17	0.002
48	5.20	--	6.97	--	22.63	--	29.17	--
72	3.37	--	6.48	--	17.56	--	24.34	--
96	3.98	0.004	6.02	0.002	20.30	0.004	19.58	0.002
144	2.18	--	4.85	--	18.21	--	31.34	--
168	1.22	--	5.60	--	12.87	--	24.34	--
196	1.06	--	4.19	--	10.96	--	21.83	--
216	0.85	--	4.85	--	7.91	--	25.33	--
240	0.68	0.06	3.99	0.002	7.63	0.008	22.60	0.004

Drug conc. determined by bioassay; MIC determined vs. VCAL1005.

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3. Activity *in vivo*

3.1 *Candida albicans*

3.1.1 Disseminated Candidiasis

Effect on Survival

The activity of anidulafungin in a mouse survival model of disseminated *C. albicans* infection was measured in ICR mice that were immunosuppressed by sublethal X-irradiation (400 r) and infected, 24 hours after irradiation, intravenously (i.v.) via the lateral tail vein (2×10^6 cfu/mouse; *C. albicans* A26-ATCC 90234) (Eli Lilly Nonclinical Pharmacology report WLC/IND9603). Mice treated intraperitoneally (i.p.) were given four doses (0, 4, 24, 48 hours) beginning within 15 minutes after inoculation of *C. albicans*. Mice treated orally were given two doses per day, the first dose beginning within 15 minutes after inoculation of *C. albicans*. In this model, all untreated mice die by day 4 after infection.

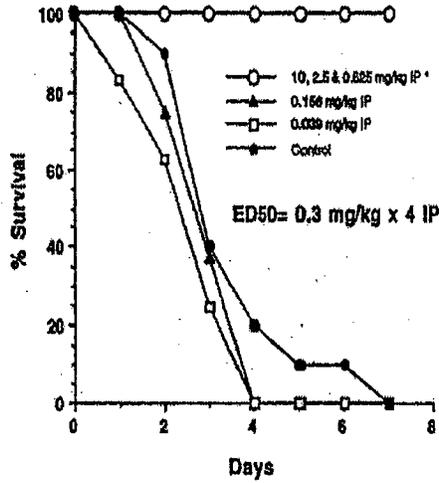
Table 29 demonstrates that an i.p. dose of 0.3 mg/kg/dose was necessary for anidulafungin to achieve 50% survival of animals. For amphotericin B and fluconazole, an i.p. dose of 4.0 and p.o. dose of 0.3 mg/kg/dose, respectively, was necessary to achieve 50% survival in mice. Anidulafungin administered orally twice daily for four days at ≥ 6.25 mg/kg significantly prolonged survival of infected mice and at 12.5 mg/kg all mice survived. Figures 8 -10 are survival curves which show that anidulafungin had 100% survival at ≥ 0.625 mg/kg/dose i.p., amphotericin B had 100% survival at 6.25 mg/kg/dose i.p., and fluconazole had 100% survival at 0.5 mg/kg/dose p.o. Anidulafungin was administered orally over two days while fluconazole was administered orally over four days. Anidulafungin was 25-fold less active when administered orally as compared with parenterally due to its low oral bioavailability in mice (see Figure 11).

Table 29: Efficacy of Anidulafungin in a Mouse Survival Model of Disseminated *C. albicans* Infection

Antifungal compound	Route (N mice per group)	Dose range (mg/kg/dose) Regimen	ED ₅₀ (mg/kg/dose)	Comment
Anidulafungin	i.p. (8)	0.039-10 0, 4, 24, 48 h	0.3	100% survival at ≥ 0.625 mg/kg/dose
Anidulafungin	p.o. (10)	1.56-12.5 2x/day for 4 d	7.8	100% survival at 12.5 mg/kg dose
Amphotericin B	i.p. (8)	0.78-12.5 0, 4, 24 h	4.0	100% survival at 6.25 mg/kg/dose
Fluconazole	p.o. (8)	0.062-1.0 2x/day for 4 d	0.3	100% survival at 0.5 mg/kg/dose

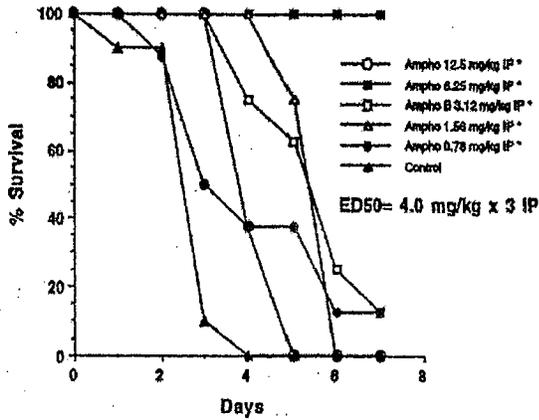
• data from Eli Lilly Report WLC/IND9603

Figure 8: Survival Curve of Anidulafungin against Disseminated *C. albicans* Infection



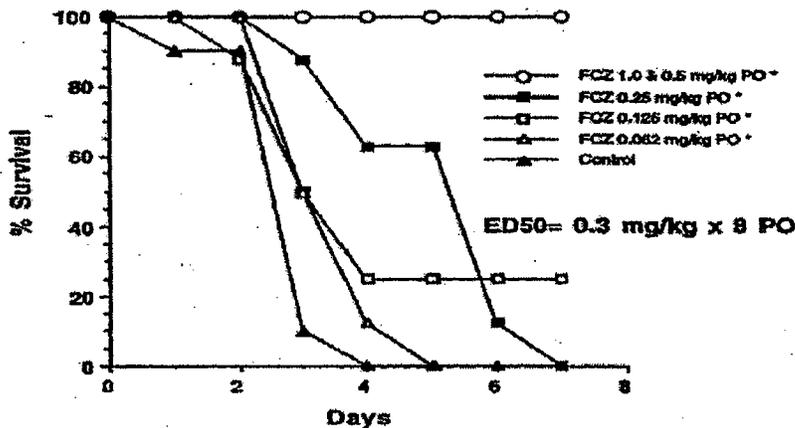
*data from Eli Lilly Report WLC/IND9603

Figure 9: Survival Curve of Amphotericin B against Disseminated *C. albicans* Infection



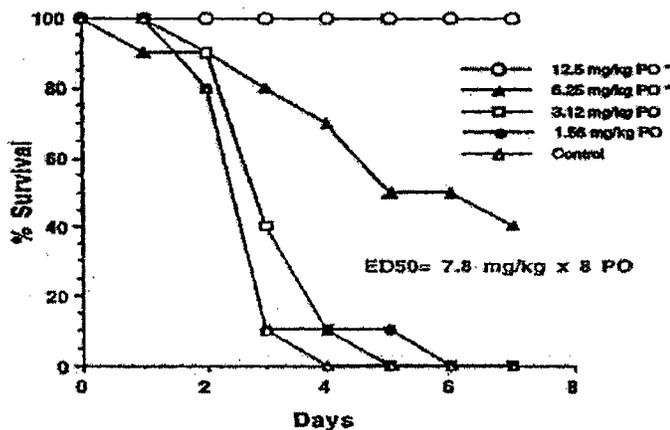
*data from Eli Lilly Report WLC/IND9603

Figure 10: Survival Curve of Fluconazole against disseminated *C. albicans* Infection



*data from Eli Lilly Report WLC/IND9603

Figure 11: Survival Curve of Oral Anidulafungin against disseminated *C. albicans* Infection



In another experiment (Eli Lilly Nonclinical Pharmacology report WLC/IND9603), the effect of delayed treatment and different dosage regimens on the activity of anidulafungin in mice with disseminated *C. albicans* infection was studied (Table 30). In this case, the ICR mice were immunosuppressed by sublethal x-irradiation and 24 hours later inoculated i.v. with 2×10^6 CFU/mouse *C. albicans* A26 - ATCC 90234. When infection was better established before treatment, by delaying treatment for different times up to 24 hours after infection, the dose at which 50% of the mice survived was <1 mg/kg/dose, even when the number of treatments was reduced to 3 or 2. When a single treatment was given 48 hours post-infection, the dose required to have 50% of the animals survive was greatly affected

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requiring >5.0 mg/kg/dose. Only one of the 24 mice receiving ≥ 0.312 mg/kg in the first three treatment regimens died during study no. 2.

Table 30: Effect of Delayed Treatment and Different Dosage Regimens on the Efficacy of Anidulafungin in the Mouse Survival Model of Disseminated *C. albicans* (Report WLC/IND9603)

	Route (N mice per group)	Dose range** (mg/kg/dose)	Regimen (h post-infection)	ED 50 (mg/kg/dose)*
Study no. 1	i.p. (10)	<u>10</u> , 2.5, 0.625, 0.156	0, 4, 24, 48	0.37
			1, 4, 24, 48	0.5
			2, 4, 24, 48	0.34
			4, 24, 48	0.93
Study no. 2	i.p. (8)	<u>5</u> , <u>1.25</u> , <u>0.312</u> , 0.078	0, 4, 24, 48	0.11
			4, 24, 48	0.10
			6, 24, 48	0.12
			24, 48	0.33
			48	>5.0

*Survival was scored at 7 days post-infection.
*data from Eli Lilly Report WLC/IND9603
** Doses resulting in 100% survival for each of the treatment regimens are underlined.

In another study (Eli Lilly Nonclinical Pharmacology Report WLC/IND9707), a comparison of the efficacy of i.p. and i.v. administration of anidulafungin and i.p. amphotericin B against disseminated *C. albicans* infection in immunosuppressed (sublethal X-irradiation) ICR mice was studied (Table 31). After sublethal x-irradiation, the ICR mice were inoculated 24 hours later with 2×10^6 CFU/mouse of *C. albicans* A26- ATCC 90234. Mice were given 3 doses of anidulafungin or amphotericin B beginning 4, 6, or 24 hours after challenge with *C. albicans*. All infected control mice given vehicle i.p. or i.v. died by day 4. The i.v. anidulafungin appeared to be more effective than when administered i.p. All mice receiving anidulafungin i.v. at 0.625 mg/kg and i.p. at 1.25 mg/kg beginning 4 or 6 hours after inoculation of *C. albicans* survived the 7-day evaluation period (see Figure 12-14). Anidulafungin and amphotericin B were highly effective when administered 4 or 6 hours after inoculation of *C. albicans* but were less effective when the first dose was delayed for 24 hours.

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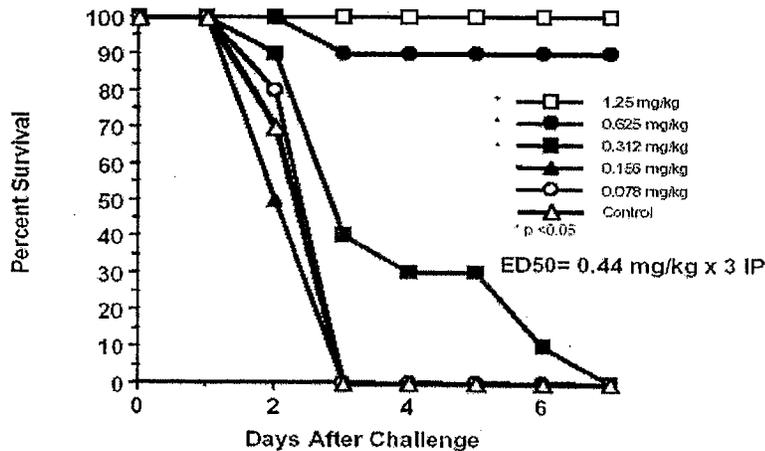
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Table 31: Efficacy of Anidulafungin in a Mouse Survival Model of Disseminated *C. albicans* Infection. Comparison of IP and IV Administration of Anidulafungin and IP Amphotericin B.

Antifungal Compound	Route (No. of mice per group)	Doses Range (mg/kg)	Timing of Doses	ED ₅₀ (mg/kg)
Anidulafungin	i.p. (10)	0.078 - 1.25	4, 24, 48 h	0.44
			6, 24, 48 h	0.41
			24, 48, 72 h	1.02
	i.v. (10)	0.078 - 0.625	4, 24, 48 h	0.29
			6, 24, 48 h	0.44
			24, 48, 72 h	0.46
Amphotericin B	i.p. (10)	0.078 - 1.25	4, 24, 48 h	0.10
			6, 24, 48 h	0.07
			24, 48, 72 h	1.25

* per Eli Lilly Nonclinical Pharmacology Report WLC/IND9707

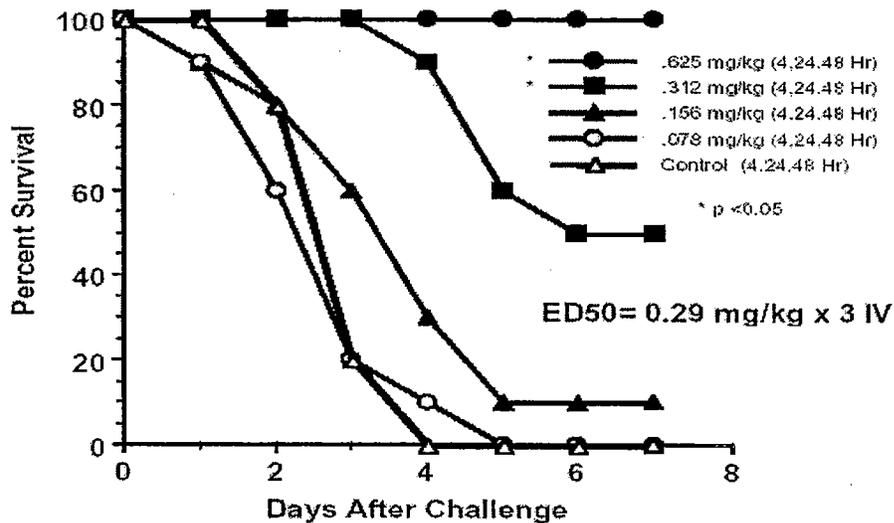
Figure 12: Survival curves of Immunosuppressed ICR Mice Infected with *C. albicans* and Treated with Different Dosages of Anidulafungin IP starting 4 hours After Challenge.



Anidulafungin was administered IP at 4, 24, and 48 hours after challenge.

* per Eli Lilly Nonclinical Pharmacology Report WLC/IND9707

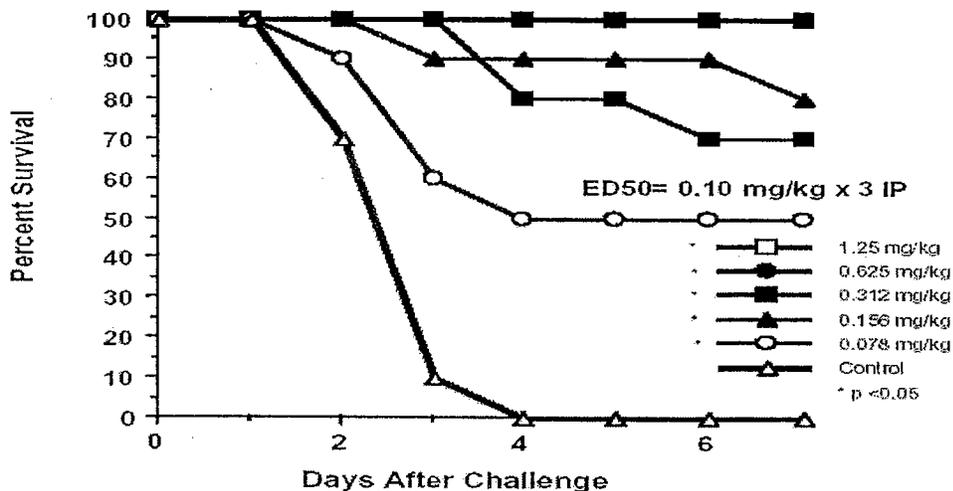
Figure 13: Survival Curves of Immunosuppressed ICR Mice Infected with *C. albicans* and Treated with Different Dosages of Anidulafungin IV starting 4 hours After Challenge



Anidulafungin was administered IV at 4, 24, and 48 hours after challenge.

* per Eli Lilly Nonclinical Pharmacology Report WLC/IND9707

Figure 14: Survival Curves of Immunosuppressed ICR Mice Infected with *C. albicans* and Treated with Different Dosages of Amphotericin B IP starting 4 hours After Challenge



Amphotericin B was administered IP at 4, 24, and 48 hours after challenge.

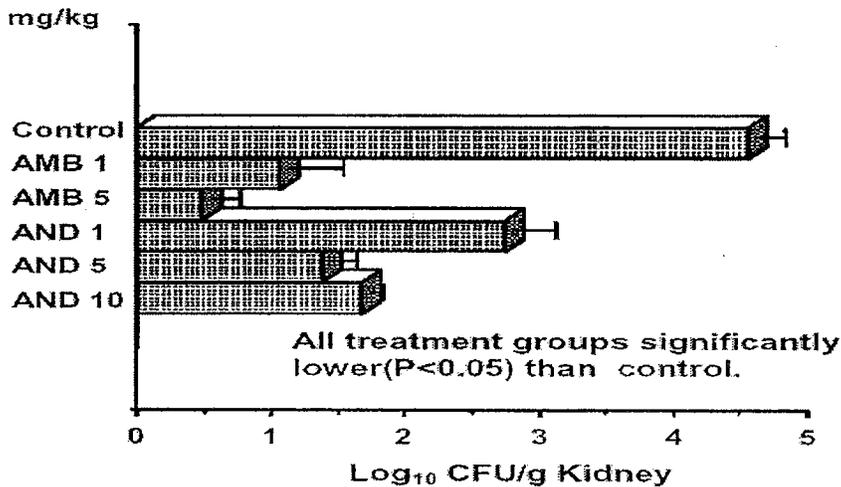
• per Eli Lilly Nonclinical Pharmacology Report WLC/IND9707

Effect on Mycological Burden

Immunocompetent (sublethal x-irradiation) ICR mice were infected i.v. with 2×10^5 CFU of *C. albicans* A26 - ATCC 90234 (Eli Lilly Nonclinical Pharmacology Report WLC/IND9707). Therapy was initiated one hour after inoculation of *C. albicans* and mice were treated twice daily for 3 days with different dosages of anidulafungin or amphotericin B. There were 10 animals in each treatment group. Mice were sacrificed 24 hours after the last dose. Kidneys and liver were homogenized and CFU determined using standard plate counts. Mean CFU per gram of tissue obtained from treated animals were compared by Student's t-test with those from control animals. The detection limits of the assays were approximately 20 CFU of *C. albicans* per mouse paired kidneys and approximately 18 CFU for the liver. Figure 15 demonstrates that anidulafungin given i.v. twice daily for 3 days at doses of 1, 5 or 10 mg/kg, beginning one hour after inoculation of *C. albicans*, reduced the number of organisms in the kidneys of mice with disseminated Candidiasis. Reduction relative to controls ranged from 2 to 3 logs.

Figure 16 demonstrates that anidulafungin given twice daily for 3 days at 10 mg/kg, beginning one hour after inoculation of *C. albicans*, reduced the number of organisms in the livers of mice with disseminated Candidiasis. Anidulafungin given i.v. was not as effective as amphotericin B given i.p. in reducing the numbers of *C. albicans* in both the livers and kidneys of infected mice.

Figure 15: Efficacy of Anidulafungin and Amphotericin B in Reducing the Kidney Load of *C. albicans* in Immunocompetent ICR Mice

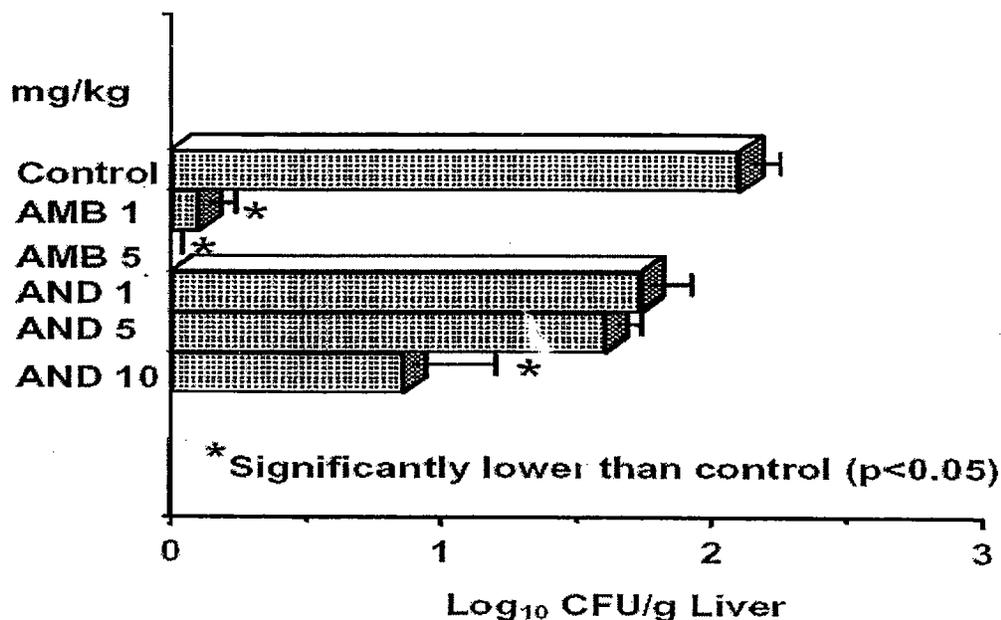


AMB = IP amphotericin B; AND = IV anidulafungin. Bars indicate SEM.

* per Eli Lilly Nonclinical Pharmacology Report WLC/IND907

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Figure 16: Efficacy of Anidulafungin and Amphotericin B in Reducing the Liver Load of *C. albicans* in Immunocompetent ICR Mice

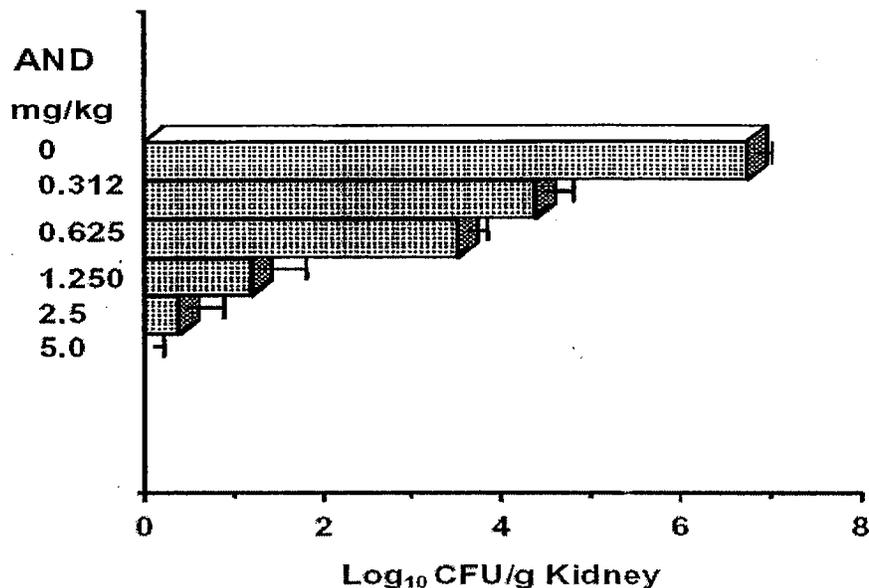


AMB = IP amphotericin B; AND = IV anidulafungin. Bars indicate SEM.

- per Eli Lilly Nonclinical Pharmacology Report WLC/IND9707

In another study, C'5-deficient DBA/2N male mice were infected i.v. with 1×10^5 CFU/mouse of *C. albicans* A26 - ATCC 90234. Treatment with different dosages (0.31 to 5 mg/kg/dose) of anidulafungin (i.p.), amphotericin B (i.p.), or fluconazole (p.o.) was initiated within 30 minutes and mice were treated twice daily for 4 days. CFU were determined in the kidneys 24 hours after the last dose. Mean numbers of CFU per gram of tissue obtained from treated animals were compared by Student's t-test with those from control animals. The detection limit was approximately 50 CFU of *C. albicans* per mouse paired kidneys. All doses of anidulafungin reduced the number of organisms in the kidneys (Figure 17). A dose-response relationship was apparent. The lowest dose of anidulafungin (0.31 mg/kg) reduced the CFU of *C. albicans* by a 2.4-log. At the highest dose of anidulafungin (5.0 mg/kg), no *C. albicans* was detected (6.75-log reduction compared to non-treated controls). Amphotericin B was also highly effective in reducing the number of organisms in the kidneys (Figure 18). Fluconazole was less effective than anidulafungin and amphotericin B (Figure 19). There was no dose-response relationship with fluconazole and the mean number of CFU's for all treatment groups was $> 10^3$.

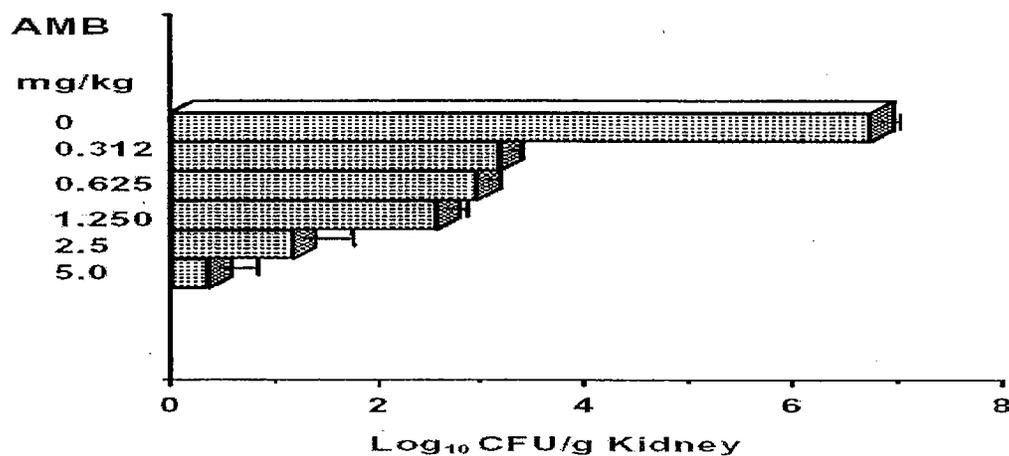
Figure 17: Efficacy of Different Anidulafungin Dosages (IP) in Reducing the Kidney Load of *C. albicans* in C'5-Deficient DBA/2N Mice.



AND = IP anidulafungin. Bars indicate SEM

* per Eli Lilly Nonclinical Pharmacology Report WLC/IND9707

Figure 18: Efficacy of Different Dosages of amphotericin B (IP) in Reducing the Kidney load of *C. albicans* in C'5-Deficient DBA/2N Mice.

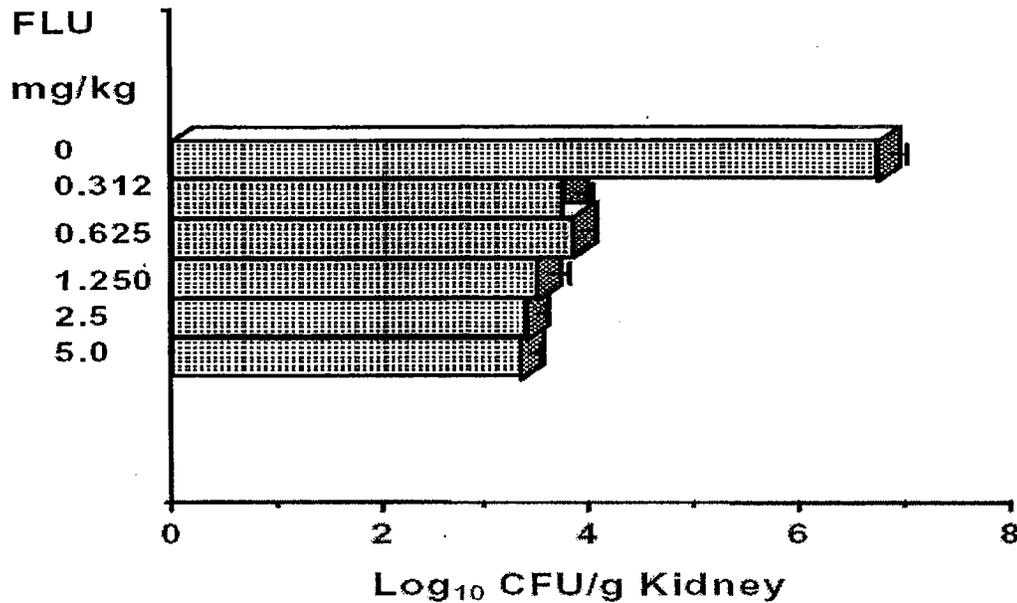


AMB = IP amphotericin B

* per Eli Lilly Nonclinical Pharmacology Report WLC/IND9707

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Figure 19: Efficacy of Different Dosages of fluconazole (PO) in Reducing the Kidney Load of *C. albicans* in C'5-Deficient DBA/2N Mice.

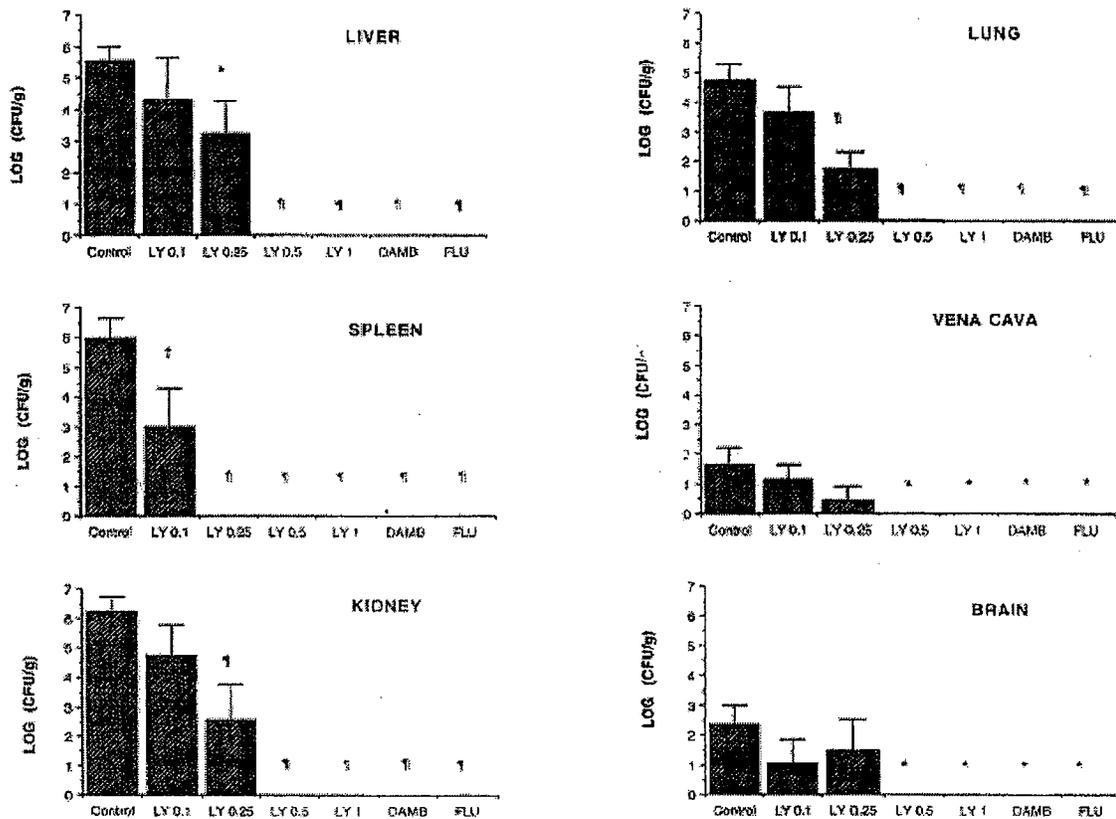


• per Eli Lilly Nonclinical Pharmacology Report WLC/IND9707

Petraitene *et al.*, 1999 (AAC, 43; 2148-2155) conducted another study in rabbits (White New Zealand) where neutropenia was induced with 5 days of cytosine arabinoside treatment (440 mg/m²/day). The neutropenic state was maintained by additional treatments every two days over the entire experiment. Ceftazidime, gentamicin, and vancomycin were administered from day 4 to prevent opportunistic bacterial infections. The rabbits were infected on day 6 with 10³ *C. albicans* blastoconidia (NIH-8621-isolated from neutropenic patient with disseminated Candidiasis) intravenously over 60 seconds, via an indwelling silastic intravenous catheter. The study groups contained 6 animals per group and included untreated controls, amphotericin B (1 mg/kg/day), fluconazole (10 mg/kg/day), and anidulafungin (0.1, 0.25, 0.5, or 1 mg/kg/day). Antifungal treatment was administered daily for 10 days by slow i.v. infusion, starting 24 hours after infection (days 7-16). On day 17, the rabbits were sacrificed and fungal tissue burden was determined. Antifungal activity was determined by the decrease in CFU in the tissues. Decrease of *C. albicans* from the tissues was dependent on the dose of anidulafungin. Rabbits treated with anidulafungin at 0.5 or 1 mg/kg/day had similar clearance of *C. albicans* from the liver, spleen, kidney, lung, vena cava, and brain to that obtained with amphotericin B and fluconazole (Figure 20). In liver, spleen, kidney, and lung, CFU/gram of tissue were reduced by approximately 5 logs at the 0.5 mg/kg/day dose of anidulafungin as compared to the untreated controls.

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Figure 20: Effect of Different Anidulafungin Doses in Reducing Organ Loads of *C. albicans* in Disseminated Infection in Persistently Neutropenic Rabbits



* per Petraitiene et al. 1999

Pharmacokinetic and Pharmacodynamic Modeling of Anidulafungin in Neutropenic Rabbit Models

Groll *et al.*, 2001 (AAC, 45; 2845-2855) examined and summarized the pharmacokinetics of healthy New Zealand White rabbits given a wide range of anidulafungin doses (0.1 to 20 mg/kg i.v.) and attempted to relate the pharmacokinetic parameters to activity against *C. albicans* in animal models with persistent neutropenia (Petraitiene *et al.*, AAC, 4: 2148-2155, 1999). Antifungal treatment was begun 24 hours after inoculation and was administered daily for a total of 10 days. Rabbits were euthanized 24 hours after the 10th dose of antifungal treatment by intravenous pentobarbital injection. Anidulafungin has predictable pharmacokinetics that is linear with dose, the drug is rapidly and widely distributed in the body, and it has a terminal half-life of elimination of 10-18 hours. Tissues taken from infected animals after 10 days of daily dosing showed substantial penetration into tissue sites relevant for treatment of invasive opportunistic infections (Table 32). The highest concentrations were in the lung and liver, followed by the spleen and kidney, and measurable concentrations were seen in the brain at doses ≥ 0.5 mg/kg. Trough anidulafungin concentrations in the lung, liver, spleen, and kidney of animals

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infected with *C. albicans* were above the *in vitro* fungicidal concentrations for the experimental isolates at doses ≥ 0.25 mg/kg or higher (Table 33). Pharmacodynamic modeling was used to assess the relationship between pharmacokinetic parameters and efficacy in neutropenic rabbits with disseminated Candidiasis, using residual fungal burden in the main target sites as the primary endpoint. Rabbits treated with anidulafungin demonstrated dose-dependent decrease of *C. albicans* from kidney tissue with 100% eradication at doses of ≥ 0.5 mg/kg/day (Table 34).

Table 32: Single and Multiple-Dose Compartmental Pharmacokinetic Parameters of Anidulafungin in Plasma of Uninfected Rabbits

Dose (mg/kg)	Pharmacokinetic parameter		
	C _{max} (mg/L)	C _{min} (mg/L)	AUC ₀₋₂₄ (mg.h/L)
<u>Single dose</u>			
0.10	0.51	<0.02	0.48
0.25	1.49	<0.02	1.50
0.5	1.70	<0.02	3.22
1	3.21	0.05	9.15
5	18.09	0.32	50.57
10	30.05	0.59	92.10
20	51.07	1.23	187.07
<u>Multiple doses</u>			
0.10	0.47	<0.02	0.71
0.25	0.99	<0.02	2.01
0.5	1.86	0.025	3.42
1	2.98	0.074	7.97
5	14.18	0.49	52.03
10	32.24	1.04	112.60
20	63.03	1.45	208.80

*per Groll et al. 2001

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Table 33: Tissue levels of anidulafungin after multiple dosing (q.d. for 10 days) in rabbits systemically infected with *C. albicans*

Dose (mg/kg/day)	Brain (mg/kg)	Aqueous humor (mg/L)	Vitreous humor (mg/L)	Choroid (mg/L)	Lung (mg/kg)	Liver (mg/kg)	Spleen (mg/kg)	Kidney (mg/kg)
0.1	<0.02	<0.02	<0.02	<0.02	0.85	0.33	0.25	<0.02
0.25	<0.02	<0.02	<0.02	<0.02	1.31	0.62	0.44	0.36
0.5	0.25	<0.02	<0.02	<0.02	2.52	1.24	0.89	0.70
1	0.41	<0.02	<0.02	<0.02	4.20	2.81	1.55	1.33
5	1.58	<0.02	0.10	0.30	17.9	16.82	9.82	6.87
10	3.91	0.06	0.18	1.47	32.6	43.76	21.74	16.92

*per Groll et al. 2001

Table 34: Effect of anidulafungin on residual fungal burden in kidney tissue and dosage related pharmacodynamic parameters in persistently neutropenic rabbits with subacute disseminated candidiasis

Dosage group (mg/kg QD)	<i>C. albicans</i> in kidney tissue (log CFU/g)	C_{max} (mg/L)	C_{min} (mg/L)	AUC ₀₋₂₄ (mg.h/L)	$T_{tau \geq MIC}$ (h)	Conc. kidney tissue (mg/kg)
0 (Control)	6.22	NA	NA	NA	NA	NA
0.1	5.68	0.66	0.03	2.28	0.90	0.17
0.25	2.58	1.41	0.04	4.39	3.50	0.36
0.5	0.00	1.95	0.09	8.25	11.58	0.70
1	0.00	3.29	0.13	12.47	16.13	1.33

*per Groll et al. 2001

3.1.2 Oropharyngeal and Esophageal Candidiasis:

Immunosuppressed female New Zealand Rabbits were infected with fluconazole-resistant *C. albicans* isolates from an HIV patient (Petraitis et al., AAC, 4; 471-479, 2001). The MICs of anidulafungin, fluconazole and amphotericin B for these isolates are found below in Table 35.

Table 35: MIC of Anidulafungin and other Antifungal Agents for Test Strains

<i>C. albicans</i> isolate	MIC (μ g/ml)		
	Anidulafungin (AM-3)	Fluconazole (RPMI)	Amphotericin B (AM-3)
NIH 105	0.015	>64	0.05
NIH 126	0.004	>64	0.05

The rabbits were immunosuppressed over the 14 day course study by daily injection of 5 mg/kg methylprednisolone. Gentamicin (40 mg/L) was administered in the drinking water to prevent bacterial infection throughout the experiment. Rabbits were orally inoculated with 2 fluconazole-resistant strains (2×10^8 blastoconidia in 1.5 mL saline) of *C. albicans* (NIH 105 and NIH 126) each day during the first week of immunosuppressive treatment. Visual observation and the presence of *Candida* in the tissues confirmed reliable oropharyngeal and esophageal Candidiasis. On Day 8, i.v. antifungal therapy was started and administered daily for 7 days. Tissues were obtained from euthanized rabbits 24 hours after administration of the seventh dose of antifungal treatment. The study groups consisted of untreated controls, anidulafungin (1, 2.5, or 5 mg/kg/day), fluconazole (2 mg/kg/day), and amphotericin B (0.3 mg/kg/day). Antifungal efficacy was determined by the decrease in CFU of *C. albicans* recovered from the tissues compared with the untreated controls. Clearance of *Candida* from tissues by anidulafungin was dose dependent (Table 36). Doses of 2.5 and 5 mg/kg/day reduced *C. albicans* CFU in the tongue, oropharynx, and esophagus, compared to the untreated controls (Table 37). No significant clearance of *C. albicans* was demonstrated with fluconazole or amphotericin B at the dosages used, which were chosen to reflect recommended human dosages for esophageal candidiasis.

Table 36: Efficacy of Anidulafungin and Other Antifungal Agents against Fluconazole-Resistant Oropharyngeal/Esophageal Candidiasis in Immunosuppressed Rabbits (*per Petraitis *et al.*, 2001)

Treatment Group	Tissue burden (log ₁₀ CFU/g tissue ± SD)					
	Controls	Anidulafungin			Fluconazole	Amphotericin B
	(N=14)	1 mg/kg/day (N=9)	2.5 mg/kg/day (N=9)	5 mg/kg/day (N=9)	2 mg/kg/day (N=9)	0.3 mg/kg/day (N=4)
Tongue	5.61 ± 0.14	5.26 ± 0.22	2.23 ± 0.74	0.84 ± 0.42	5.23 ± 0.28	4.80 ± 0.31
Oropharynx	4.22 ± 0.17	3.03 ± 0.17	1.61 ± 0.42	0.26 ± 0.26	4.23 ± 0.20	3.16 ± 0.35
Esophagus	4.55 ± 0.19	3.16 ± 0.62	0.77 ± 0.51	0.00 ± 0.00	4.68 ± 0.66	3.84 ± 0.38

Table 37: Concentrations of Anidulafungin and other Antifungal Agents in Gastrointestinal Organs, Saliva, and Mucosa in Rabbits with Oropharyngeal/esophageal Candidiasis

Treatment group ^a	Concentration (mg/kg or mg/L) ^b		
	Esophagus	Saliva	Plasma
Anidulafungin 1 mg/kg/day	0.564 ± 0.149	0.017 ± 0.007	1.653 ± 0.365
Anidulafungin 2.5 mg/kg/day	1.276 ± 0.273	0.024 ± 0.011	3.623 ± 0.882
Anidulafungin 5 mg/kg/day	2.402 ± 0.401	0.046 ± 0.022	7.426 ± 1.616
Fluconazole 2 mg/kg/day	3.577 ± 0.787	Not detectable	1.357 ± 0.129
Amphotericin B 2 mg/kg/day	0.020 ± 0.007	Not detectable	0.995 ± 0.056

^aThere were nine animals in each group.

^bAll values are expressed as mean ± SEMs. The lower limits of quantitation in plasma and saliva were 0.020 mg/L for anidulafungin, 0.5 mg/L for fluconazole, and 0.040 mg/L for deoxycholate amphotericin B; the lower limits of quantitation in tissues were 0.200 mg/kg for anidulafungin, 1.5 mg/kg for fluconazole, and 0.160 mg/kg for amphotericin B.

- per Petraitis *et al.*, 2001

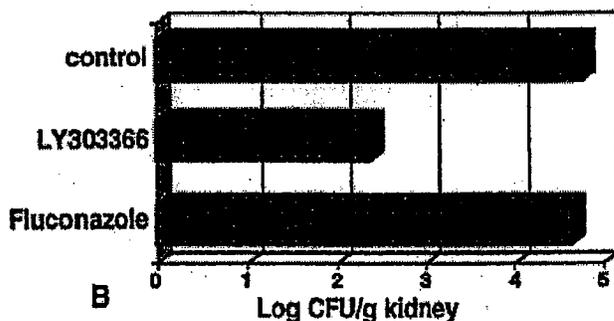
The table above demonstrates that the concentrations of anidulafungin in tissues were proportional to the dose administered.

3.2 *Candida krusei*

In a preliminary study, orally administered anidulafungin (16 mg/kg twice daily for 4 days) was effective in reducing the fungal burden in the kidneys of immunocompetent ICR mice that had been previously infected intravenously with 2×10^5 CFU of fluconazole-nonsusceptible *C. krusei* strain 6K7831 (Nonclinical Pharmacology Report WLC/IND 9603) (see Figure 21). Tissue samples were taken 24 hours after the last dose of antifungal treatment. Additional studies are being conducted to more clearly define the activity of anidulafungin against fluconazole non-susceptible *Candida spp.*

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Figure 21: Efficacy of Anidulafungin against Fluconazole Non-susceptible *C. krusei* Infection in Mice



3.3 *Aspergillus fumigatus*

3.3.1 Disseminated Aspergillosis

Effect of Survival:

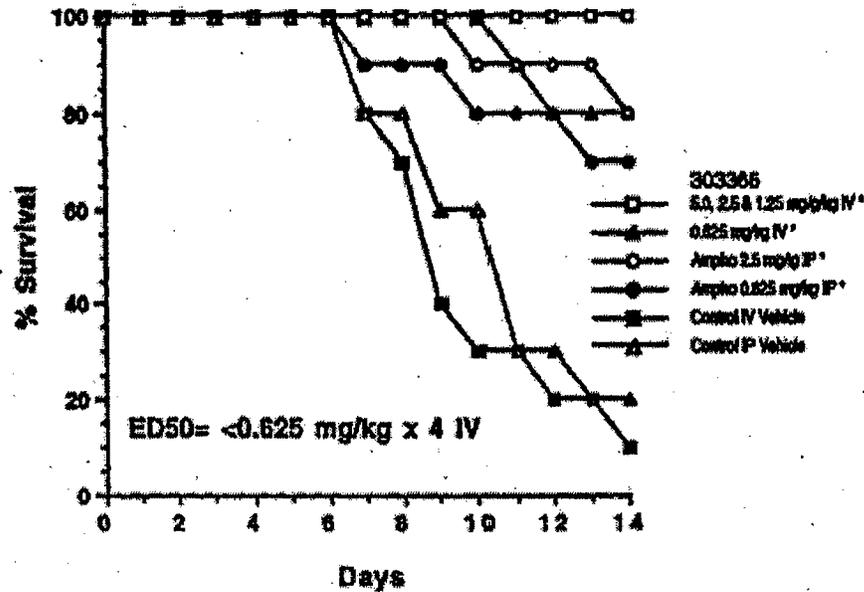
Complement-deficient DBA/2N mice were immunosuppressed with dexamethasone starting 4 days before infection and continuing for up to 14 days after infection for the survivors (Eli Lilly Non-clinical Pharmacology Report WLC/IND 9604). Dexamethasone was administered in the drinking water at a concentration calculated to deliver approximately 1.2 mg/kg/day. The mice were inoculated i.v. with 100 conidia of *A. fumigatus* strain WMI (clinical isolate obtained from [

]) This inoculum kills 80-100% of mice by days 5-8 post-infection. Infection was verified by visualization of dichotomously branched hyphae in lungs, kidneys, liver, and pericardium using microscopy. Treatment was initiated 24 hours after infection with the administration of anidulafungin once daily for 10 days i.p. or i.v. on alternate days 1, 3, 5, and 7 in different experiments. Amphotericin B was administered i.p. on alternate days 1, 3, 5, and 7. There were 10 mice in each group. Anidulafungin appeared to be as effective as amphotericin B when administered i.v. in this animal model.

Doses of <0.625-1.25 mg/kg/day i.v. prolonged the survival of the mice compared to the controls. All mice survived to day 14 post-challenge with ≥ 1.25 mg/kg/dose of i.v. anidulafungin and 80% survival was obtained with 2.5 mg/kg/dose of i.p. amphotericin B (see Figure 22).

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Figure 22: Survival Curve Demonstrating the Efficacy of Anidulafungin Against Disseminated *A. fumigatus* Infection in Dexamethasone Immunosuppressed DBA/2N mice

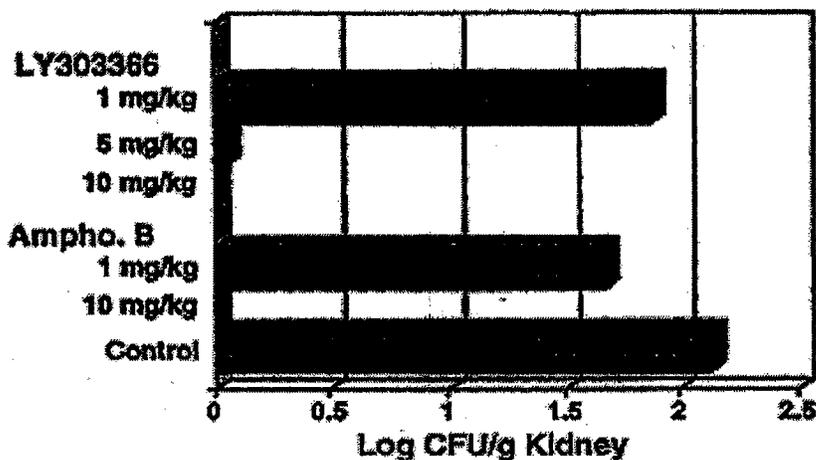


* per Eli Lilly Non-clinical Pharmacology Report WLC/IND 9604

Effect on Mycological Burden:

Immunocompetent ICR mice (sublethal xirradiation) were challenged i.v. with 1×10^5 conidia of *A. fumigatus* strain WM1 (Eli Lilly Non-clinical Pharmacology Report WLC/IND9604). Intraperitoneal treatment with different doses of anidulafungin or amphotericin B was initiated within 30 minutes of challenge. Anidulafungin was administered daily for 8 days and amphotericin B was administered on days 1, 3, 5, and 7. The animals were sacrificed and paired kidneys were homogenized to determine CFU at 24 hours after the last treatment. Both drugs cleared the kidneys of viable organisms at 10 mg/kg/dose. At the 5 mg/kg/dose of anidulafungin, low numbers of CFU were detected (see Figure 23).

Figure 23: Antifungal Efficacy of Anidulafungin Against Disseminated *A. fumigatus* Infection as Measured by Recovery of Organisms from Kidneys of ICR Mice



• per Eli Lilly Non-clinical Pharmacology Report WLC/IND9604

In another study, the activity of anidulafungin in murine models of invasive Aspergillosis with amphotericin B-susceptible and -refractory isolates was examined (Verweij et al., AAC, 42: 873-878, 1998). Four doses (1, 2.5, 10, and 25 mg/kg of body weight) of anidulafungin were compared with amphotericin B (0.5 to 5 mg/kg) in a temporarily neutropenic murine model of invasive Aspergillosis against an amphotericin B-susceptible (AF210) (patient successfully treated with amphotericin B) and an amphotericin B-resistant (AF65) (patient failed therapy with amphotericin B) strain of *Aspergillus fumigatus*. Both strains had similar MICs.

Virus-free male CD-1 mice were immunosuppressed with cyclophosphamide (200 mg/kg) and infected 3 days later by injection into lateral tail veins with $1-5 \times 10^5$ conidia per mouse. Treatment started 18 hours after infection and lasted for 10 days. Anidulafungin at 1, 2.5, 10 or 25 mg/kg/day was given once daily intravenously for 10 days, and amphotericin B at 0.5, 2, and 5 mg/kg was given once daily intraperitoneally for 10 days, or only on days 1, 2, 4, and 7 (at 5 mg/kg). Kidneys and lungs from survivors were cultured on day 11. Control mice in both experiments had 90 to 100% mortality (Table 39). Anidulafungin, at doses ≥ 2.5 mg/kg/day, increased the survival times of mice infected with either strain ($P < 0.05$) and was comparable to amphotericin B (Figures 24-25). Anidulafungin also reduced the fungal burden in the lungs and kidney (Table 38). The results for anidulafungin were similar for both strains and indicated potential useful activity against amphotericin B susceptible and resistant isolates.

Table 38: Culture Results for Lungs and Kidneys of Mice Infected with *A. fumigatus* AF210 and AF65

Treatment ^a	AF210			AF65		
	No. of survivors/ total no. of mice	Mean CFU (10 ³) ^b in:		No. of survivors/ total no. of mice	Mean CFU (10 ³) ^b in:	
		Lungs	Kidneys		Lungs	Kidneys
i.p. control	0/10	3.1 (0)	330 (0)	1/10	16.1 (7.8)	344 (171.8)
i.v. control	0/10	3.1 (0)	330 (0)	0/10	20 (0)	430 (0)
AB (mg/kg)						
0.5/day	1/10	2.8 (0.9)	297 (97.8)	4/10	12.2 (9.5)	269 (198.7)
2/day	7/10	0.9 (1.4)**	101 (149.7)**	1/10	18.0 (6.0)	388 (126.0)
5, in 4 doses	10/10	0.6 (1.2)**	3.5 (4.3)**	3/10	14.1 (9.0)	306 (189.3)
5/day	8/10	1.0 (1.3)**	66 (131.6)**	1/10	18.0 (5.9)	419 (33.0)
LY (mg/kg/day)						
1	3/10	2.4 (1.2)	271 (118.0)	2/10	16.1 (7.7)	365 (130.0)
2.5	7/10	0.9 (1.4)**	109 (144.7)**	8/10	4.6 (7.7)**	182 (177.3)*
10	8/10	0.6 (1.2)**	77 (126.4)**	7/10	7.2 (8.8)**	135 (192.6)*
25	8/10	0.9 (1.4)**	127 (139.3)**	8/10	7.0 (8.9)**	116 (164.1)*

^a i.p., intraperitoneal; i.v., intravenous; AB, amphotericin B; LY, LY303366.

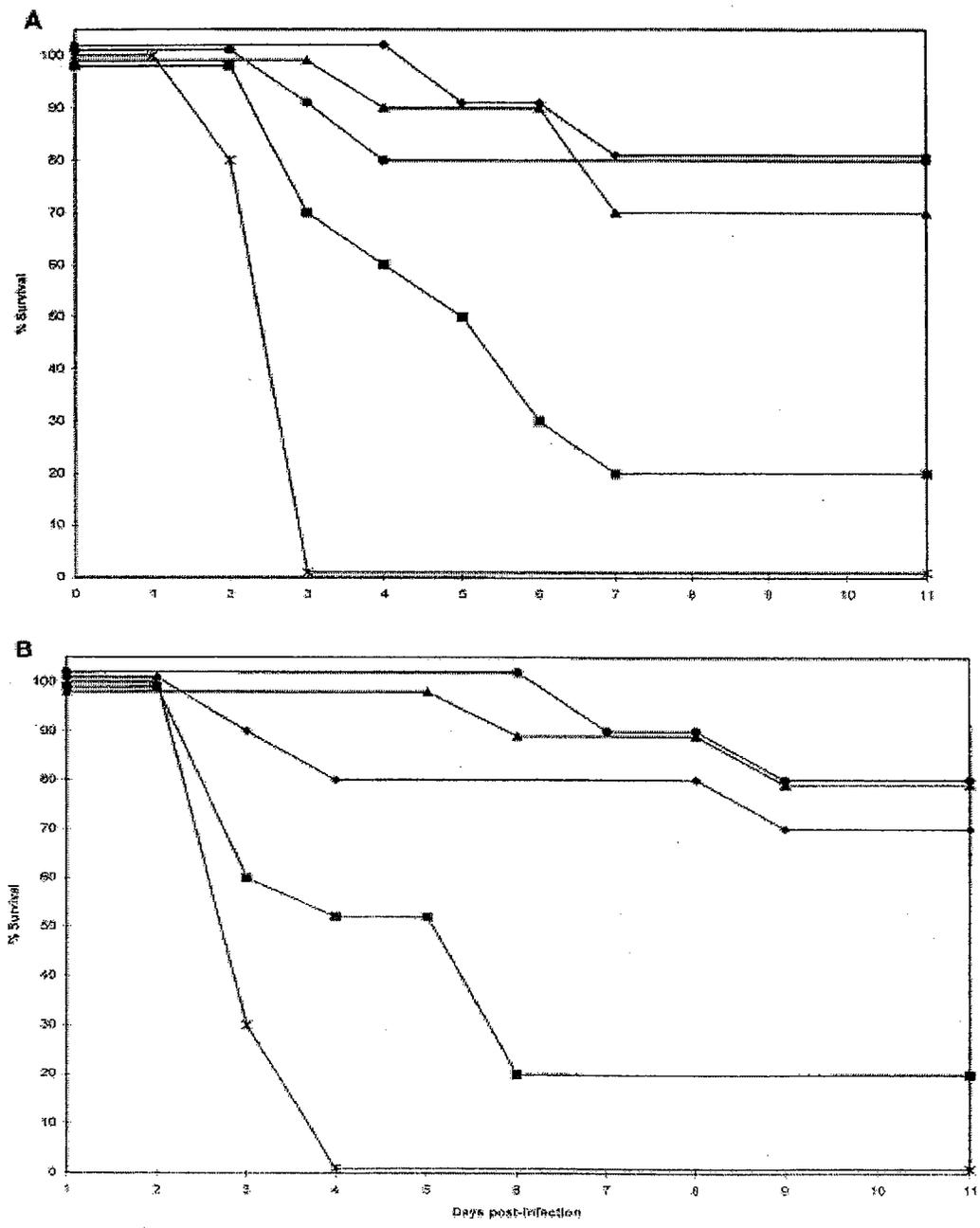
^b Numbers in parentheses represent standard deviations. *, $P < 0.05$ (comparison with controls); **, $P < 0.01$ (comparison with controls).

* per Verweij et al., 1998

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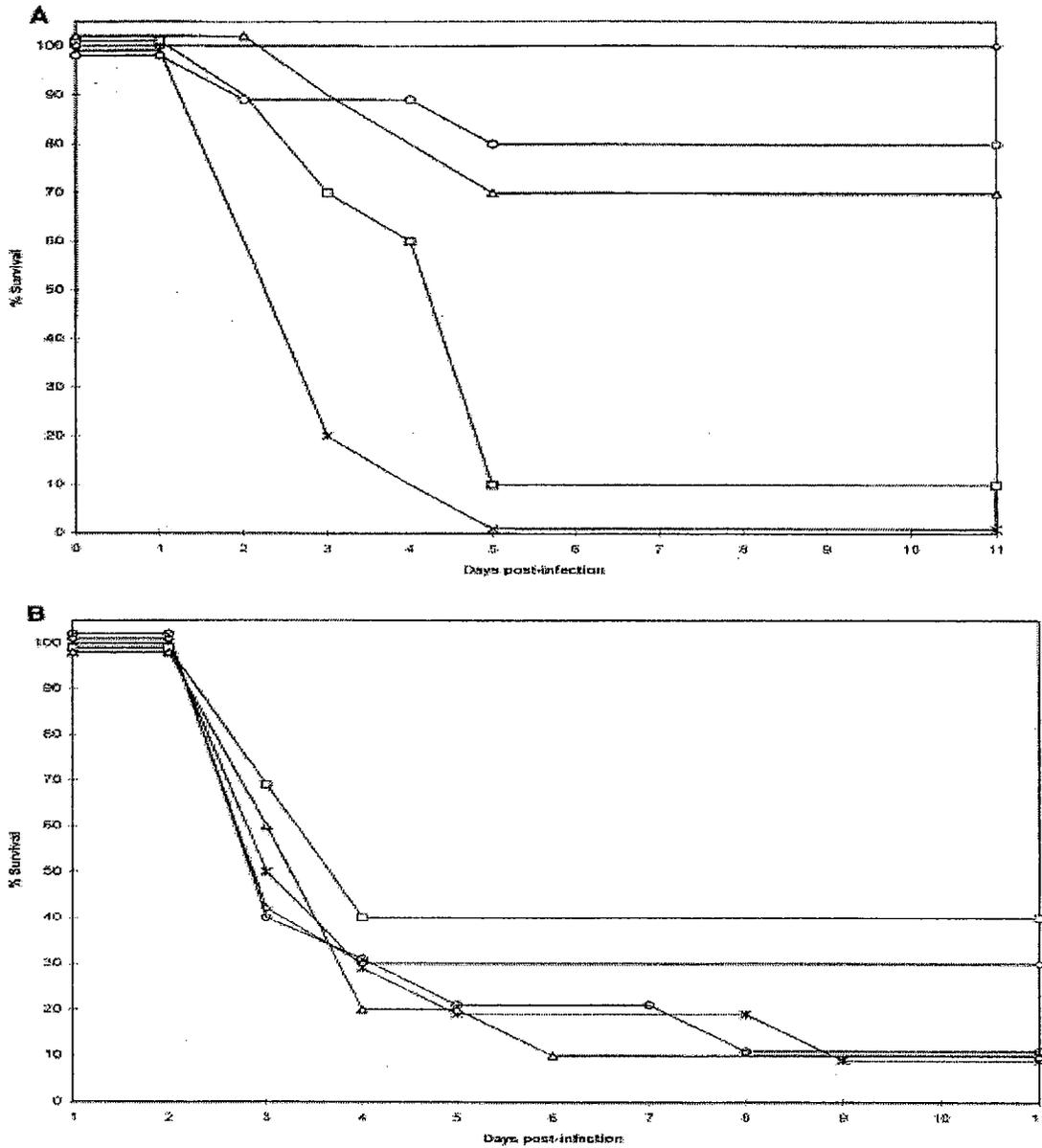
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Figure 24: Survival of CD-1 Mice Infected with (A) AF210 (Amphotericin B-susceptible) or (B) AF65 (amphotericin B-resistant) and Treated with Anidulafungin



Survival for CD-1 mice infected with isolate AF210 (A) or AF65 (B) and treated with Anidulafungin at 1 mg/kg/day (n), 2.5 mg/kg/day (?), 10 mg/kg/day (♦), or 25 mg/kg/day (?), or given 2.5% (wt/vol) Polysorbate 80 solvent intravenously (*)

Figure 25: Survival of CD-1 Mice Infected with (A) AF210 (Amphotericin B-susceptible) or (B) AF65 (amphotericin B-resistant) and Treated with Amphotericin B

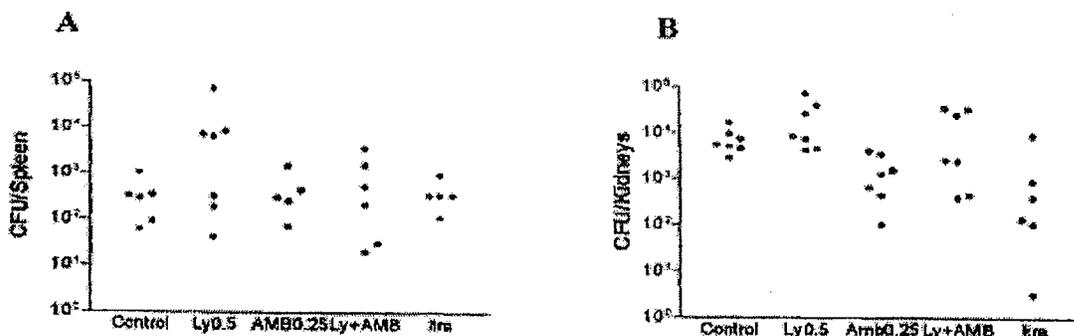


Survival for CD-1 mice infected with isolate AF210 (A) or AF65 (B) and treated with amphotericin B at 0.5 mg/kg/day (□), 2 mg/kg/day (△), 5 mg/kg given on days 1, 2, 4, and 7 (◇), or 5 mg/kg/day (O), or given dextrose intraperitoneally (*) (per Verweij et al., 1998)

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The activity of anidulafungin against itraconazole-susceptible and -resistant isolates in murine models of invasive Aspergillosis was studied (Graybill and Najvar, 1999, Eli Lilly, Inc. Supported Studies, [1]. Three clinical isolates of *Aspergillus fumigatus* were used. One was obtained from the fungus testing laboratory [2] (itraconazole susceptible). The MIC at 24 hours of incubation was 0.125 µg/mL and at 48 hours was 0.5 µg/mL as performed by the NCCLS method modified for filamentous fungi. The other two itraconazole-resistant isolates were obtained from [3]. The MIC for the itraconazole-resistant strain NCPF7909 at 24 hours was 2 µg/mL and 48 hours 4 µg/mL. The MIC for strain NCPF7100 was 2 µg/ml at 24 hours and the 48 hour MIC was omitted from the report. Male ICR mice were immunosuppressed with intravenous 5-fluorouracil (150 mg/kg) and cyclophosphamide (200 mg/kg) one day prior to intravenous infection. Inocula ranged from 9 x 10⁴ to 3 x 10⁵ conidia per mouse. One day after inoculation, treatment was begun with water (orally), itraconazole (30 mg/kg, 3 times per day orally), and various doses of amphotericin B (i.p.), or anidulafungin (i.v.) for 7 days. Tissue burdens were examined the day after treatment ceased. Survival was evaluated through day 30. Anidulafungin did not have any effect on the tissue burden of the spleen and kidneys of mice infected with itraconazole-susceptible or -resistant *A. fumigatus* (see Figure 26-27). However, anidulafungin was effective at doses of 0.5-1.0 mg/kg/day in prolonging survival in itraconazole-susceptible and -resistant strains. Amphotericin B at 1.5 mg/kg prolonged survival in mice infected with any strain (Figures 28-29). Itraconazole was not effective against the resistant isolates used in this study.

Figure 26: Tissue Burden of Spleen (A) and Kidneys (B) in mice infected i.v. with 8.4x10⁵ Itraconazole-Susceptible *A. fumigatus* and treated from day 1 through 7 after infection. Mice were given anidulafungin 0.5 mg/kg, amphotericin B 0.25 mg/kg, both drugs or neither drug (per Graybill and Najvar, 1999)



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Figure 27: Tissue Burden of Itraconazole-Resistant *A. fumigatus* in the kidneys (A) and spleen (B) after infection with 2.8×10^5 CFU and treatment with anidulafungin, amphotericin B 0.25 mg/kg, both drugs, neither drug or itraconazole 30 mg/kg 3 times daily (per Graybill and Najjar, 1999)

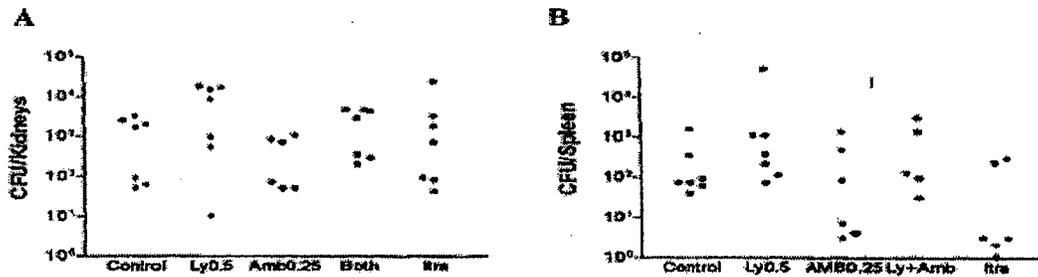
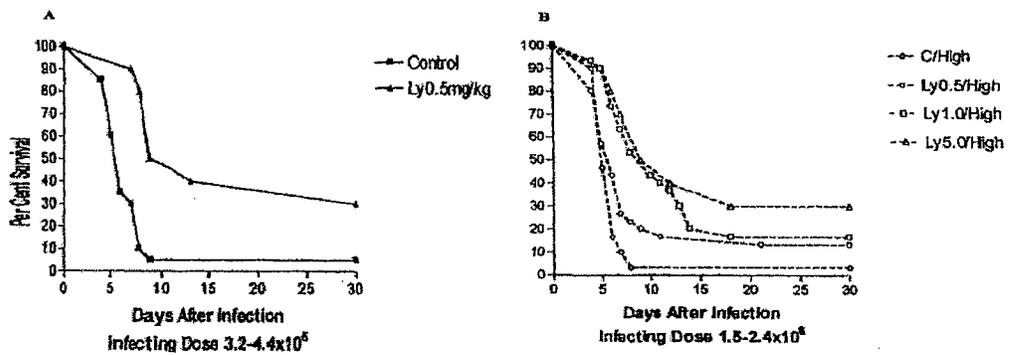


Figure 28: Survival after Infection IV with *A. fumigatus* (Itraconazole-susceptible) and treatment with Anidulafungin (per Graybill and Najjar, 1999)



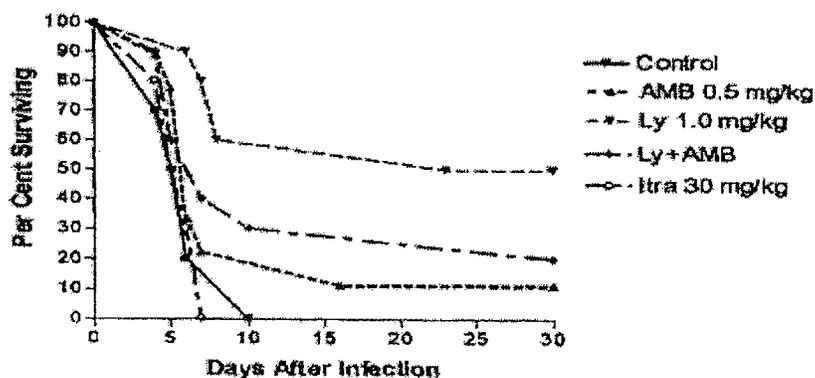
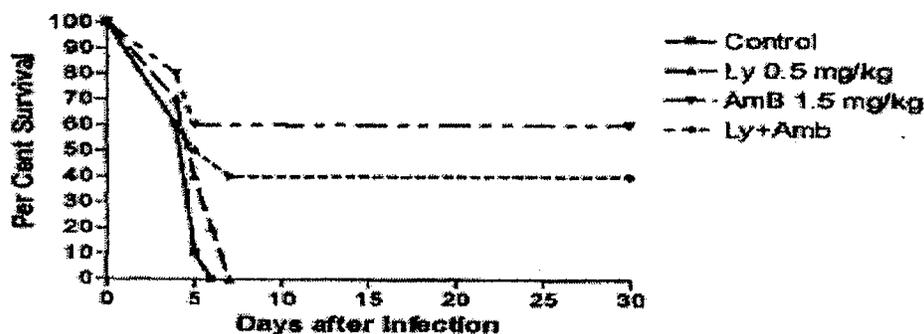
A: Low Dose Infection. Two studies were combined. Infecting doses were 3.3×10^5 and 4.2×10^5 CFU/mouse.

B: High Dose Infection. Three studies were combined. Infecting doses were 1.7×10^6 , 1.5×10^6 , and 2.4×10^6 CFU/mouse.

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Figure 29: Survival after Infection IV with *A. fumigatus* (Itraconazole-resistant) and treatment with Anidulafungin (per Graybill and Najvar, 1999)



3.3.2 Pulmonary Aspergillosis:

Groll *et al.*, (AAC, 45: 2845-2855, 2001) measured the pharmacodynamics in neutropenic rabbits with pulmonary Aspergillosis. Neutropenia was induced in rabbits as described earlier for disseminated candidiasis and maintained for 16 days (Petraitis *et al.*, AAC, 45: 471-479, 2001). On day 2 of the experiment, rabbits were intratracheally inoculated with a defined 200-250 μ l inoculum containing 10^8 conidia of *Aspergillus fumigatus* strain (NIH 4215 with an anidulafungin MIC of 0.13 μ g/mL) obtained from a neutropenic patient with a fatal case of pulmonary Aspergillosis. Antifungal therapy was begun 24 hours after intratracheal inoculation and administered daily throughout the experiments for a maximum of 12 days. Surviving rabbits were euthanized 24 hours after the 12th dose of antifungal

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treatment. Anidulafungin had no apparent effect on residual fungal burden, despite improvement in survival and reduction in pulmonary tissue injury. The beneficial effects were independent of dosage and no concentration-effect or exposure-effect relationships were observed for residual *Aspergillus* burden in lung tissue or survival of rabbits. Although other indicators demonstrated efficacy of anidulafungin in this infection model, no pharmacodynamic conclusions could be made because the CFU did not decline in a dose-dependent manner (Table 39).

Table 39: Effect of anidulafungin on residual fungal burden in kidney tissue and dosage related pharmacodynamic parameters in persistently neutropenic rabbits with invasive pulmonary aspergillosis (Groll *et al.* 2001)

Dosage group* (mg/kg)	<i>A. fumigatus</i> in lung tissue (log CFU/g)	C _{max} (mg/L)	C _{min} (mg/L)	AUC ₀₋₂₄ (mg.h/L)	T _{1au} ≥ 3x MIC (h)	Conc lung tissue (mg/kg)
0 (Control)	1.77	NA	NA	NA	NA	NA
1 QD	1.92	3.26	0.17	13.88	11.21	6.69
5 QD	2.36	15.76	1.17	78.00	23.71	34.61
10 QD	1.60	32.25	1.96	164.22	24.00	47.74
5 BID	1.96	17.84	2.73	142.21	24.00	33.65
20 QD	2.31	73.28	3.19	359.03	24.00	112.43
10 BID	2.18	39.91	6.65	326.70	24.00	67.67

* QD once-daily, BID twice-daily

The activity of anidulafungin against invasive Aspergillosis in the persistently neutropenic rabbit model was measured (Petraitis *et al.* AAC, 46: 1857-1869,1998). Immunosuppressed female New Zealand White rabbits were inoculated endotracheally with 1×10^8 *A. fumigatus* conidia (NIH isolate 4215). Immunosuppression was with cytosine arabinoside, initiated before infection and continued throughout the treatment period. Daily i.v. antifungal therapy (amphotericin B at 1 mg/kg/day or anidulafungin at 1, 5, 10, or 20 mg/kg/day) was begun 1 day after inoculation and continued for 12 days (see Table 40). The rabbits were euthanized 24 hours after the last dose of antifungal therapy. In the anidulafungin treated rabbits, survival was prolonged at the 1 mg/kg/day and 10 mg/kg/day dosages by about 2-3 days. However, such an effect was not observed at 5 or 20 mg/kg/day. Anidulafungin also reduced Aspergillosis-mediated pulmonary injury as measured by pulmonary lesion scores and total lung weight. However, there was no improvement in the clearance of *A. fumigatus* from the lungs after treatment with anidulafungin, in contrast to amphotericin B. Histological examination of the anidulafungin-treated lung tissue revealed a dose-related alteration in the cell wall morphology of *Aspergillus* hyphae (see Figure 30).

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Table 40: Survival of Persistently Neutropenic Rabbits with Primary Pulmonary *Aspergillosis* Treated with Amphotericin B or Anidulafungin Compared to Untreated Controls

Treatment group	Survival (no. of days)				P value ^b
	Mean \pm SEM	Median	Range	95% CI ^a	
Control (n = 16)	7.12 \pm 0.72	6.5	2-13	5.59-8.66	
LY1 (n = 8)	9.62 \pm 1.05	10.0	4-13	7.14-12.11	0.04
LY5 (n = 8)	8.50 \pm 0.33	8.5	7-10	7.73-9.27	0.09
LY10 (n = 16)	9.75 \pm 0.75	10.5	5-13	8.15-11.35	0.03
LY20 (n = 16)	7.06 \pm 0.45	6.0	5-10	6.10-8.02	0.90
AmB (n = 8)	8.38 \pm 0.78	8.0	6-13	6.54-10.21	0.26

^a 95% CI, 95% confidence interval.

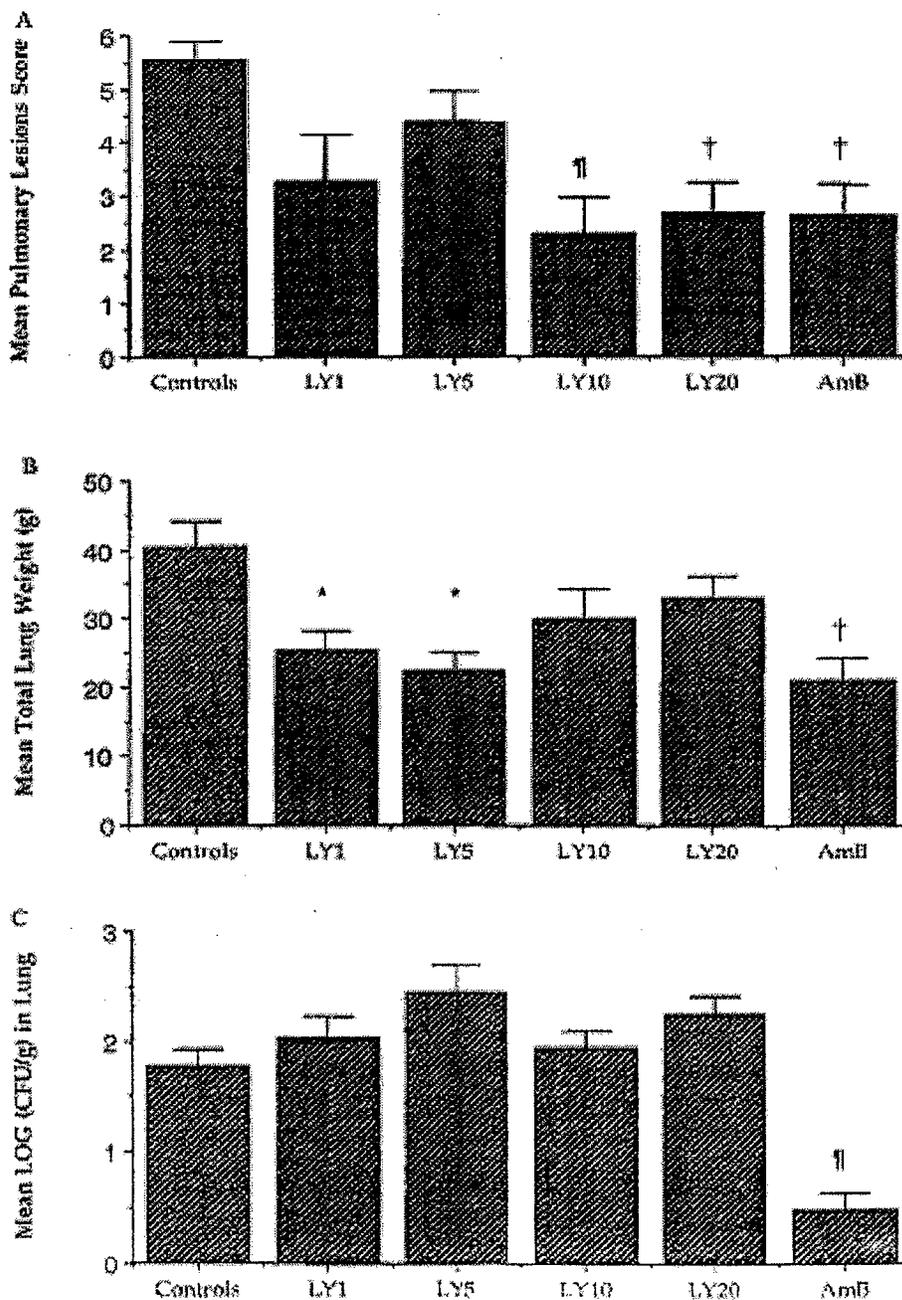
^b P values comparing survival to the value for the control group by Mann-Whitney U test.

* per Petraitis et al. 1998

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Figure 30: Response of Primary Pulmonary *Aspergillosis* in Persistently Neutropenic Rabbits to Antifungal Therapy Measured by Mean Pulmonary Hemorrhage Score (A), Mean Lung Weight (B), and Mean Pulmonary Tissue Concentration of Organism (C) in Untreated Controls and in Rabbits Treated with Anidulafungin (1, 5, 10, 20 mg/kg/day) and amphotericin B (1 mg/kg/day)



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Similar results were reported in another study by Roberts *et al.* (AAC, 44: 3381-3388, 2000) using immunosuppressed (single i.v. dose of cyclophosphamide/daily subcutaneous dose of triamcinolone) New Zealand White rabbits that were infected with $1-3 \times 10^6$ (lethal inoculum) or $1-3 \times 10^5$ conidia (sublethal inoculum) of *A. fumigatus*. Rabbits were given daily i.v. doses of anidulafungin (5 mg/kg/day or 10 mg/kg/day) or amphotericin B (1.0 mg/kg/day) for 6 days beginning on the day after infection. Surviving rabbits were euthanized 72 hours after the last dose of antifungal treatment. This treatment prevented death of infected rabbits over the 10-day duration of the study but did not eliminate *Aspergillus* organism from the organ tissues. In contrast, 3 of the 4 control animals died during the study. Semiquantitative cultures of kidney, liver, lung, and brain tissues showed an effect of anidulafungin on the appearance of colonies but not on the recovery of organisms from these tissues. Unlike amphotericin B, anidulafungin did not significantly reduce the tissue burden, except in the sublethally challenged rabbits treated with the 10 mg/kg/day dose (Table 41-42).

Table 41: Results of Organ Cultures with Intravenously Administered Anidulafungin begun 24 hours after Challenge

Inoculum	LY-303366 dose, in mg/kg (no. of rabbits cultured) ^a	Colony counts (mean log ₁₀ CFU/g of tissue ± SE)				No. of rabbits with positive cultures/no. cultured			
		Liver	Lung	Kidney	Brain	Liver	Lung	Kidney	Brain
Lethal	None (4)	3.36 ± 0.5	2.11 ± 0.04	2.78 ± 0.5	1.66 ± 0.5	4/4	4/4	4/4	4/4
	10 (8)	2.72 ± 0.4	2.64 ± 0.3	2.10 ± 0.5	1.10 ± 0.4	8/8	7/8	6/8	5/8
	5 (7)	2.30 ± 0.6	1.86 ± 0.4 ^d	1.60 ± 0.5	0.92 ± 0.4	7/7	5/7	4/7	3/7
	AmB 1.0 (3)	0.0 ^b	0.75 ± 0.6	0.26 ± 0.2 ^b	0.0	0/3 ^c	1/3	0/3 ^c	1/3
Sublethal	None (4)	3.25 ± 0.3	1.71 ± 0.5	1.88 ± 0.5	1.27 ± 0.7	4/4	3/4	2/4	3/4
	10 (7)	1.60 ± 0.5	1.42 ± 0.6	1.56 ± 0.4	0.90 ± 0.5	1/7 ^c	5/7	4/7	1/7
	5 (8)	2.10 ± 0.6	2.01 ± 0.5	1.69 ± 0.5	1.41 ± 0.3	5/8	6/8	5/8	2/8
	AmB 1.0 (3)	0.10 ± 0.08 ^b	0.20 ± 0.2	0.0 ^b	0.95 ± 0.4	0/3 ^c	1/3	1/3	0/3

^a AmB, amphotericin B.
^b P < 0.05 compared to controls (Wilcoxon rank sum test).
^c P < 0.05 compared to controls (Fisher's exact test).
^d P < 0.05 compared to LY-303366 at 10 mg/kg (Wilcoxon rank sum test).

Table 42: Results of Organ Cultures with Intravenously Administered Anidulafungin begun 48 hours prior to challenge

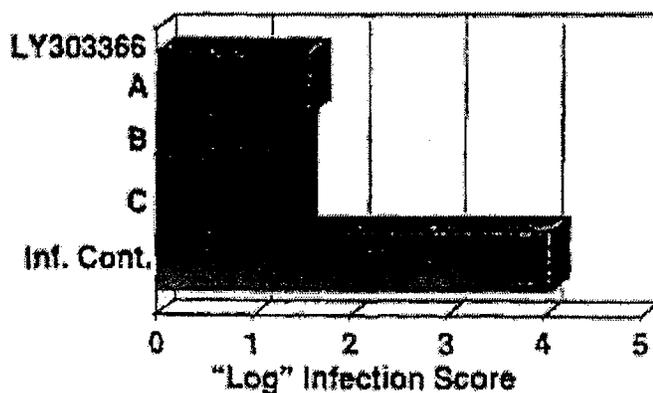
Inoculum	LY-303366 dose, in mg/kg (no. of rabbits cultured) ^a	Colony counts (mean log ₁₀ CFU/g of tissue ± SE)				No. of rabbits with positive cultures/no. cultured			
		Liver	Lung	Kidney	Brain	Liver	Lung	Kidney	Brain
Lethal	None (4)	2.99 ± 0.3	3.16 ± 0.2	3.10 ± 0.3	1.86 ± 0.8	4/4	4/4	4/4	3/4
	10 (6)	2.31 ± 0.5	2.84 ± 0.4	1.85 ± 0.6	0.54 ± 0.2	5/6	5/6	3/6	1/6 ^d
	5 (6)	3.18 ± 0.4	3.04 ± 0.4	2.38 ± 0.5	1.81 ± 0.5	6/6	6/6	6/6	5/6
	AmB 1.0 (3)	0.0 ^b	0.36 ± 0.2 ^b	1.31 ± 0.6	0.10 ± 0.08	0/3 ^c	0/3 ^c	1/3	0/3
Sublethal	None (4)	2.82 ± 0.5	1.58 ± 0.2	1.08 ± 0.2	1.01 ± 0.5	3/4	3/4	4/4	1/4
	10 (6)	1.92 ± 0.4	2.12 ± 0.5	1.00 ± 0.4	0.61 ± 0.3	5/6	4/6	5/6	2/6
	5 (6)	2.01 ± 0.6	1.81 ± 0.5	0.95 ± 0.4	0.56 ± 0.3	6/6	3/6	1/6 ^{c,e}	1/6
	AmB 1.0 (4)	0.08 ± 0.07 ^b	0.73 ± 0.3	0.42 ± 0.2	0.23 ± 0.1 ^b	1/4	1/4	2/4	1/4

^a AmB, amphotericin B.
^b P < 0.05 compared to controls (Wilcoxon rank sum test).
^c P < 0.05 compared to controls (Fisher's exact test).
^d P < 0.05 compared to LY-303366 at 5 mg/kg (Fisher's exact test).
^e P < 0.05 compared to LY-303366 at 10 mg/kg (Wilcoxon rank sum test).

3.4 *Pneumocystis carinii*

Activity of anidulafungin against *Pneumocystis carinii* was measured in immunosuppressed Lewis Rats (Boylan and Current, *Inf. Immunity*, **60**: 1589-1597, 1992). Rats were immunosuppressed by daily administration of methylprednisolone acetate and infected intratracheally with two inoculations of *P. carinii* (0.1 ml of S-DMEM containing 10^6 organisms administered 48 hours apart) and were assigned to control groups or to therapy or prophylaxis treatment groups. Animals used to screen drugs for prophylactic activity against *P. carinii* pneumonia started receiving therapy 24 hours after initial *P. carinii* inoculation and animals harboring a 2-week-old *P. carinii* infection were used to screen drugs for therapeutic activity. Both the therapeutic and prophylactic treatments were continued until week 5 of *P. carinii* infection. The efficacy of drugs against *P. carinii* pneumonia was determined by evaluating the lungs for severity of *P. carinii* infections at necropsy at week 5. The results in Figure 31 show that oral administration of anidulafungin 5 mg/kg once daily for 4 days reduced the number of cysts in the lungs of heavily infected, immunosuppressed rats by more than 99%. Prophylactic oral administration of 1 mg/kg twice daily for 4 weeks resulted in > 90% reduction in all life-cycle forms (data not shown) (Turner & Current, 1997, Chapter 10, Echinocandin Antifungal Agents, Lilly Research Laboratories, Eli Lilly and Company; Current *et al.*, 1993, Abstract # 358, Conference on AntimicroAgentsChem., New Orleans, LA).

Figure 31: Mean Infection Scores of *P. carinii* Cysts in Homogenates of Lung Tissues in Four-day Cyst Reduction Model



A= animals receiving a single 10mg/kg i.v. dose 4 days prior to necropsy

B= animals receiving a single 5 mg/kg i.v. dose 4 days prior and daily 5 mg/kg oral doses on days 1-4 prior to necropsy

C= animals receiving daily oral doses on days 1-4 prior to necropsy.

- Infected controls received no therapy for *P. carinii* pneumonia
- LY303366=anidulafungin

In another study by Bartlett *et al.*, 1998 (*Experimental Biology*, Abstract #4563), BALB/c mice were immunosuppressed with antibody directed to L3T4+ lymphocytes and transtracheally inoculated with

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10^6 *P. carinii* organisms after 14 days of antibody suppression. Infections were allowed to develop for 3 weeks, after which treatment was initiated. Mice were treated with anidulafungin i.p. for 6 weeks after infection. Of the mice treated with anidulafungin at 2.0 mg/kg/day, only one of 10 animals had any detectable organisms by Giemsa staining of lung impression smears. The authors have stated that in mice treated with 0.5 mg/kg/day of anidulafungin, low numbers of organisms were detected in 4 of 10 animals, while the other animals appeared sterile. However, the data were not available for review.

Bartlett, *et al.* (AAC, 40: 1811-1816, 1996), conducted studies using a dexamethasone-immunosuppressed rat model to determine the effect of anidulafungin on the ultrastructure of trophic forms of *P. carinii* in the lungs. After 1 week of immunosuppression, the rats were inoculated intratracheally with 10^6 *P. carinii* organisms. Infections were allowed to develop for 3 weeks. Eight heavily infected rats were then treated i.v. once daily with 2.5 mg/kg anidulafungin and another 8 heavily infected rats were not treated and served as controls. Two rats from the control group and two from the anidulafungin-treated group were sacrificed on days 1, 3, 5, and 7, and their lungs fixed and processed for morphologic observations by transmission electron microscopy. It is suggested that anidulafungin may interfere with the export of surface glycoprotein by the trophic forms of *P. carinii* based upon culture studies where after 4 days of exposure to anidulafungin, surface glycoprotein was associated with internalized tubular elements. Similar cytoarchitectural changes were observed in trophic forms of *P. carinii* in the lungs of the rats treated for 4-8 days with anidulafungin demonstrating that such cellular changes may not be just an artifact of culture. After 4 days of treatment with anidulafungin, cysts were practically eliminated from the lungs.

4. Drug Resistance

In vitro

A serial passage experiment was performed using a strain of *C. albicans* (A26) and the potential for development of resistance to anidulafungin was compared with fluconazole and amphotericin B. At each passage, MICs were determined using tube dilution methodology (macro-dilution). Anidulafungin and amphotericin B were tested in AM-3 media, and fluconazole in RPMI 1640. Incubation at each phase was for 48 hours at 35°C; initial inoculum was 5×10^4 total cells. At each passage, 0.05 mL of broth was transferred from the highest concentration that had growth to a new set of two-fold dilution tubes. This was repeated for 13 transfers over 26 days. The MICs at each passage shown in Figure 32 indicate no more than a 2 – fold increase of the anidulafungin MIC. However, the fluconazole MIC increased from 0.25 µg/mL to 16 µg/mL within 6 passages (12 days). The amphotericin MIC remained at 0.06 µg/mL. The results of this experiment indicate a lower potential for resistance development to anidulafungin or amphotericin than to fluconazole. However, the clinical significance of this *in vitro* finding is not known.

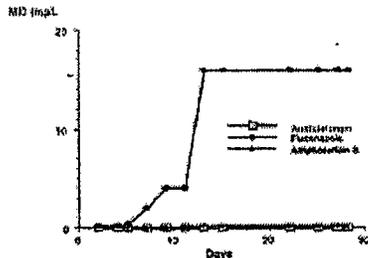


Figure 32: Development of antifungal resistance in *C. albicans* A26 upon serial passage

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In vivo

There were no studies conducted to measure the potential for development of resistance *in vivo*.

5. Cross-resistance

In vitro

Activity Against Fluconazole Resistant Yeast

A study by Espinel-Ingroff (J Clin Micro, 36 (10), 2950-2956, 1998) measured the activity of anidulafungin against fluconazole resistant strains of fungi. Table 7 demonstrates the activity of anidulafungin against fluconazole resistant strains of yeast included in the MSG studies and Table 5b shows the results against 10 fluconazole resistant isolates as defined by NCCLS criteria (Espinel-Ingroff, J Clin Micro, 36 (10), 2950-2956, 1998). The anidulafungin MICs were two 2 fold dilutions lower than caspofungin (0.25 µg/mL for anidulafungin and 1 µg/mL for caspofungin).

In another study, the activity of anidulafungin against 43 isolates of *Candida* spp. considered to be fluconazole resistant, isolated from HIV infected patients was examined (Chavez *et al.*, J Antimicrob Chemo, 44:697-700, 1999). These isolates were stated to have fluconazole MICs > 64 µg/mL. It is unclear from Table 9 as to how many isolates from each species were with a fluconazole MIC of >64 µg/mL. *Candida* spp. with fluconazole MICs >64 µg/mL exhibited low anidulafungin MICs.

Another Spanish study compared the activity of anidulafungin, against 156 fluconazole nonsusceptible (MIC ≥16 µg/mL) clinical isolates collected at 51 different hospitals between 1995 and 2000 (Cuenca-Estrella, *et al.*, J Antimicrob Chemo, 46; 475-477, 2000). Table 7 shows the MIC₅₀, MIC₉₀, and MIC range of the isolates in µg/mL. Against these 156 fluconazole nonsusceptible isolates, anidulafungin had low MICs for the test isolates of *C. albicans* (0.0002-0.015 µg/mL), *C. glabrata* (<0.0002-0.25 µg/mL), *C. krusei* (<0.0002-0.5 µg/mL) and *C. tropicalis* (<0.0002-0.12 µg/mL). There were too few isolates of *C. parapsilosis* (n=5) and *C. guilliermondii* (n=3) to comment.

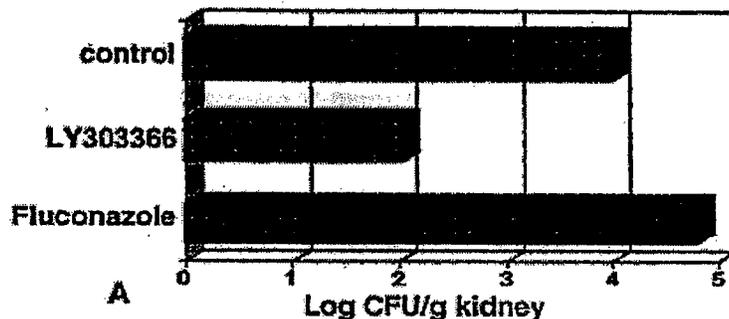
The clinical significance of these *in vitro* results is not known.

In vivo

Activity Against Fluconazole-Resistant *C. albicans*

Orally administered anidulafungin (16 mg/kg twice daily for 4 days) was effective in reducing the fungal burden in the kidneys of immunocompetent ICR mice that had been previously infected intravenously with 2×10^5 CFU of fluconazole-resistant *C. albicans* CA4 strain (Nonclinical Pharmacology Report WLC/TND 9603). A higher dose level (twice ED₅₀ for *C. albicans* A26) was used because anidulafungin is poorly absorbed in mice (<5% oral bioavailability). Treatment with fluconazole at twice the ED₅₀ for *C. albicans* A26 did not reduce the number of *C. albicans* recovered from the kidneys (Figure 33).

Figure 33: Efficacy of Anidulafungin against Fluconazole-Resistant *C. albicans* Infection in Mice



In another study, anidulafungin was effective in reducing fluconazole-resistant *C. albicans* in the tissue of immunosuppressed rabbits in a model of oropharyngeal and esophageal candidiasis (Tables 36-37).

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Activity against fluconazole-nonsusceptible *C. krusei*

Orally administered anidulafungin was effective in reducing the fungal burden in the kidneys of immunocompetent mice infected with fluconazole-nonsusceptible *C. krusei* (Figure 21).

Activity against amphotericin B-resistant *A. fumigatus*

Anidulafungin, at doses of ≥ 2.5 mg/kg/day within 18 hours after infection, increased the survival times and decreased the mycological burden (lungs and kidneys) of immunosuppressed mice infected with amphotericin B-susceptible and –refractory *A. fumigatus* isolates (Figures 24-25 and Table 38).

Activity against Itraconazole-resistant *A. fumigatus*

Anidulafungin, at doses of 0.5-1.0 mg/kg/day, was effective in prolonging the survival of immunosuppressed mice infected with itraconazole-susceptible and –resistant *A. fumigatus* isolates but was not effective at reducing the mycological burden in the spleen and kidneys (Figures 26-29).

6. Drug Combinations

6.1 Activity *in vitro* Against *Candida* spp.

In an ICAAC abstract by Karlowsky *et al.*, (1997, Toronto) 4 isolates each of *C. albicans*, *C. glabrata*, and *C. tropicalis* in addition to 2 isolates of *C. krusei* were tested against anidulafungin in combination with amphotericin B, 5FC, fluconazole, itraconazole and ketoconazole using the macrodilution checkerboard method. The authors stated that the activity of anidulafungin was additive or indifferent when combined with other antifungal agents against all isolates except 1 isolate of *C. glabrata* which demonstrated synergy with itraconazole and 4 isolates of *C. tropicalis* which demonstrated antagonism with ketoconazole. Complete methods and raw data were not included in the abstract for an independent review.

Roling *et al.* (Diag Micro and Inf Dis, **43**: 13-17, 2002) in their report measured the activity of caspofungin and anidulafungin alone and in combination with fluconazole at different time intervals. Two isolates each of *C. albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis* and *C. neoformans* were included. NCCLS M27A microdilution methods were used for susceptibility testing (RPMI 1640, starting inoculum $0.5 \times 10^3 - 2.5 \times 10^3$ CFU/mL, 35° C, 48 hours for *Candida* spp., 72 hours for *C. neoformans*). Endpoints for all agents were interpreted as 80% reduction in growth. Starting inoculum for time kill procedures was $1 \times 10^5 - 5 \times 10^5$ CFU/mL. Drug concentrations tested: 20 µg/mL fluconazole + 2 µg/mL of either caspofungin and anidulafungin; 0.5 µg/mL fluconazole + 0.007 µg/mL of either caspofungin and anidulafungin. At predetermined time points (0, 2, 4, 8, 12 and 24 hours) following addition of antifungal, 0.1 mL sample was removed and serially diluted. Colony counts were determined after incubation at 35° C for 24-48 hours. Cidal activity was defined as $>3 \log_{10}$ (99.9%) reduction in growth from starting inoculum. Synergy was defined as $>2 \log_{10}$ CFU/mL increase in activity produced by the combination as compared to the most active agent alone. Antagonism was defined as a decrease in activity $>2 \log_{10}$ CFU/mL of the combination compared with the most active agent alone. Indifference was defined as $<2 \log_{10}$ CFU/mL increase or decrease in activity of the combination compared with the

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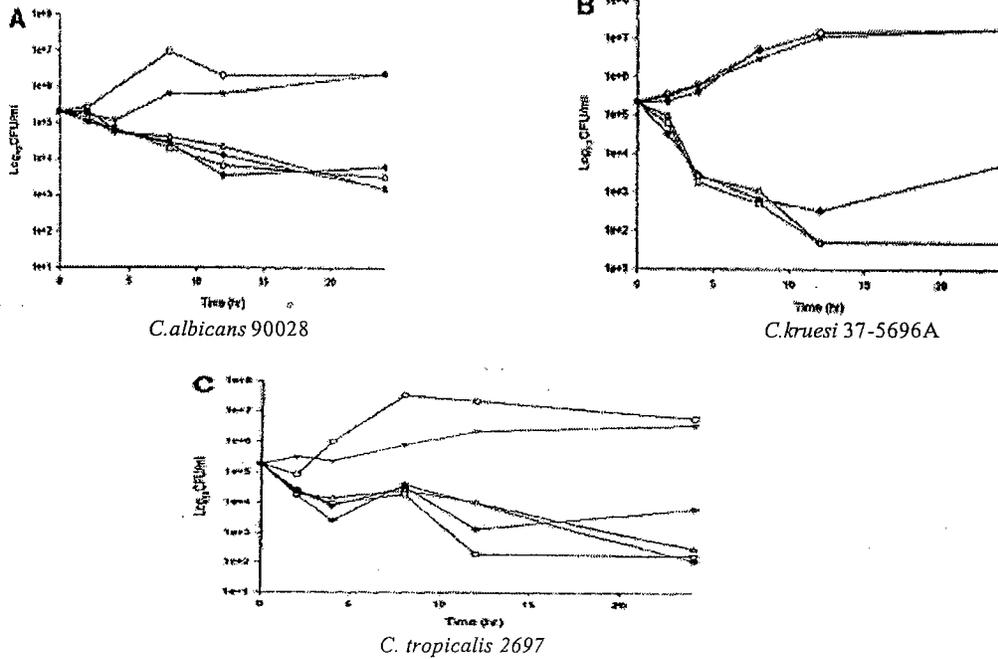
activity of the most active agent alone. Table 43 shows the MIC data for the individual strain, Figure 34 the time kill curves. In the time kill experiments, combinations of anidulafungin and fluconazole resulted in indifference against the *Candida* spp. The echinocandins alone showed little antifungal activity against the isolates of *C. neoformans*. Combinations of fluconazole and echinocandins showed no improvement of activity over fluconazole alone against *C. neoformans*.

Table 43: MIC data for each strain

Microorganism	Fluconazole (µg/ml)	Anidulafungin (µg/ml)	Caspofungin (µg/ml)
<i>C. albicans</i>			
90028	0.5	0.015	0.03
0Y31.5	0.25	0.015	0.03
<i>C. glabrata</i>			
350	>128	0.06	0.03
582	4	0.045	0.03
<i>C. krusei</i>			
37-5696A	64	0.12	0.25
6238	64	0.12	0.25
<i>C. tropicalis</i>			
2697	>128	0.03	0.03
3829	0.5	0.05	0.03
<i>C. neoformans</i>			
887.002	8	>2	>2
1041.027	2	>2	>2

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Figure 34: o control, ▽ flucon20, □ anid2, ● caspo2, △ flucon20+anid2, ● flucon20+casp2



6.2 Activity against other fungal species

In a study by Rex *et al.* (ICAAC abstract M1816, 2002), the *in vitro* activity of anidulafungin and micafungin in combination with amphotericin B against *Aspergillus* spp. and *Fusarium* spp. was measured against 29 isolates of *Aspergillus* spp. and 10 isolates of *Fusarium* spp. NCCLS M38 microdilution methods were utilized in a checkerboard manner. Combinations were tested in RPMI 1640 with 2% glucose and in AM3 medium with 2% glucose. Plates were read at 24, 48, and 72 hours. Complete methods were not described for an independent review. The authors state that the combination of anidulafungin and micafungin with amphotericin B exhibited synergistic to indifferent results against both species regardless of the media used. There was no antagonism observed.

In the reports reviewed there was no *in vitro* antagonism observed between anidulafungin and other systemic antifungals. These data suggest that combination therapy with anidulafungin and other systemic antifungal agents may be useful. However, the clinical significance of such an effect has not been measured.

In Vivo

Candida spp

There were no studies conducted to measure the activity of anidulafungin in combination with other antifungal agents *in vivo*.

Aspergillus fumigatus

In the study by Graybill and Najvar, 1999 (Eli Lilly, Inc. Supported Studies, Σ J), effect of a combination of amphotericin B and anidulafungin against itraconazole-susceptible and -resistant isolate of *Aspergillus fumigatus* was evaluated. Anidulafungin alone was effective at prolonging survival at the 1 mg/kg dose and amphotericin B at the 1.5 mg/kg dose against itraconazole-susceptible strain (Figure 28). However, anidulafungin did not have any effect on the tissue burden in the kidneys or spleen of the itraconazole susceptible and resistant *A. fumigatus* strains (Figures 26-27). The combination of anidulafungin and amphotericin B had no additive or antagonistic effect on fungal burden. Additional studies were done at a lower infective dose of 3×10^5 CFU/mouse which showed similar effectiveness of therapy compared with controls. Figure 29 demonstrates that the combination of amphotericin B and anidulafungin against itraconazole resistant *A. fumigatus* prolonged survival.

IV. CLINICAL MICROBIOLOGY

The sponsor has conducted 4 clinical trials in patients with *Candida* infections. VER002-4 for esophageal candidiasis (EC), XBAF, a supportive study in EC, VER002-6 for invasive candidiasis (IC), and VER002-11 for fluconazole refractory mucosal candidiasis (currently ongoing).

1. Esophageal Candidiasis

The safety and efficacy of anidulafungin for the treatment of EC in patients >18 years of age was examined in a pivotal phase 3 multinational clinical trial (VER002-4). The study was conducted at sites in the United States (including Puerto Rico), South Africa, Argentina and Thailand. Patients with proven EC (endoscopy positive and smear positive for yeast) were enrolled. Patients were randomly assigned to receive 14-21 days of anidulafungin (100 mg loading dose/50 mg/day maintenance dose plus oral placebo) or oral fluconazole (200 mg loading dose/100 mg maintenance dose plus IV placebo). Endoscopic, clinical and microbiological assessments were determined at baseline, end of therapy (EOT) and at the sign of clinical recurrence or at the follow up visit (two weeks after EOT). Microbiological assessment was based on cultures of endoscopic lesions. Exclusions included evidence of systemic fungal infection (except for study indication), esophageal biopsy with HSV or CMV, or systemic antifungal therapy one week prior to enrollment. Please refer to the Medical Officer's review for other study details.

The primary evaluation endpoint was endoscopic response at EOT in the clinically evaluable population.

Clinical responses at EOT were defined as:

Cure: Absence of symptoms, additional systemic antifungal treatment not required for treatment of study condition

Improvement: Less severe symptoms compared to baseline evaluation, additional systemic antifungal therapy not required for treatment of study condition

Failure: No significant improvement in symptoms or additional systemic antifungal therapy required

Indeterminate: Evaluation not able to be made

Mycologic responses at EOT were defined as follows:

Proven eradication: Culture negative for *Candida* spp. present at baseline

Presumed eradication: Culture results not available, patient had no visible lesions on endoscopy

Proven persistence: Baseline *Candida* spp. present with no previous eradication

Presumed persistence: Culture results not available, patient has visible lesions on endoscopy

Unable to determine: No culture result, endoscopic result is indeterminate

Patients with a new yeast species identified at EOT:

Superinfection: New *Candida* spp. identified at EOT, visible lesions on endoscopy

Colonization: New *Candida* spp. at EOT, no visible lesions on endoscopy

Success: Baseline *Candida* spp. eradicated or presumed eradicated, no superinfection or colonization

Failure: Proven or presumed persistence or not able to determine for one or more of the baseline *Candida* spp., or superinfection

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A total of 601 patients with EC (proven or probable) were enrolled in this study. Of these, 448 patients completed the study, 442 were in the mycological intent to treat (MITT) group of which 366 patients were microbiologically evaluable (ME) at EOT. Table 44 shows a summary of the patient populations throughout the study. Some of the patients shown to have both anidulafungin and fluconazole in their plasma were excluded for the purpose of this review. The anidulafungin treatment arm contained 180 patients, fluconazole arm had 186 patients that were ME at EOT.

Table 44: Summary of Patient Populations

Population	Anidulafungin	Fluconazole	Total
ITT	300	301	601
Clinically Evaluable at EOT ^a	249	255	504
Clinically Evaluable at FU	233	229	462
Microbiological ITT ^a	219	223	442
Microbiologically Evaluable at EOT ^{bc}	180	186	366
Microbiologically Evaluable at FU ^b	168	167	335

^aPatient population that had a baseline *Candida* isolate and received at least one dose of study drug. ^bMicrobiologically evaluable patients are both MITT and clinically evaluable. ^cPrimary evaluation timepoint

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The majority of patients in the ME group were infected with a single yeast [95% of the anidulafungin patients (171 out of 180 patients) and 93.5% (174 out of 186 patients) in the fluconazole group]. The per patient mycologic response in the ME population data showed that the clinical cure at the EOT was the same in both groups; whereas at FU failures were higher in the anidulafungin arm than in the fluconazole arm (Tables 45 and 46). A majority of the patients in both the anidulafungin and fluconazole groups were infected with *C. albicans*. There were very few patients with pathogens other than *C. albicans* in the study groups (11 in anidulafungin arm, 16 in fluconazole group). It is of note that the 2 cases of *C. krusei* in the fluconazole arm were proven eradication and the single case in the anidulafungin arm was proven persistence thereby suggesting lack of activity against *C. krusei*. The mycologic response was comparable in both arms. There was no correlation between the endoscopic responses at EOT in the ME population and the anidulafungin or fluconazole MICs. There were few patients with fluconazole non-susceptible baseline isolates (2 *C. albicans*, 3 *C. krusei* and 5 *C. glabrata* that were S-DD) in both arms. It is of note that patients with the isolates showing higher fluconazole MICs did not fail fluconazole therapy. At FU (14 days post treatment), 74/168 (44%) patients in the anidulafungin arm were clinically cured; whereas 135/177 (76.3%) patients in the fluconazole arm were cured. Most superinfections in this group were due to a *Candida* isolate that was not further speciated (*Candida* spp.), while the majority of clinical failures were due to recurrence of a baseline isolate that had previously been eradicated. Here again, there was no correlation between clinical outcome and anidulafungin or fluconazole MICs.

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Table 45: Anidulafungin Clinical and Mycological Response at EOT¹ (180 patients)

Baseline Yeast	Clinical Success – EOT		Clinical Failure – EOT
	ME (MR) *	PE (MR)	P (MR)
<i>C. albicans</i> (169)	145 (86.3%) ¹	2 (1.2%)	22 (13.1%)
<i>C. glabrata</i> (1)	-	-	1 (100%)
<i>Candida</i> spp.(1)	1 (100%)	-	-
<i>C. albicans</i> + <i>C. krusei</i> (1)	-	-	1 (100%)
<i>C. albicans</i> + <i>C. glabrata</i> (7)	6 (85.7%)	1 (14.3%)	-
<i>C. albicans</i> + <i>C. tropicalis</i> (1)	1 (100%)	-	-
Total (180)	156/180 (86.7%)		24/180 (13.3%)

() = number of patients *(MR) = mycologic response ME = proven mycologic eradication

PE = presumed mycologic eradication P = proven persistence

¹ = one patient colonized with *C. glabrata*, one patient superinfection with *Candida* spp.(not included in tabulation)

Table 46 : Fluconazole Clinical and Mycological Response at EOT (186 patients)

Baseline Yeast	Clinical Success – EOT ¹		Clinical Failure – EOT ²	
	ME (MR)*	PE (MR)	P (MR)	PP
<i>C. albicans</i> (170)	145 (85.3%) ¹	4 (2.4%)	9 (5.3%)	1 (0.6%)
<i>C. glabrata</i> (2)	1 (50%)	-	1 (50%)	-
<i>Candida</i> spp. (2)	2 (100%)	-	-	-
<i>C. albicans</i> + <i>C. krusei</i> (2)	2 (100%)	-	-	-
<i>C. albicans</i> + <i>C. glabrata</i> (9)	8 (88.9%)	1 (11.1%)	-	-
<i>C. albicans</i> + <i>C. tropicalis</i> (1)	1 (100%)	-	-	-
Total (186)	163/186 (87.6%) (8 patients not included – see ¹)		11/186 (5.9%) (4 patients not included – see ²)	

() = number of patients (MR)* = mycologic response ME = proven mycologic eradication

PE = presumed mycologic eradication P = proven persistence PP = presumed persistence

¹ one patient colonized with *C. tropicalis*, one patient colonized with *C. parapsilosis*, 4 patients colonized with *C. glabrata*, one patient superinfected with *C. krusei*, one patient superinfected with *C. glabrata* (not included in tabulation).

² one patient ME with *C. krusei* superinfected but failed clinically. Three patients ME with *C. glabrata* superinfected but failed clinically (not included in tabulation). 11 patients not included in tabulations

The sponsor had collected multiple isolates from patients enrolled in the clinical trial at different sites. The results in Table 49 show the number and per cent of different *Candida* spp. isolated at baseline for each of the treatment groups and populations. *C. albicans* was the predominating organism regardless of population or treatment arm. The majority of clinical isolates came from South Africa (Table 50). Table 51 lists the MICs against baseline isolates from the MITT population against a panel of antifungal drugs. Anidulafungin and caspofungin were tested both in RPMI 1640 broth and in AM3 broth. MICs in AM3 media generally were lower than in RPMI. There was no correlation of MIC with clinical or mycologic outcome.

Table 47: Anidulafungin Clinical and Mycological Response at FU (168 patients)

Yeast	Clinical Success - FU			Clinical Failure - FU			
	ME (MR)*	PE (MR)	C (MR)	P (MR) (MR)	PP (MR)	PR(MR)	S
<i>C. albicans</i> ¹ (154)**	72 (46.8%) (0.6%)	2 (1.3%)	1	10 (6.5%)	4 (2.6%)	66 (42.9%)	-
<i>Candida</i> spp. (11)**	-	-	1 (9%)	- (100%)	-	-	11
<i>C. krusei</i> (1)	-	-	-	- (100%)	-	-	1
<i>C. albicans</i> + <i>C. glabrata</i> (1)	-	-	-	-	-	1 (100%)	-
<i>C. albicans</i> + <i>C. pelliculosa</i> (1)	-	-	-	-	-	1 (100%)	-
Total (168)	74/168 (44.0%)**			94/168 (56%)			

() = number of patients ¹isolates unable to determine (ITT only) * = mycologic response ME = proven mycologic eradication PE = presumed mycologic eradication C = colonization P = proven persistence PP = presumed persistence PR = proven recurrence S = superinfection ** does not include colonization numbers

Table 48: Fluconazole Clinical and Mycological Response at FU (167 patients)

Yeast	Clinical Success - FU		Clinical Failure - FU	
	ME (MR)*	PE (MR)	P (MR)	PR(MR)
<i>C. albicans</i> (163)	123 (75.5%)	2 (1.2%)	6 (3.7%)	32 (19.6%)
<i>Candida</i> spp. (2)	2 (100%)	-	-	-
<i>C. krusei</i> (2)	1 (50%)	-	-	1 (50%)
<i>C. glabrata</i> (10)	7 (70%)	-	-	3 (30%)
Total (177)	135/177 (76.3%)		42/177 (24%)	

() = number of patients * = mycologic response ME = proven mycologic eradication PE = presumed mycologic eradication P = proven persistence PR = proven recurrence

Table 49: Number and % of different *Candida* spp. at baseline

Population	Species	Treatment Arm		
		Anidulafungin	Fluconazole	Total
Microbiological ITT population n (%)	Total isolates	229	237	466
	<i>C. albicans</i>	212 (92.6)	213 (89.9)	425 (91.2)
	<i>C. glabrata</i>	11 (4.8)	13 (5.5)	24 (5.2)
	<i>C. krusei</i>	1 (0.4)	2 (0.8)	3 (0.6)
	<i>C. lusitanae</i>	0	1 (0.4)	1 (0.2)
	<i>C. pelliculosa</i>	0	1 (0.4)	1 (0.2)
	<i>C. tropicalis</i>	1 (0.4)	2 (0.8)	3 (0.6)
	<i>Candida</i> sp.	4 (1.7)	5 (2.1)	9 (1.9)
Microbiologically evaluable at end of therapy population n (%)	Total isolates	189	198	387
	<i>C. albicans</i>	177 (93.7)	182 (91.9)	359 (92.8)
	<i>C. glabrata</i>	8 (4.2)	11 (5.6)	19 (4.9)
	<i>C. krusei</i>	1 (0.5)	2 (1.0)	3 (0.8)
	<i>C. tropicalis</i>	1 (0.5)	1 (0.5)	2 (0.5)
	<i>Candida</i> sp.	2 (1.1)	2 (1.0)	4 (1.0)
Microbiologically evaluable at follow-up population n (%)	Total isolates	177	178	355
	<i>C. albicans</i>	165 (93.2)	163 (91.6)	328 (92.4)
	<i>C. glabrata</i>	8 (4.5)	10 (5.6)	18 (5.1)
	<i>C. krusei</i>	1 (0.6)	2 (1.1)	3 (0.8)
	<i>C. tropicalis</i>	1 (0.6)	1 (0.6)	2 (0.6)
	<i>Candida</i> sp.	2 (1.1)	2 (1.1)	4 (1.1)

Isolates indicated as "*Candida* sp." were not identified to species level.

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Table 50: Anidulafungin activity (by country) against baseline pathogens (MITT)

Country	Species*	N	MIC (mg/L)		
			MIC ₅₀	MIC ₉₀	Range
Argentina	<i>Candida albicans</i>	43	0.12	0.5	0.015 - 2
	<i>Candida glabrata</i>	1			0.25
	<i>Candida krusei</i>	3			0.25 - 1
	<i>Candida pelliculosa</i>	1			0.015
	Total	48	0.25	0.5	0.015 - 2
Puerto Rico	<i>Candida albicans</i>	2			0.06 - 0.12
	Total	2			0.06 - 0.12
South Africa	<i>Candida albicans</i>	297	0.12	0.5	0.015 - 1
	<i>Candida glabrata</i>	12	0.25	0.25	0.06 - 0.25
	<i>Candida lusitanae</i>	1			1
	<i>Candida tropicalis</i>	1			0.12
	Total	311	0.12	0.25	0.015 - 1
Thailand	<i>Candida albicans</i>	79	0.25	0.5	0.015 - 1
	<i>Candida glabrata</i>	11	0.25	0.25	0.12 - 0.5
	<i>Candida tropicalis</i>	2			0.12
	Total	92	0.25	0.5	0.015 - 1
United States	<i>Candida albicans</i>	3			0.12 - 0.25
	Total	3			0.12 - 0.25
VER002-J Total	<i>Candida spp.</i>	456	0.12	0.5	0.015 - 2

* Only isolates that were received at the central reference laboratory and had MIC determinations are included.

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Table 51: Baseline Isolate MICs - MITT

	MIC of Various Anti-Fungal Agents for Baseline Isolates From Mycological Intent-to-Treat Population					
	Anidulafungin Arm		Fluconazole Arm		Total	
	MIC ₅₀	MIC range	MIC ₅₀	MIC range	MIC ₅₀	MIC range
All species		229		237		466
Anidulafungin RPMI	0.25	0.015, 1	0.5	0.015, 2	0.5	0.015, 2
Anidulafungin AM-3	0.0035	0.0035, 0.015	0.007	0.0035, 0.03	0.007	0.0035, 0.03
Caspofungin RPMI	0.25	0.03, 1	0.5	0.015, 2	0.5	0.015, 2
Caspofungin AM-3	0.06	0.007, 0.12	0.06	0.007, 0.25	0.06	0.007, 0.25
Fluconazole	0.5	0.12, 256	1.0	0.12, 128	0.5	0.12, 256
Itraconazole	0.12	0.015, 16	0.25	0.015, 2	0.12	0.015, 16
Voriconazole	0.03	0.007, 4	0.06	0.007, 1	0.03	0.007, 4
Ketoconazole	0.03	0.007, 2	0.06	0.007, 8	0.03	0.007, 8
Amphotericin B	1	0.25, 2	1	0.5, 2	1	0.25, 2
5-Fluorocytosine	1	0.06, 128	1	0.06, 128	1	0.06, 128
<i>C. albicans</i>		212		213		425
Anidulafungin RPMI	0.25	0.015, 1	0.5	0.015, 2	0.5	0.015, 2
Anidulafungin AM-3	0.0035	0.0035, 0.015	0.0035	0.0035, 0.03	0.0035	0.0035, 0.03
Caspofungin RPMI	0.25	0.03, 1	0.5	0.015, 2	0.5	0.015, 2
Caspofungin AM-3	0.06	0.007, 0.12	0.06	0.007, 0.25	0.06	0.007, 0.25
Fluconazole	0.5	0.12, 8	0.25	0.12, 128	0.5	0.12, 128
Itraconazole	0.12	0.015, 0.5	0.12	0.015, 2	0.12	0.015, 2
Voriconazole	0.015	0.007, 0.25	0.015	0.007, 1	0.015	0.007, 1
Ketoconazole	0.03	0.007, 0.5	0.03	0.007, 8	0.03	0.007, 8
Amphotericin B	1	0.25, 2	1	0.5, 2	1	0.25, 2
5-Fluorocytosine	1	0.06, 128	1	0.06, 128	1	0.06, 128

Data Source: Table 2.7

Note: MIC values are in mg/L, all values are presented from the 48-hour reading. Endpoints were MIC-0 (complete inhibition) for anidulafungin, caspofungin and amphotericin B, MIC-2 (50% inhibition) for the azoles and 5-fluorocytosine.

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In another clinical trial (XBAF), the efficacy of anidulafungin was measured in a phase 2 dose ranging study in patients with EC. Thirty six patients were enrolled; 33 of which had AIDS (19 patients in the anidulafungin 50mg loading dose followed by 25mg once daily for 14 – 21 days, and 17 patients in the 70mg/35mg dose group). Mycologic response was determined using culture results and endoscopic grade. Please refer to the Medical Officer's review for more details. Twenty seven patients were ME at EOT. Table 52 shows the clinical response data by pathogen. The treatment groups had overall similar clinical success rates. Here again, a majority of the patients had *C. albicans* infections.

Table 52: Clinical Trial XBAF Clinical response by pathogen

Yeast	Clinical Success – EOT Mycologic*		Clinical Failure – EOT Mycologic*	
	50mg/25mg	70mg/35mg	50mg/25mg	70mg/35mg
<i>C. albicans</i> n=31 (17/14) ¹	7 (41.2%) *C=1,P=1,E=4, PE=1	7 (50%) *C=4,E=2,PE=1	10 (58.8%) *C=4,I=1,P=5	7(50%) * P=1,PE=1,C=2, I=3
<i>Candida</i> spp. n=1 (1/0)	-	-	1(100%) *P=1	-
<i>T. beigelii</i> n=1	-	-	-	1(100%) *E=1
<i>C. glabrata</i> + <i>C.albicans</i> n=2 (1/1)	-	-	1 (50%) *PE=1	1 (50%) *I=1
No pathogen isolated (0/1)	-	-	-	1(100%) *I =1
Total (19/17)	7 (36.8%)	7 (41.2%)	12 (63.2%)	10 (58.8%)

(¹) = number of patients/dose group in the 50mg. arm/70mg. arm

Clinical Success = improvement, cure Clinical failure = failure, indeterminate

n= number of isolates

*E=eradication, P=persistence, I=indeterminate, C=colonization, PE=presumed eradication

2. Invasive Candidiasis/Candidemia

Clinical Trial VER002-6 was a supportive phase 2 dose ranging study of the efficacy of three different doses of anidulafungin (100/50 mg [loading dose/maintenance dose], 150/75 mg or 200/100 mg of anidulafungin per day) in the treatment of invasive candidiasis. There was no comparator drug used. Twenty four study sites from the US were included. Patients were given IV anidulafungin for up to 42 days. To be included in the study patients must have had culture or microscopic evidence of *Candida* spp., documented candidemia or culture of another normally sterile site. One hundred and twenty patients were enrolled (40 patients per group); 83 patients were evaluable at EOT, 68 patients (55%) were evaluable at follow up (the primary efficacy time point). Clinical and microbiological outcomes were determined at baseline, EOT, FU (2 weeks after EOT or earlier if relapse or another systemic fungal agent was initiated), or as clinically indicated. In the MITT group of 116 patients (4 of the 120 enrolled patients were excluded due to no baseline pathogen recovered) 94% had candidemia, 10% had positive cultures from tissue and 4% had positive cultures from both blood and tissue. Table 53 shows a summary of the patient populations. Microbiological evaluation was based on culture of one or more normally sterile sites per patient. All clinical isolates were sent to the reference laboratory at the [] for identification to species level and for *in vitro* susceptibility testing by the NCCLS M27A broth microdilution methods. Patients that were enrolled on the basis of

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a positive yeast culture that was subsequently identified as organisms other than *Candida* spp. were withdrawn from the study. The primary endpoint was response at FU in the evaluable population (combining clinical and microbiological outcomes). Microbiological response was determined by the presence or absence of *Candida* spp. Microbiological success: eradication, presumed eradication (culture results not available however clinical response was success). Please refer to Medical Officer's review for more details.

Microbiological responses are shown in Tables 54 and 55. The majority of the patients at EOT were presumed success while at FU most successes were proven (blood cultures were required at FU). Clinical success rates did not correlate with MICs for all species.

Table 53: Summary of Patient Populations

Population	Anidulafungin dose (loading dose/ daily dose)			Total
	100 mg/ 50 mg	150 mg/ 75 mg	200 mg/ 100 mg	
	ITT	40	40	
MITT	37	40	39	116
Evaluable at EOT	25	30	28	83
Evaluable at Follow-up*	18	26	24	68

* Primary efficacy time point

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Table 54: Per Patient Microbiological Responses

Analysis Population	100 mg/50 mg	150 mg/75 mg	200 mg/100 mg	Total
MICROBIOLOGICAL ITT POPULATION AT END-OF-THERAPY				
Success (n, %)	[N=37] 28 (75.7)	[N=40] 32 (80.0)	[N=39] 32 (82.1)	[N=116] 92 (79.3)
Proven Eradication (n, %)	7 (18.9)	5 (12.5)	12 (30.8)	24 (20.1)
Presumed Eradication (n, %)	21 (56.0)	27 (67.5)	20 (51.3)	68 (58.2)
Failure (n, %)	9 (24.3)	8 (20.0)	7 (17.9)	24 (20.7)
Proven Persistence (n, %)	2 (5.4)	0	1 (2.6)	3 (2.7)
Presumed Persistence (n, %)	2 (5.4)	1 (2.5)	3 (7.7)	6 (5.2)
Superinfection (n, %)	1 (2.7)	1 (2.5)	0	2 (1.7)
Unable to Determine (n, %)	5 (13.5)	6 (15.0)	3 (7.7)	14 (12.1)
MICROBIOLOGICAL ITT POPULATION AT FOLLOW-UP				
Success (n, %)	[N=37] 23 (62.2)	[N=40] 27 (67.5)	[N=39] 23 (59.0)	[N=116] 73 (61.2)
Proven Eradication (n, %)	15 (40.5)	24 (60.0)	18 (46.2)	57 (48.7)
Presumed Eradication (n, %)	8 (21.6)	3 (7.5)	5 (12.8)	16 (13.5)
Failure (n, %)	12 (32.2)	13 (32.5)	16 (41.0)	41 (35.0)
Proven Persistence (n, %)	1 (2.7)	0	1 (2.6)	2 (1.7)
Presumed Persistence (n, %)	2 (5.4)	1 (2.5)	3 (7.7)	6 (5.2)
Proven Recurrence (n, %)	0	1 (2.5)	0	1 (0.9)
Superinfection (n, %)	1 (2.7)	1 (2.5)	0	2 (1.7)
Unable to Determine (n, %)	12 (32.4)	10 (25.0)	12 (30.8)	34 (29.3)
EVALUABLE AT END OF THERAPY POPULATION				
Success (n, %)	[N=25] 21 (84.0)	[N=30] 28 (93.3)	[N=26] 25 (96.2)	[N=81] 74 (91.4)
Proven Eradication (n, %)	3 (12.0)	7 (23.3)	7 (26.9)	17 (20.9)
Presumed Eradication (n, %)	18 (72.0)	21 (70.0)	18 (68.3)	57 (69.5)
Failure (n, %)	4 (16.0)	2 (6.7)	1 (3.8)	7 (8.6)
Proven Persistence (n, %)	1 (4.0)	0	1 (3.8)	2 (2.4)
Presumed Persistence (n, %)	2 (8.0)	1 (3.3)	2 (7.7)	5 (6.0)
Superinfection (n, %)	1 (4.0)	1 (3.3)	0	2 (2.4)
EVALUABLE AT FOLLOW-UP POPULATION				
Success (n, %)	[N=19] 14 (73.7)	[N=20] 15 (75.0)	[N=24] 18 (75.0)	[N=63] 47 (74.6)
Proven Eradication (n, %)	11 (57.9)	12 (60.0)	13 (54.2)	36 (57.0)
Presumed Eradication (n, %)	3 (15.8)	3 (15.0)	5 (20.8)	11 (17.2)
Failure (n, %)	4 (21.1)	4 (20.0)	6 (25.0)	14 (22.2)
Proven Persistence (n, %)	1 (5.3)	0	1 (4.2)	2 (3.2)
Presumed Persistence (n, %)	2 (10.5)	1 (5.0)	2 (8.3)	5 (7.9)
Proven Recurrence (n, %)	0	1 (5.0)	0	1 (1.6)
Superinfection (n, %)	1 (5.3)	1 (5.0)	0	2 (3.2)
Unable to Determine (n, %)	0	1 (5.0)	0	1 (1.6)

n = Number of patients with response data; N = Number of patients in the dose group and population.
Note: Patients with episodes of persistence at EOT were presumed persistent at follow-up if no data at follow-up.

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Most of the patients had a single baseline *Candida* spp. Of the MITT population at baseline, 9 patients had mixed infections due to 2 *Candida* spp. and 1 patient had 3 different species. In the ME population of 68 patients at FU, 63 of the 68 patients had a single baseline *Candida* spp. and 5 patients had mixed infection with 2 *Candida* spp. *Candida albicans* was the predominating pathogen, followed by *C. glabrata*. In the ME group at FU, six different species were isolated at baseline: *Candida albicans* (n=34), *C. glabrata* (n=21), *C. krusei* (n=4), *C. parapsilosis* (n=7), *C. tropicalis* (n=6), *C. dubliniensis* (n=1). Since some patients had multiple isolates, the total patients with different *Candida* species in this population was 73 (39 isolates or 53% were species other than *C. albicans*). Non-*albicans* yeast were isolated from 57% of the evaluable patients at FU; 50% were either infected or co-infected with *C. albicans*.

The success rates for *C. albicans*, the most frequently isolated organism, and *C. glabrata*, the second most common isolate, were similar among the 3 treatment groups at EOT and FU (Table 55). Numbers for other organisms were small. Because cultures were required only if clinically indicated at EOT, most successes were presumed rather than proven. There was no relationship between clinical success at FU and the anidulafungin MIC.

Table 55: Clinical response at EOT and FU by organism

EVALUABLE AT END OF THERAPY POPULATION, END OF THERAPY VISIT					
Baseline Species	Outcome	100 mg/50 mg	150 mg/75 mg	200 mg/100 mg	Total
All species		[N=29]	[N=31]	[N=31]	[N=91]
	Success (n, %)	24 (82.7)	29 (93.5)	28 (90.3)	81 (89.4)
	Proven Eradication (n, %)	4 (14.3)	7 (22.6)	8 (25.8)	19 (21.1)
	Presumed Eradication (n, %)	20 (71.4)	22 (71.0)	20 (64.5)	62 (68.3)
	Failure (n, %)	4 (14.3)	2 (6.5)	3 (9.7)	9 (10.0)
<i>Candida albicans</i>		[N=29]	[N=31]	[N=31]	[N=91]
	Success (n, %)	13 (100.0)	14 (45.2)	14 (45.2)	37 (40.7)
	Proven Eradication (n, %)	2 (20.0)	4 (28.6)	4 (28.6)	8 (8.8)
	Presumed Eradication (n, %)	9 (80.0)	9 (64.3)	10 (70.4)	29 (31.9)
	Failure (n, %)	0	1 (7.1)	3 (9.7)	4 (4.4)
<i>Candida dubliniensis</i>		[N=2]	[N=0]	[N=0]	[N=2]
	Success (n, %)	1 (100.0)	0	0	1 (100.0)
	Proven Eradication (n, %)	1 (100.0)	0	0	1 (100.0)
	Failure (n, %)	0	0	0	0
	Presumed Persistence (n, %)	0	0	0	0
<i>Candida lusitana</i>		[N=2]	[N=0]	[N=0]	[N=2]
	Success (n, %)	1 (100.0)	0	0	1 (100.0)
	Proven Eradication (n, %)	1 (100.0)	0	0	1 (100.0)
	Failure (n, %)	0	0	0	0
	Presumed Persistence (n, %)	0	0	0	0
<i>Candida glabrata</i>		[N=9]	[N=7]	[N=11]	[N=27]
	Success (n, %)	8 (88.9)	7 (100.0)	11 (100.0)	26 (96.3)
	Proven Eradication (n, %)	2 (22.2)	0	4 (36.4)	6 (22.2)
	Presumed Eradication (n, %)	6 (66.7)	7 (100.0)	7 (63.6)	20 (74.1)
	Failure (n, %)	1 (11.1)	0	0	1 (3.7)

n = Number of patients with isolates of the species with the indicated outcome.
 N = Number of patients with isolates of the species in the dose group.
 Data Source: Appendix 14, Table 14.5 Parts 3 and 4; Appendix 18, Listing 18.17.1

EVALUABLE AT END OF THERAPY POPULATION, END OF THERAPY VISIT					
Baseline Species	Outcome	100 mg/50 mg	150 mg/75 mg	200 mg/100 mg	Total
<i>Candida krusei</i>		[N=2]	[N=3]	[N=0]	[N=5]
	Success (n, %)	1 (50.0)	2 (66.7)	0	3 (60.0)
	Proven Eradication (n, %)	0	1 (33.3)	0	1 (20.0)
	Failure (n, %)	1 (50.0)	1 (33.3)	0	2 (40.0)
	Presumed Persistence (n, %)	0	0	0	0
<i>Candida parapsilosis</i>		[N=4]	[N=3]	[N=1]	[N=8]
	Success (n, %)	3 (75.0)	3 (100.0)	1 (100.0)	7 (87.5)
	Proven Eradication (n, %)	0	2 (66.7)	1 (100.0)	3 (37.5)
	Failure (n, %)	1 (25.0)	0	0	1 (12.5)
	Presumed Persistence (n, %)	0	0	0	0
<i>Candida tropicalis</i>		[N=1]	[N=4]	[N=2]	[N=7]
	Success (n, %)	0	4 (100.0)	2 (100.0)	6 (85.7)
	Proven Eradication (n, %)	0	0	1 (50.0)	1 (14.3)
	Failure (n, %)	1 (100.0)	0	0	1 (14.3)
	Presumed Persistence (n, %)	0	0	0	0

MICROBIOLOGICAL ITT POPULATION, FOLLOW-UP VISIT						
Baseline Species	Outcome	100 mg/50 mg	150 mg/75 mg	200 mg/100 mg	Total	
All species		[N=42]	[N=43]	[N=42]	[N=127]	
	Success (n, %)	22 (52.4)	28 (65.1)	24 (57.1)	74 (58.3)	
	Proven Eradication (n, %)	19 (45.2)	25 (58.1)	20 (47.6)	64 (50.4)	
	Presumed Eradication (n, %)	3 (7.1)	3 (7.0)	4 (9.5)	10 (7.8)	
	Failure (n, %)	20 (47.6)	15 (34.9)	16 (38.1)	51 (40.2)	
	Proven Persistence (n, %)	4 (9.5)	2 (4.7)	4 (9.5)	10 (7.9)	
	Proven Recurrence (n, %)	0	1 (2.3)	0	1 (0.8)	
	Unable to Determine (n, %)	15 (35.7)	12 (27.9)	12 (28.5)	40 (31.5)	
	<i>Candida albicans</i>		[N=20]	[N=20]	[N=22]	[N=62]
		Success (n, %)	11 (55.0)	13 (65.0)	12 (54.5)	36 (58.1)
Proven Eradication (n, %)		9 (45.0)	12 (60.0)	8 (36.4)	29 (46.8)	
Presumed Eradication (n, %)		2 (10.0)	1 (5.0)	4 (18.2)	7 (11.3)	
Failure (n, %)		9 (45.0)	7 (35.0)	10 (45.5)	26 (41.9)	
Proven Persistence (n, %)		0	1 (5.0)	4 (18.2)	5 (7.9)	
Proven Recurrence (n, %)		0	1 (5.0)	0	1 (1.6)	
Unable to Determine (n, %)		9 (45.0)	5 (25.0)	6 (27.3)	20 (32.3)	
<i>Candida dubliniensis</i>			[N=1]	[N=0]	[N=0]	[N=1]
		Success (n, %)	1 (100.0)	0	0	1 (100.0)
	Proven Eradication (n, %)	1 (100.0)	0	0	1 (100.0)	
<i>Candida lusitana</i>		[N=1]	[N=0]	[N=0]	[N=1]	
	Failure (n, %)	1 (100.0)	0	0	1 (100.0)	
<i>Candida glabrata</i>		[N=11]	[N=10]	[N=15]	[N=36]	
	Success (n, %)	7 (63.6)	6 (60.0)	10 (66.7)	23 (63.9)	
	Proven Eradication (n, %)	6 (54.5)	5 (50.0)	8 (53.3)	19 (52.8)	
	Presumed Eradication (n, %)	1 (9.1)	1 (10.0)	2 (13.3)	4 (11.1)	
	Failure (n, %)	4 (36.4)	4 (40.0)	5 (33.3)	13 (36.1)	
	Unable to Determine (n, %)	1 (9.1)	0	0	1 (2.8)	

n = Number of patients with isolates of the species with the indicated outcome.
 N = Number of patients with isolates of the species in the dose group.

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Table 55 (cont'd):

		[N=2]	[N=3]	[N=0]	[N=5]
<i>Candida krusei</i>	Success (n, %)	0	1 (33.3)		1 (20.0)
	Proven Eradication (n, %)	0	1 (33.3)		1 (20.0)
	Failure (n, %)	2 (100.0)	2 (66.7)		4 (80.0)
	Presumed Persistence (n, %)	1 (50.0)	1 (33.3)		2 (40.0)
	Unable to Determine (n, %)	1 (50.0)	1 (33.3)		2 (40.0)
<i>Candida parapsilosis</i>	Success (n, %)	3 (50.0)	4 (100.0)	3 (100.0)	8 (72.7)
	Proven Eradication (n, %)	2 (50.0)	3 (75.0)	1 (100.0)	7 (63.6)
	Presumed Eradication (n, %)	2	1 (25.0)	0	1 (9.1)
	Failure (n, %)	2 (50.0)	0	0	3 (27.3)
	Presumed Persistence (n, %)	1 (25.0)	0	0	1 (9.1)
	Unable to Determine (n, %)	2 (33.3)	0	0	2 (18.2)
<i>Candida species</i>	Success (n, %)	[N=0]	[N=0]	[N=1]	[N=1]
	Proven Eradication (n, %)			1 (100.0)	1 (100.0)
<i>Candida lusitana</i>	Success (n, %)	[N=1]	[N=6]	[N=3]	[N=10]
	Proven Eradication (n, %)	0	4 (66.7)	2 (66.7)	6 (60.0)
	Failure (n, %)	1 (100.0)	2 (33.3)	1 (33.3)	4 (40.0)
	Presumed Persistence (n, %)	1 (100.0)	0	0	1 (10.0)
	Unable to Determine (n, %)	0	2 (33.3)	1 (33.3)	3 (30.0)

n = Number of patients with isolates of the species with the indicated outcome.
 N = Number of patients with isolates of the species in the dose group.

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3. Fluconazole refractory mucosal candidiasis

VER002-11 is an ongoing study of the safety and efficacy of anidulafungin in patients with fluconazole refractory mucosal candidiasis. The sponsor has submitted results for 5 patients enrolled to date (all were considered clinical successes at EOT). All patients had advanced HIV infection and AIDS, OPC and 2 of the patients also had EC. Fluconazole refractory disease was defined as active oral/oropharyngeal with or without esophageal clinical disease that failed to resolve following a 14 day course of fluconazole at a dose of 200 mg/day. Three patients had baseline isolates of *C. albicans*, 2 patients had both *C. albicans* and *C. glabrata*. Data is too limited to allow a definitive assessment of anidulafungin in patients with fluconazole refractory mucosal candidiasis. Table 56 demonstrates the clinical, endoscopic and microbiological outcome for the five patients enrolled.

Table 56: VER002-11 Data: Patient 8-001=*C.albicans*, 5-001=*C.albicans*+*C.glabrata*, 2-001=*C.albicans*, 8-002=*C.albicans*+*C.glabrata*, 2-002=*C.albicans*

Patient	Clinical Outcome		Endoscopic Outcome		Per-pathogen Microbiological Outcome	
	EOT	FU	EOT	FU	EOT	FU
8-001	Cure	Cure	Cure	Indeterminate	Proven eradication	Presumed eradication
5-001	Improvement	Deterioration	N/A	N/A	Proven persistence	Proven persistence
2-001	Cure	Recurrence	N/A	N/A	Proven persistence	Presumed persistence
8-002	Improvement	Cure	Improvement	Indeterminate	Proven persistence ^a	Proven persistence ^b
					Proven eradication ^b	Proven eradication ^b
2-002	Improvement	Deterioration	N/A	N/A	Proven persistence	Proven persistence

a Outcome for *C. albicans*
 b Outcome for *C. glabrata*
 EOT=end of therapy; FU=follow-up; N/A=not applicable (patient did not have esophageal candidiasis)

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Table 57 shows the clinical and mycological cures by species for all four clinical studies combined at EOT.

Table 57: Clinical, all 4 clinical studies combined (EOT)

Yeast	Anidulafungin		Fluconazole	
	Clinical Success	Mycologic eradication	Clinical Success	Mycologic eradication
<i>C. albicans</i>	197/243*(81)	191/243(79%)	149/170*(88)	158/170(93%)
<i>C. glabrata</i>	26/28* (93%)	26/28(93%)	1/2*(50%)	2/2(100%)
<i>C. krusei</i>	3/5 (60%)	4/5(80%)	0/0	0/0
<i>C. tropicalis</i>	6/7 (86%)	6/7(86%)	0/0	0/0
<i>C. parapsilosis</i>	7/8 (88%)	7/8(88%)	0/0	0/0
<i>C. famata</i>	1/1 (100%)	1/1(100%)	0/0	0/0
<i>C. dubliniensis</i>	1/1 (100%)	1/1(100%)	0/0	0/0
<i>Candida</i> spp.(not speciated)	1/2 (50%)	1/1(100%)	2/2(100%)	0/0
<i>C. albicans</i> + <i>C. glabrata</i>	9/10 (90%)	8/10(80%)	9/9(100%)	9/9(100%)
<i>C. albicans</i> + <i>C. tropicalis</i>	1/1 (100%)	1/1(100%)	1/1(100%)	1/1(100%)
<i>C. albicans</i> + <i>C. krusei</i>	0/2 (0%)	0/1(0)	0/2 (0%)	2/2(100%)
<i>T. beigelii</i>	0/1 (0%)	1/1(100%)	0/0	0/0
Total	252/309 (82%)	247/307(80%)	162/186(87%)	172/184(93%)

*Does not include mixed infections

V. The Label

1. Label Proposed by the sponsor

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REVISIONS

REVISIONS TO BE EVALUATED

2. Comments

The sponsor has proposed to describe the activity of anidulafungin against several *Candida* species in the microbiology section of the label. Although the sponsor has measured the activity of anidulafungin against other *Candida* spp., in the absence of standardized *in vitro* susceptibility methods the clinical significance of such *in vitro* testing is not known. At the present time the criteria for listing of species is limited to those against which the correlation of *in vitro* testing with activity *in vivo* has been established. Based on the review of clinical and animal studies, anidulafungin is active against *C. albicans* and *C. glabrata*.

The sponsor has proposed [

] However, the clinical indication being pursued at the present time is for the treatment of esophageal candidiasis. [

] will be misleading and promote off label use.

It would be useful to cross reference the Microbiology section in the Indications and Usage as well as the Clinical Study sections of the package insert.

3. FDA's version of the label:

(Please note that the additions are double underlined and the deletions are striked out)

Mechanism of action

Anidulafungin is a semi-synthetic echinocandin with antifungal activity.

Anidulafungin selectively inhibits glucan synthase, an enzyme present in fungal, but not mammalian cells. This results in inhibition of 1,3- β -D-glucan, an essential component of the fungal cell wall and

Activity in vitro

Anidulafungin is active in vitro against *Candida* *albicans* and *C. glabrata*,

MICs were determined according to the National Committee for Clinical Laboratory Standards (NCCLS) approved method M27-A.

Susceptibility testing methods for echinocandins have not been standardized and anidulafungin breakpoints have not been established. The relationship between clinical response and *in vitro* activity remains to be elucidated.

Activity in vivo

Parenterally administered anidulafungin was effective against *Candida* *albicans* in immunocompetent and/or immunosuppressed mice and rabbits with disseminated infection as measured by prolonged survival and reduction in mycological burden in target organs.

Anidulafungin also reduced the mycological burden of fluconazole-resistant *C. albicans* in an oropharyngeal/esophageal infection model in immunosuppressed rabbits.

Drug Resistance

Emergence of resistance to anidulafungin in vivo has not been studied.

Anidulafungin was active *in vitro* and *in vivo* against fluconazole-resistant *C. albicans*. Cross resistance with other echinocandins has not been studied.

VI. RECOMMENDATIONS:

The NDA submission is approvable with respect to microbiology pending an accepted version of the label.

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Microbiologist, HFD-590

Susan Peacock
Microbiologist, HFD-590

CONCURRENCES:

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