# CENTER FOR DRUG EVALUATION AND RESEARCH

***APPLICATION NUMBER:***

**22404Orig1s000**

# MICROBIOLOGY REVIEW(S)

**MICROBIOLOGY REVIEW**
**DIVISION OF SPECIAL PATHOGEN AND TRANSPLANT PRODUCTS**

| | | |
|---|---|---|
| **NDA #:** 22-404 | **REVIEWER** | **:** Lynette Berkeley |
| (SDN9 - original) | **CORRESPONDENCE DATE** | **:** 3-29-10 |
| | **CDER RECEIPT DATE** | **:** 3-29-10 |
| | **REVIEW ASSIGN DATE** | **:** 3-29-10 |
| | **REVIEW COMPLETE DATE** | **:** 3-29-10 |

**SPONSOR:** BioAlliance Pharma
% Beckoff Associates Inc.,
Commerce Plaza II, Ste 300,
7400 West 110th Street,
Overland Park, Kansas 66210

**DRUG CATEGORY:** Antifungal

**INDICATION:** Treatment of oropharyngeal candidiasis

**DOSAGE FORM:** Tablets

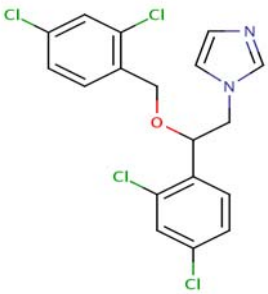
**PRODUCT NAMES:**

**a. PROPRIETARY:** Oravig®

**b. NONPROPRIETARY:** Miconazole

c. **CHEMICAL:** 1-[(2RS)-2-[2,4-Dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-H-
Imidazole

**STRUCTURAL FORMULA:**




Molecular weight:416.13
Molecular formula: $C_{18}H_{14}C_{l4}N_2O$

**SUPPORTING DOCUMENTS**: IND # 69578

## Table of Contents

## 1. Executive Summary

The applicant has proposed to add statements such as [redacted] in the product labeling. The studies submitted in support of such a claim include testing of 3 strains of *Candida* species *(*1 of *C. albicans* and 2 of *C. glabrata)* with a MIC of 64 µg/mL. Miconazole MICs against these 3 isolates were ≤ 0.5 µg/mL. The number of isolates is very small and clinical relevance of such a finding is not known.

The applicant also proposes [redacted] for which clinical relevance is not known, in the package insert. However, it is the Division policy to list only those species for which clinical relevance is known. Therefore, in the absence of such information, only those [redacted] for which clinical relevance is known are included in the labeling.

## 2. Introduction and Background

The applicant has proposed to add the following changes to the draft labeling that was proposed by the Division.

## 3. The Labeling

The applicant has proposed the following changes to the microbiology subsection of the proposed labeling. The additions are double underlined and deletions striked out.

### 3.1. Applicant's proposal

12.4 Microbiology

Mechanism of Action

Miconazole inhibits the enzyme cytochrome P450 14α-demethylase which leads to inhibition of ergosterol synthesis, an essential component of the fungal cell membrane. Miconazole also affects the synthesis of triglycerides and fatty acids and inhibits oxidative and peroxidative enzymes, increasing the amount of reactive oxygen species within the cell.

Activity *in vitro* and *in vivo*

[redacted]

Drug Resistance

*In vitro* studies have shown that some *Candida* strains that demonstrate reduced susceptibility to one antifungal azole may also exhibit reduced susceptibility to other azoles suggesting cross-resistance. [redacted]

Clinically relevant resistance to systemically utilized triazoles may occur in *Candida* species. Resistance may occur by multiple mechanisms such as changes in amino acids and/or in the regulation of the target enzyme and of a variety of efflux pump proteins. Multiple mechanisms may co-exist in the same isolate.

Resistance breakpoints, correlating *in vitro* activity with clinical efficacy, have not been established for miconazole.

**3.2. Summary of study reports and comments:**

A summary of the two study reports not included in the original NDA review (for details see microbiology review dated 12/16/09) is as follows:

**(i) Study report CMM-06-17 (Ghannoum et al. 2006):** The *in vitro* susceptibility of 25 strains of 6 *Candida spp*. (*C. albicans, C. dubliniensis, C. glabrata, C. krusei. C. parapsilosis* and *C. tropicalis*, provided by the Center for Medical Mycology) was determined against miconazole and other antifungal drugs by the CLSI method (M27A2). The comparators tested were amphotericin B, caspofungin, clotrimazole, fluconazole, itraconazole, nystatin and voriconazole. Of the 25 strains of *Candida* species one strain of *C. albicans* (8283) and two strains o*f C glabrata* (2723, 8576) had MIC of 64 µg/mL. The results in Table 1 show that miconazole MIC against all strains tested including fluconazole resistant strains ranged from 0.06 to 0.5 µg/mL. The number of fluconazole resistant strains tested is limited (n=3). Also, the clinical relevance of such a finding is unknown.

Table 1. Comparison of miconazole and fluconazole against fluconazole resistant and strains showing elevated fluconazole MICs.

| Isolate | Miconazole (µg/ml) | Fluconazole (µg/ml) |
|---|---|---|
| *C. albicans* 2341 | 0.5 | 16 |
| *C. albicans* 2591 | 0.5 | 16 |
| *C. albicans* 2599 | 0.5 | 8.0 |
| *C. albicans* 2623 | 0.25 | 8.0 |
| *C. albicans* 2677 | 1.0 | 32 |
| *C. albicans* 2701 | 1.0 | 16 |
| *C. albicans* 2707 | 0.5 | 8.0 |
| *C. albicans* 8280 | 1.0 | 8.0 |
| *C. albicans* 8283 | 0.25 | 64 |
| *C. glabrata* 1910 | 0.25 | 16 |
| *C. glabrata* 1911 | 0.25 | 16 |
| *C. glabrata* 2654 | 0.25 | 16 |
| *C. glabrata* 2655 | 0.125 | 8.0 |
| *C. glabrata* 2723 | 0.25 | 64 |
| *C. glabrata* 8566 | 0.125 | 16 |
| *C. glabrata* 8576 | 0.5 | 64 |
| *C. glabrata* 8658 | 0.125 | 8.0 |
| *C. parapsilosis* 8148 | 0.25 | 16 |
| *C. krusei* 9415 | 0.25 | 16 |
| *C. krusei* 9418 | 0.5 | 8.0 |
| *C. krusei* 9423 | 0.12 | 16 |
| *C. krusei* 9425 | 0.5 | 16 |
| *C. krusei* 9427 | 0.5 | 32 |
| *C. krusei* 9428 | 0.5 | 16 |
| *C. krusei* 9481 | 0.25 | 16 |
| *C. krusei* 9538 | 0.12 | 16 |
| *C. krusei* 9541 | 0.25 | 16 |
| *C. krusei* 9572 | 0.5 | 16 |
| *C. krusei* 9574 | 0.06 | 16 |
| *C. krusei* 9575 | 0.06 | 8.0 |



**(ii) Study CMM-06-21 (Ghannoum et al. 2006):**

The effect of repeated exposure of *Candida spp*. in the presence of miconazole*, in vitro*, on the development of resistance was determined; fluconazole was included as a comparator for testing. The testing was done according to CLSI method (M27A2). The isolates were selected from the culture collection at the Center for Medical Mycology, Ohio. Two strains each of *C. albicans, C. glabrata and C. tropicalis* were tested. The author states that 1 of the strains from each species was identified as being resistant to fluconazole and the other was susceptible. However, the actual fluconazole MICs were not specified. From the initial MIC determination a sub-inhibitory concentration that was 0.5 the MIC and showing 50% inhibition as compared to the growth concentration that contained no antibiotic was transferred to potato dextrose agar (PDA) and incubated at $35^0$C for 24 hours. The pellet of cells from the 0.5 MIC culture was removed and transferred to the PDA plate. After incubation, the yeast cells were harvested and diluted with sterile saline to a concentration of $10^7$ determined by a hemacytometer. One milliliter of the $10^7$ concentration of cells was added to a tube that containing 10 mL of one of the four concentrations of miconazole (0.5, 1, 2, 4) times the concentration of miconazole MIC and incubated at $35^0$C for 24 hours. The tubes were centrifuged at 3,000

RPM for 10 minutes, all liquid was removed and replaced by 0.6 mL of sterile saline. An aliquot of 100µL from each tube was plated on to PDA and incubated under the conditions as described above. Additionally, 100ul of the sediment was added to a second 10 mL set of concentrations of miconazole and the cycle repeated for a total of 15 passages *in vitro*. This procedure was also followed for fluconazole. Resistance was defined as ≥ 3 fold dilution over the initial MIC. Results in Tables 2 and 3 show that there was no increase in MIC after 15 passages of 5/6 strains of the *Candida spp.* to miconazole. The only strain that appeared to develop resistance was *C. albicans* 8479 with a 4 to 5 fold increase in MIC after repeat passages. In the fluconazole group, *C. albicans* 8479 also showed an increase in MIC but only slightly. Overall, the only strain that appeared to develop resistance was *C. albicans* 8479 and that was to miconazole.

Table 2. Miconazole MIC values in µg/mL for *Candida spp.* isolates following repeated exposure to miconazole

| Isolate | Initial MICON MIC | MICON MIC following final passage | | | |
|---|---|---|---|---|---|
| | | 4 MIC | 2 MIC | MIC | .5 MIC |
| C. albicans 8479 | 0.008 | 0.25 | 0.25 | 0.5 | 0.25 |
| C. albicans 8283* | 0.5 | 0.25 | 0.12 | 0.25 | 0.25 |
| C. glabrata 8683 | 0.008 | 0.25 | 0.03 | 0.03 | 0.03 |
| C. glabrata 8576* | 0.5 | 0.5 | 0.5 | 0.25 | 0.5 |
| C. tropicalis 8679 | 0.06 | 0.06 | 0.03 | 0.06 | 0.06 |
| C. tropicalis 2395* | 0.12 | 0.5 | 0.25 | 0.25 | 0.25 |

* Fluconazole-resistant strains

Table 3. Fluconazole MIC values in µg/mL for *Candida spp.* isolates following repeated exposure tofluconazole

| Isolate | Initial FLU MIC | FLU MIC following final passage | | | |
|---|---|---|---|---|---|
| | | 4 MIC | 2 MIC | MIC | .5 MIC |
| C. albicans 8479 | 0.12 | 2.0 | 2.0 | 2.0 | 2.0 |
| C. glabrata 8683 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 |
| C. tropicalis 8679 | 0.12 | 0.5 | 0.25 | 0.25 | 0.25 |

The study reports referenced by the sponsor do not support the changes proposed by the sponsor

### 3.3. FDA's version of the labeling

### 12.4 Microbiology

Mechanism of Action

Miconazole inhibits the enzyme cytochrome P450 14α-demethylase which leads to inhibition of ergosterol synthesis, an essential component of the fungal cell membrane.

Miconazole also affects the synthesis of triglycerides and fatty acids and inhibits oxidative and peroxidative enzymes, increasing the amount of reactive oxygen species within the cell.

Activity *in vitro* and *in vivo*

Miconazole is active against *Candida albicans, C. parapsilosis* and *C. tropicalis.* Correlation between minimum inhibitory concentration (MIC) results *in vitro* and clinical outcome has yet to be established.

Drug Resistance

*In vitro* studies have shown that some *Candida* strains that demonstrate reduced susceptibility to one antifungal azole may also exhibit reduced susceptibility to other azoles suggesting cross-resistance. Clinically relevant resistance to systemically utilized triazoles may occur in *Candida* species. Resistance may occur by multiple mechanisms such as changes in amino acids and/or in the regulation of the target enzyme and of a variety of efflux pump proteins. Multiple mechanisms may co-exist in the same isolate.

Resistance breakpoints, correlating *in vitro* activity with clinical efficacy, have not been established for miconazole.

## 4. Conclusions

The study reports referenced by the applicant include testing of only 3 fluconazole resistant strains with an MIC of 64 µg/mL. Also, the clinical relevance of such an effect is unknown. The changes proposed by the applicant are not supported by the limited information available for review.

## 5. Recommendations

The study reports referenced by the applicant do not support the addition of statements proposed by the applicant on March 29, 2010. The NDA submission should be approved pending an accepted version of the labeling.

*Lynette Berkeley*
Lynette Berkeley, Ph.D, M.T. (ASCP)
Microbiologist, DSPTP

**CONCURRENCES:**
DSPTP /MicroTL *Shukal Bala* Signature 3/30/10 Date
CC:
DSPTP/Original NDA
DSPTP/PM/Judith Milstein

| Application Type/Number | Submission Type/Number | Submitter Name | Product Name |
|---|---|---|---|
| NDA-22404 | ORIG-1 | BIOALLIANCE PHARMA | Lauriad (miconazole (b) (4) tablet) |

---

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

---

/s/

---

LYNETTE Y BERKELEY
04/02/2010
Addendum to General Microbiology review

SHUKAL BALA
04/05/2010

# Product Quality Microbiology Review

**22 JANUARY 2010**

**NDA:** 22-404 amendment

**Drug Product Name**
**Proprietary:** Lauriad (b) (4) Buccal tablets
**Non-proprietary:** miconazole

**Review Number:** 2

## Dates of Submission(s) Covered by this Review

| Submit | Received | Review Request | Assigned to Reviewer |
|---|---|---|---|
| 14 January 2010 | 15 January 2010 | N/A | N/A |

## Submission History (for amendments only)

| Submit Date(s) | Microbiology Review # | Review Date(s) |
|---|---|---|
| 5 February 2009 | 1 | 4 December 2009 |
| 15 June 2009 | 1 | 4 December 2009 |

## Applicant/Sponsor

**Name:** BioAlliance Pharma
**Address:** 49 Boulevard du General Martial Valin, 75015 Paris, France
**Representative:** Lavonne M. Patton, Ph.D. (Authorized US Agent)
**Telephone:** 913-451-3955

**Name of Reviewer:** Bryan S. Riley, Ph.D.

**Conclusion:** Recommended for approval

# Product Quality Microbiology Data Sheet

**A.** **1.** **TYPE OF SUBMISSION:** Amendment to an original NDA

**2.** **SUBMISSION PROVIDES FOR:** Response to Product Quality Microbiology Deficiency

**3.** **MANUFACTURING SITE:** (b) (4)

**4.** **DOSAGE FORM, ROUTE OF ADMINISTRATION AND STRENGTH/POTENCY:** Non-sterile (b) (4) tablet for buccal administration, 50 mg/tablet

**5.** **METHOD(S) OF STERILIZATION:** N/A

**6.** **PHARMACOLOGICAL CATEGORY:** anti-fungal agent for treatment of oropharyngeal candidiasis

**B.** **SUPPORTING/RELATED DOCUMENTS:** Product Quality Microbiology review of NDA 22-404 (review dated 4 December 2009).

**C.** **REMARKS:** This was an eCTD submission. This amendment was in response to a product quality microbiology deficiency provided to the applicant on 23 December 2009.

**filename:** N022404R2.doc

# Executive Summary

**I. Recommendations**

**A. Recommendation on Approvability –** This submission is recommended for approval on the basis of product quality microbiology.

**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 –** The drug product is a non-sterile buccal adhesive tablet with microbial limit specifications.

**B. Brief Description of Microbiology Deficiencies –** N/A

**C. Assessment of Risk Due to Microbiology Deficiencies –** N/A

**III. Administrative**

**A. Reviewer's Signature _____**
Bryan S. Riley, Ph.D.

**B. Endorsement Block _____**
James L. McVey, NDMS Team Leader

**C. CC Block**
N/A

2 pages have been wthheld in full as B(4) CCI/TS immediately following this page

| Application Type/Number | Submission Type/Number | Submitter Name | Product Name |
|---|---|---|---|
| NDA-22404 | ORIG-1 | BIOALLIANCE PHARMA | Lauriad (miconazole [redacted] (b) (4) tablet) |

---

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

---

/s/

---

BRYAN S RILEY
01/25/2010

JAMES L MCVEY
01/25/2010
I concur.

**MICROBIOLOGY REVIEW**
**DIVISION OF SPECIAL PATHOGEN AND TRANSPLANT PRODUCTS**

**NDA #:** 22,404
(SDN9 - original)

**REVIEWER** : Lynette Berkeley
**CORRESPONDENCE DATE** : 06-17-09
**CDER RECEIPT DATE** : 06-18-09
**REVIEW ASSIGN DATE** : 10-15-09
**REVIEW COMPLETE DATE:** 12-16-09

**SPONSOR:** BioAlliance Pharma
% Beckoff Associates Inc.,
Commerce Plaza II, Ste 300,
7400 West 110th Street,
Overland Park, Kansas 66210

**DRUG CATEGORY:** Antifungal

**INDICATION:** Treatment of oropharyngeal candidiasis

**DOSAGE FORM:** Tablets

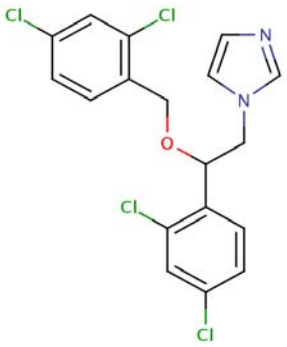
**PRODUCT NAMES:**

**a. PROPRIETARY:** Oravig

**b. NONPROPRIETARY:** Miconazole

c. **CHEMICAL:** 1-[(2RS)-2-[2,4-Dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-H-Imidazole

**STRUCTURAL FORMULA:**




Molecular weight: 416.13
Molecular formula: $C_{18}H_{14}C_{l4}N_2O$

**SUPPORTING DOCUMENTS**: IND # 69578

**TABLE OF CONTENTS**

## 1. EXECUTIVE SUMMARY

Miconazole is an imidazole antifungal agent. It has two identified mechanisms of action. The major mechanism is inhibition of activity of cytochrome P 450 dependent 14 α–demethylase which results in reduction of ergosterol and consequently the synthesis of the fungal cell membrane. A less well known mechanism of action is the inhibition of peroxidases resulting in a build up of reactive oxygen species which are toxic to the cell and eventually result in cell death.

*In vitro* susceptibility to miconazole was tested against > 1500 isolates of *Candida albicans* and other *Candida* species by the CLSI M27 A3 method. Overall, the *C. albicans* $MIC_{50}$ and $MIC_{90}$ values were 0.1 µg/mL and 1 µg/mL, respectively. Both $MIC_{50}$ and $MIC_{90}$ were <1.0 µg/mL against the other *Candida spp.* tested, with the exception of *C. guilliermondii, C. krusei and C. tropicalis*. Against *C. krusei* species the $MIC_{50}$ and $MIC_{90}$ values were higher (2.0 µg/mL and 4.0 µg/mL, respectively).

The effect of different doses of miconazole ointment topically and orally was measured in a cutaneous *Candida albicans* infection model. The guinea pigs were treated in separate groups with each formulation for 14 days. The animals were given prophylactic treatment of the same formulations orally 2 days prior to infection and for topical treatment one day after inoculation with *C. albicans.* The outcome of the infection was assessed both microscopically and by culture. Oral treatment with miconazole administered 2 days prior to infection was more effective that topical application one day post-infection. However, topical treatment with miconazole was more effective than placebo.

The applicant proposes to use miconazole buccal tablets (50 mg) to treat oropharyngeal candidiasis (OPC) in HIV patients. The effect of once daily miconazole treatment was compared with that of 10 mg of clotrimazole delivered 5 times daily for 14 days. A second study involved the treatment of patients with head and neck cancer with miconazole buccal tablets or miconazole gel and the third study was a noncomparator study in HIV patients treated with miconazole buccal tablets.

The pivotal study BA2004/01/04 comprised of 476 patients in the PP population. A majority of the patients were infected with *C. albicans* (85%) followed by *C. tropicalis* (8%). The miconazole $MIC_{90}$ values against *C. albicans* at baseline in the two treatment arms were similar. The MICs at the TOC visit were also similar to that of baseline isolates. The primary endpoint was clinical cure and mycological eradication was the secondary endpoint. In patients infected with *C. albicans* the clinical cure rate was 65% for the miconazole arm and 74% for the clotrimazole arm. The mycological cure rates were similar (29% and 25 % respectively). There was no correlation between the clinical and mycological cure rates.

Study BA2002/ 01/02 compared the efficacy of buccal tablet and the miconazole gel. Two hundred and ten patients from France and North America were enrolled in the trial and three patients were lost to follow-up. The clinical cure rates were similar in the two treatment arms. However, the mycological response (69%) was better in patients treated with the gel compared to the buccal tablet (47%).

Study BA 2002/01/03 was designed to evaluate the clinical efficacy in HIV patients with oropharyngeal candidiasis once daily Oravig 50 mg for 14 days. Nineteen patients were enrolled in the study. Of these 19 patients, 18 were infected with *C. albicans* and one was dually infected with *C. albicans* and *C. glabrata.* Seventeen patients (89%) were clinically cured.

Overall, there was no correlation between the rates of mycological eradication and clinical cures. However, all three of the studies support efficacy of miconazole BT for the treatment of OPC in patients infected with

*C. albicans*, *C. tropicalis* and *C. parapsilosis*. For changes to the microbiology section of the labeling see section 7.3.

## 2. INTRODUCTION AND BACKGROUND

The subject of this NDA is Oravig (miconazole) for the treatment of oropharyngeal candidiasis (OPC). The applicant has proposed to administer 50 mg buccal tablet (BT), of miconazole, to the upper gum region once daily for 14 days. The tablet will adhere to the upper gum just above the incisor tooth and will dissolve gradually providing a continuous release of the miconazole into the buccal cavity. Miconazole is approved for the topical treatment of intravaginal and dermal fungal infections.

Miconazole, a synthetic imidazole, is a white (b) (4) powder; (b) (4)

ORAVIG was designed to deliver high miconazole saliva concentrations. The single administration of ORAVIG containing 50 mg of miconazole to 18 healthy volunteers provided mean salivary maximum concentrations of 15 mcg/mL at 7 hours after application of the tablet and AUC (0-24h) of 55 µg/mL
In healthy volunteers, the duration of adhesion was on average 15 hours following a single dose administration of ORAVIG 50 mg. Plasma concentrations of miconazole were below the lower limit of quantification in 59 HIV patients and in 157/162 (97%) samples from healthy volunteers following single dose administration of ORAVIG 50 mg. Measurable plasma concentrations in healthy subjects ranged from 0.5 to 0.83 µg/mL. In healthy volunteers, the terminal half-life was 24 hours following systemic administration. There are no active metabolites of miconazole (for details see Clinical pharmacology review by Dr. Yoriko Harigaya).

**Biology of oropharyngeal candidiasis:**
Oropharyngeal candidiasis, a common opportunistic infection in immuno-compromised subjects, is caused by a variety of *Candida species* the most common of which is *Candida albicans*. Other *Candida* species involved in oral candidiasis are *C. dubliniensis, C. glabrata, C. parapsilosis, C. tropicalis, C. pseudotropicalis* and *C. krusei. Candida* species comprise a part of the normal flora of 20 % to 60 % of healthy individuals. In immuno-compromised patients such as HIV infected individuals however, abnormal growth of the fungus can occur in the oral cavity and other parts of the body. The majority of cancer patients, particularly those who have undergone chemotherapy or radiotherapy are often colonized by *Candida* species.

Many asymptomatic patients present with asymptomatic colonization of *Candida spp.* and treatment does not always lead to fungal eradication. The incidence of *Candida spp.* colonization is as high as 93% in those persons while the incidence of oral candidiasis in immuno-competent patients varies from 17% to over 29%. The diagnosis of oral candidiasis is based on signs and symptoms, direct examination under the microscope, and positive culture.

# 3. PRECLINICAL/NONCLINICAL MICROBIOLOGY

## 3.1. Mechanism of action

**Effect on ergosterol synthesis:**
VandenBossche et al., 1985, identified the points in the biosynthetic pathway of ergosterol synthesis that were inhibited by the azole antifungals. Ergosterol is important for maintaining the integrity of fungal cell membrane. Accumulation of 14 methylated sterols, one of the intermediates in the synthesis of ergosterol from lanosterol was shown in a number of yeasts and molds treated both *in vitro* and *in vivo* with miconazole, ketoconazole, imazalil, paraconazole, terconazole and itraconazole. Figure 1 shows the points at which ergosterol synthesis is inhibited from 14 methyl, 24 methylene ergosterol, 4, 14-dimethylzymosterol and 24-methylene dihydrolanosterol, leading to the built up of intermediates such as 14 methylated sterols. When *Ustilago maydis* was treated with ectaconazole, another sterol metabolite, 14α-methyl-ergosta 8, 24- (28)-dien-3β, 6 α diol was found to accumulate in the yeast cell. This same sterol was found to have accumulated in *Candida albicans* after incubation with 0.005 µg/mL of ketoconazole. This indicates that azoles inhibit the conversion of lanosterol to ergosterol. Overall, there is a build up of precursors and a decrease in ergosterol.

Another study by Walsh et al. (1982), using an ergosterol deficient strain of *U. avenae* (erg-40 mutant), showed that when this mutant was incubated separately with ectaconazole and miconazole there was no inhibition of the production of wild type sporidia thereby suggesting that these antifungal agents specifically target ergosterol.

A study by Nozawa et al. (1986), examined ergosterol biosynthesis in *C. albicans* and *Trichophyton mentagrophytes* in the presence of miconazole. The fungal cells were incubated for 30 minutes in the presence of miconazole. After incubation with miconazole $^{14}$C-acetate was added for two hours to label the cells and the distribution of $^{14}$C radioactivity was measured. Various unsaponifiable components were measured by gas liquid chromatography. The results showed approximately 50 % of the total radioactivity in lanosterol from *C. albicans* while in *T. mentagrophytes* there was an increase in 24-methylendiahydrolanosterol (74.8%) instead of lanosterol. The incorporation of $^{14}$C acetate into ergosterol in the drug treated cells was ≤ 10% of that produced in cells not treated with miconazole. The results showed that incubation of *C. albicans* with miconazole led to a decrease in ergosterol synthesis by ≤10% of the drug-free control cultures.

Gibbons et al., 1979, showed that miconazole at a concentration of 0.1 µg/mL resulted in a 25% decrease in ergosterol synthesis. Details of experimental design were not available for review.

**Effect on cytochrome P450:**
The effect of miconazole on cytochrome P450 from yeast and rat liver enzyme was measured **(**VandenBossche et al., 1985). For preparation of yeast cytochrome P450, *C. albicans,* strain R.V. 4688, was grown at 37°C on sabouraud's agar. After 64 hours incubation on a 37$^0$C shaker, 0.2 mL of culture was transferred to casein hydrolysate-yeast extract-glucose medium (CYG-medium). Miconazole nitrate (R 14889; concentration was not stipulated), dissolved in dimethylsulfoxide (DMSO) was added to 100 mL of CYG to which 0.2 mL aliquots of yeast culture of approximately $10^9$ cells were added. Miconazole was added either immediately or 4 hours after inoculation and growth was stopped 1, 4, 16 or 24 h after the addition of drug. The number of cells at each time point was counted by a Coulter counter. Then 100 mL of CYG medium incubated with 0.136 µg/mL of sodium acetate and 2.5 µCi of radioactivity labeled sodium acetate were incorporated. The cells were homogenized and the supernatant subjected to thin layer chromatography (TLC) and the amount of radioactivity emitted was determined by Scintillation spectrometer. The sterols were identified by TLC, mass spectrometry, saponification and acetylation. The bubbling of yeast cells with carbon monoxide prior to addition of miconazole reduced the sensitivity of the yeast cytochrome P 450 by about 8 times **(**VandenBossche et al., 1982, 1983, 1984). The authors state that in the presence of 17 µg/mL of miconazole and rat liver cells a 50% reduction in the height of the Soret peak was observed, which is 150 times the concentration needed to achieve the same result in yeast cytochrome P450 at 448 nm. This corresponds to a much higher effect of miconazole on yeast than on cholesterol synthesis in rat liver **(**VandenBossche et al., 1978).

In another study the effect of miconazole (16.7 µg/mL) on cytochrome P450 was measured by the difference of carbon monoxide (CO) release. It was found that there was a 50% decrease in the height of the Soret band of the carbon monoxide compound of rat liver microsomal cytochrome P450 (VandenBossche et al., 1985, Arjuna et al., 2000). The same phenomenon was observed when yeast cells from *S. cerevisiae* microsomes were tested (VandenBossche et al., 1984), thus showing that miconazole is active by inhibiting cytochrome P450 enzymes and therefore causing defects in the *C. albicans* cell membrane. A spectral change occurs in the interaction of the nitrogen heterocycles with cytochrome P450 because of the binding of $N_3$ to the catalytic heme iron atom at the site occupied by the exchangeable sixth

ligand (Dawson et al., 1982). The miconazole inhibition of ergosterol synthesis results from the interference with cytochrome P450 – dependent 14α-demethylase.

**Effect on peroxidase and endogenous reactive oxygen species:**

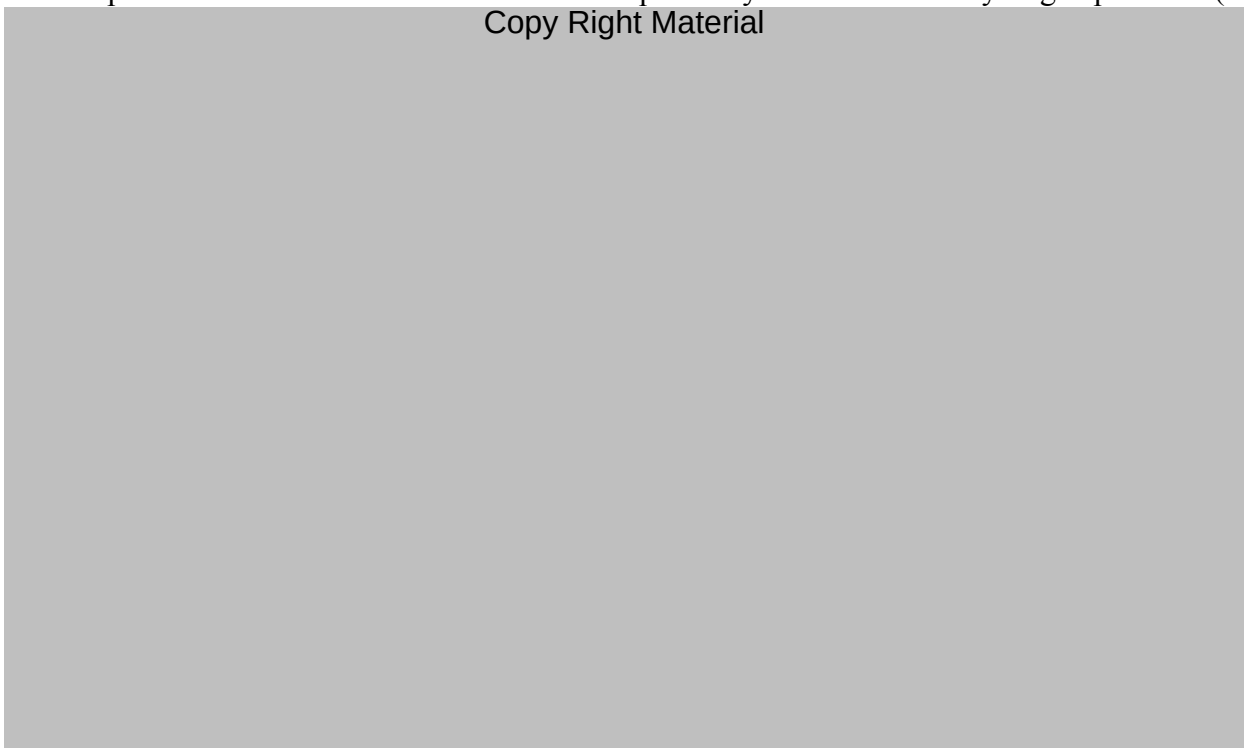
Fothergill, A.W. (2006) reported on a mechanism of action for miconazole that is not reported for other imidazoles. This mechanism relates to the inhibition of peroxidases in the yeast cells. In miconazole treated cells it has been found that peroxidases accumulate within the fungal cell. Because peroxidase is toxic to the cell this activity results in cell death. Using the methodology of Borgers et al. (1977), one loopful of *C. albicans* grown in 4 mL of sabouraud's broth was inoculated into CYG at a concentration of $2x10^5$ cells/mL and grown in a shaking incubator at $37^0$C for 24 hours. Some of the cultures were incubated with miconazole; drug free controls were included. Miconazole was added at a concentration of either 0.4 to 4.0 µg/mL immediately after inoculation of the yeast cells which were harvested 24 hours later. The cultures were centrifuged and pellets resuspended in a few drops of rat serum, and fixed in cold 3% gluteraldehyde. The pellets were allowed to solidify for 15 minutes at $4^0$C, frozen and sectioned and then fixed again for 5 minutes at $4^0$C. They were then washed in buffer supplemented with sucrose and incubated in an enriched medium for 2 hours at $37^0$C. After this the cells were fixed with 2% $OsO_4$, then dehydrated and embedded and examined microscopically either unstained or counter stained with lead citrate.

In the untreated cells the fine granular precipitate of cerous perhydroxide that was found in both the cristae of the mitochondria and the central vacuole was weak. In the presence of 0.4 µg/mL of miconazole, the precipitate in the cristae was moderate while the precipitate in the central vacuole was abundant. However, in the presence of 4.0 µg/mL concentration of miconazole the enzymatic activity was greater in the mitochondria and appeared as clumps in the swollen matrices of the mitochondria whereas activity in the vacuoles was decreased or unrecognizable probably due to decreased metabolic activity and cell damage. Overall, the cells were severely damaged. The vacuoles became distorted and filled with cytoplasmic material. The damage likely resulted from the inhibition of the activity of oxidative enzymes required to prevent the build up of peroxides during the oxidative process.

Kobayashi et al. (2002), investigated the significance of endogenous reactive oxygen species (ROS) produced by *Candida* species, *C. albicans* (ATCC 24433), *C. glabrata* (ATCC2001) and *C. tropicalis* (ATCC750) and clinical isolates of *C. albicans* and six strains of *C. glabrata* incubated with miconazole. The minimum inhibitory concentrations (MIC) were determined by the CLSI method (M27-A3). The *Candida* species had been identified with CHROMagar *Candida* (Kanto Chemical) and stored at $-70^0$C until testing. Miconazole and fluconazole were stored in stocks of 2 and 10 mg/mL and diluted as appropriate when ready to use in buffered (morpholinepropanesulfonic acid-MOPS) RPMI 1640 medium. Pyrrolidinedithiocarbamate (PDTC), an antioxidant, was kept as stock at 0.1 M in phosphate-buffered saline (PBS) which did not contain calcium and magnesium [PBS (-)] and diluted in buffered RPMI 1640 medium. Endogenous ROS were measured by a fluorometric assay using 2', 7'-dichlorofluorescin diacetate (DCFH-DA; Molecular Probes, Inc.). The cells were suspended in PBS and adjusted to a McFarland 0.5 standard then centrifuged, washed and first incubated in either the presence or absence of pyrrolidinedithiocarbamate (PDTC; Sigma) for 1 hour. After the first incubation period, the cells were reincubated with 1.25 µg/mL miconazole or fluconazole at 37°C for an additional 1 hour then 0.5 µg/mL of fluorescein DCFH-DA, in PBS, was added. The cell suspensions were placed in separate wells of a 96-well fluoroplate (FB; Wako Chemical Industry), and the fluorescence intensities (FIs) of the resuspended cells were measured with a Spectrafluor instrument (SLT-Labinstruments) at 485 nm / 538 nm; every 10 min for 4 hours.

The ROS was measured by subtracting the results of the sample cells incubated with DCFH-DA from the results of cells incubated without DCFH-DA. Both groups of cells were incubated with either 1.25 µg/mL miconazole or fluconazole. The results in Figure 2 show the time course curve of fluorescence intensity over a period of four hours. The results suggest an increase in ROS by one of the strains of *C. albicans* (ATCC 24433) incubated with miconazole + DCHF-DA compared to incubation with DCHF-DA alone. This showed that because of incubation with miconazole there is a build-up of ROS (e.g., superoxide, hydrogen peroxide) in the yeast cell. The amount of the ROS increased with increasing time after treatment showing a lag in the build up of the toxic by- product. The build up occurred because the activity of enzymes such as oxidases, catalases, superoxide dismutases that break down of these toxic substances was inhibited. The results in Figure 3 show a concentration dependent increase in ROS production in the presence of miconazole. The ROS that is primarily accumulated is hydrogen peroxide (Arjuna et al., 2000).





This study also calculated the MIC of the antifungal agents miconazole and fluconazole using a colormetric microdilution methos (ASTY; Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo, Japan), The method utilized 100µL of a 0.5 Mc Farland standard concentration of yeast cells in RPMI 1640. that contained 17.5 µg/mL resazurin and a colormetric reagent. The suspensions were added to microdilution plates that contained different concentrations of the relevant dried antifungal agent. The plates were incubated at $35^0$C for 48 hours and then read for a change in color that indicated the level of growth of the fungus compared to growth in a control well. The miconazole MIC for *C. albicans* (ATCC 24433) was 0.125 µg/mL while that for *C. glabrata* (ATCC 2001) was 0.25 µg/mL and for *C. tropicalis* (ATCC 750) 2.0 µg/mL. The incubation with PDTC the antioxidant decreased the cytostatic action of miconazole (see Figures 4 and 5). This indicates that ROS might be an important mediator of the antifungal activity of miconazole. When the production of ROS, by 10 clinical isolates of *C. albicans* and *C. glabrata* was tested, the presence of ROS was detected in all of the yeasts incubated with miconazole. A strong inverse correlation ($r = 0.088$), $P < 0.01$) between the MIC and the amount of ROS was reported Figure 5. (Kobayashi et al., 2002).

**Effect on fatty acid synthesis:**
McGinnis et al., 1996, state that the high level of free fatty acids present in fungi and gram positive bacteria are responsible for the susceptibility of some fungi and gram positive bacteria to the imidazoles. Mammalian cells and gram negative bacteria are more resistant to imidazoles because they contain lower levels of free fatty acids. Moreover, at high concentrations, miconazole in addition to affecting ergosterol synthesis also affects the synthesis of triglycerides and fatty acids thereby resulting in more rigid cells that divide more slowly than normal. Details of the experimental design and results were not available for review.

**Effect on chitin synthesis:**
The depletion of miconazole might also cause the activation of chitin synthesis **(**VandenBossche et al., 1983) and cause uncoordinated hyphal growth. *C. albicans* (57012) cells were inoculated into CYG to obtain a concentration of 2.3 x $10^5$ cells /mL. Immediately after inoculation, the cell cultures were individually exposed to 0.04 µg/mL (stated to be fungistatic) and 42 µg/mL (stated to be fungicidal) concentrations of miconazole and incubated as shake cultures at $37^0$C. Untreated controls were incubated under the same conditions. The cells were harvested after 24 hours fixed in osmium tetroxide washed, dehydrated, impregnated in 100% amylacetate and examined by scanning electron miscroscopy. Yeast cells were also treated and embedded in epon and examined by transmission electron microscopy. The untreated cells were smooth walled, spherical to elongate in shape they were separate and showed polar buds. The cells exposed to 0.04 µg/mL had convoluted edges, were interconnected into clusters and had several bud scars. The results suggest that the miconazole affected cell division. The yeast cells treated with 42 µg/mL miconazole tended to maintain their original shape but were partially covered with vesicular material which was also present between the individual cells and appeared to be broken off of the intact cells. The vesicular material was attributed to the fungal cell wall. Figure 6 is a depiction of the effect of various concentration of miconazole on *C. albicans* cells.

**Effect on influx of protons:**
Miconazole was found to cause a rapid release of potassium, amino acids, proteins, from strains of *C. albicans* and a concomitant influx of protons **(**VandenBossche et al., unpublished data). The details of the method and results were not available for review.

**3.2. Activity *in vitro*** ( Spectrum of Activity)

The i*n vitro* activity of miconazole was measured in United Kingdom against several species of *Candida* using The FUNGITEST (Sanofi Diagnostics Pasteur, FDA approved) broth dilution method as well as the CLSI (M27-A3) method of the antifungal drugs. Davey et al., 1998, tested 200 isolates that comprised of 50 isolates each of *Candida albicans* and *C. glabrata*, 20 each of *C. krusei*, *C. parapsilosis*, *C. tropicalis*, and *C. neoformans* and 10 each of *C. kefyr* and *C. lusitaniae*. Of the 200 isolates, 180 were recent clinical isolates, submitted to the Mycology Reference Laboratory, Bristol, United Kingdom, where identification and susceptibility testing were performed. The remaining 20 isolates were obtained from the United Kingdom National Collection of Pathogenic Fungi. Quality control testing was performed by the inclusion of two reference strains, *C. parapsilosis (*ATCC 90018) and *C. krusei* (ATCC 6258), in each batch of broth microdilution tests. Isolates were retrieved from storage in liquid nitrogen and were sub-cultured twice on sabouraud's dextrose agar plates (Ovoid) supplemented with 0.5% (wt/vol.) chloramphenicol. Prior to testing, subcultures on sabouraud's dextrose agar were incubated at 35°C for 24 hours (*Candida spp.*) or 48 hours (*C. neoformans*).

Specific identification was obtained by three methods (1) morphology on corn meal agar plates (Ovoid, Unipart Limited, Basingstoke, England), (2) API 20C (BioMerieux UK Limited, Basingstoke, England) yeast identification systems and (3) Auxacolor (Sanofi Diagnostics Pasteur).

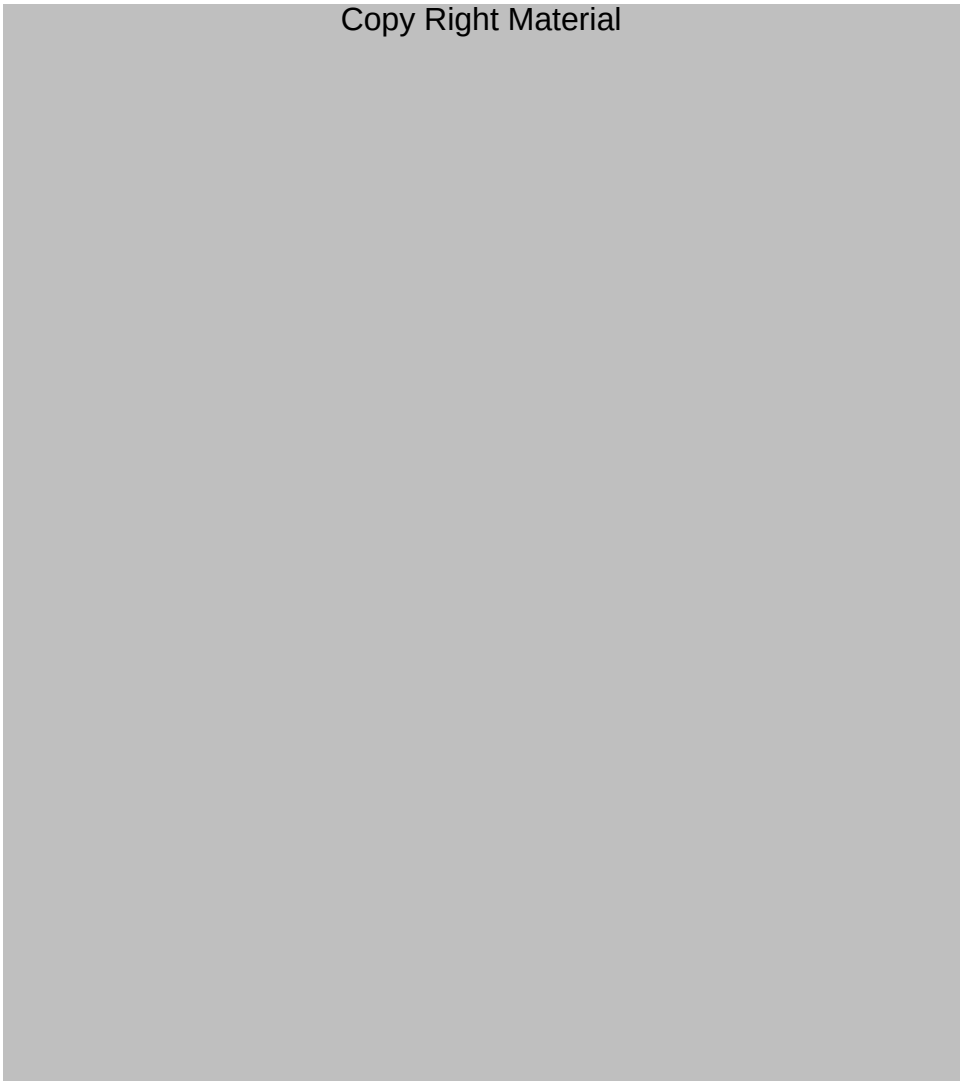
For the CLSI broth microdilution method (M27-A3) miconazole was first dissolved in dimethyl sulfoxide then further diluted in RPMI 1640 with L-glutamine but without bicarbonate (b) (4) The RPMI 1640 was supplemented with glucose (2%), and buffered to pH 7.0 with 35 mg/mL morpholinopropanesulfonic acid (MOPS; (b) (4) The final concentration for miconazole ranged from 0.125 to 64 mg/mL. The yeast cells were suspended in RPMI 1640 medium to a concentration of about 0.5 x $10^3$ to 2.5 x $10^3$ cells/mL. The 96 well round bottom microtiter plates were incubated at 35°C for 48 hours.

For the FUNGITEST method, cell suspensions were prepared in sterile distilled water and were adjusted to a turbidity corresponding to a 1.0 McFarland standard then further diluted in RPMI 1640 to a cell

concentration of $10^3$ cells/mL. A volume of 100 µL of cell suspension was placed in each well of the microplate (Sanofi Diagnostics Pasteur) and incubated at 37°C for 48 hours for *Candida spp.* The authors stated that the two susceptibility tests agreed 84% of the time. Table 1 shows the miconazole MICs for various species of yeasts as measured by the CLSI method. The $MIC_{50}$ and $MIC_{90}$ values against *C. krusei* were higher compared to other *Candida spp.; C. lusitaniae* and *C. kefyr* were the most sensitive species.

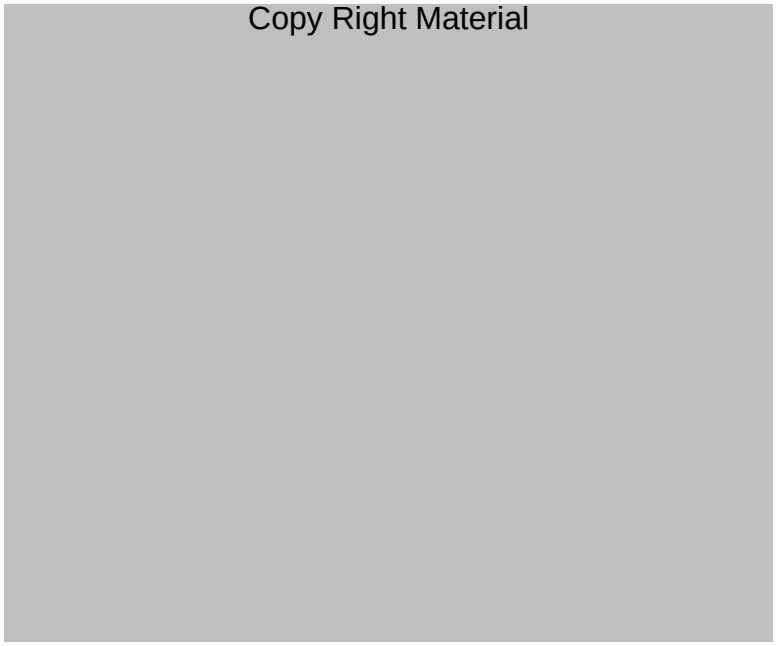


Table 2 shows the results of *in vitro* susceptibilities of the fungal isolates tested to miconazole and other antifungal drugs using the CLSI broth microdilution method and Table 3 shows the percentage of agreement between the FUNGITEST and CLSI microdilution tests.




In another study (Blignaut et al., 2002), *in vitro* activity of miconazole was measured by the CSLI method against 330 oral isolates of *Candida spp.* from HIV/AIDS patients and 224 isolates from non-HIV infected individuals in South Africa. The oral *Candida spp.* isolates from HIV infected individuals were distributed as follows (302 *C. albicans*, 17 *C. krusei*, 4 *C. tropicalis*, 3 *C. parapsilosis*, 2 *C. glabrata*, and 2 *C. dubliniensis*). Of the 224 *Candida spp.* isolates from non-HIV infected individuals 164 were *C. albicans*, 21 *C. krusei*, 8 *C. dubliniensis*, 8 *C. lusitaniae*, 6 *C. parapsilosis*, 7 *C. glabrata*, 5 *C. tropicalis*, 2 *C. rugosa*, 2 *C. guilliermondii*, and 1 *C. kefyr*. Specific identification was made by germ tube and Vitek and stored in 20% glycerol at $-70^0$C. The concentration of yeast cells used was prepared spectrophotometrically and approximated 0.5 to 2.5 $x10^3$ cells /mL. Miconazole was obtained from Sigma Chemical Co. (St Louis, Mo) and the stock solution was diluted in dimethyl sulfoxide (DMSO), polyethylene glycol (PEG) or water. The working solution was diluted in RPMI 1640 medium (Sigma Chemical Co., St Louis, Mo), buffered with MOPS to pH 7.0. Serial dilutions of the antifungal agent, at 2X final concentration, were dispensed into microdilution trays with a Quick Spense II System (Dynatech Laboratories, Chantilly, Va.). Trays were wrapped and stored at -70°C.

The authors stated that the susceptibility tests were incubated at $35^0$C and read at both 24 and 48 hours with the aid of a reading mirror. However, the results included in the publication were based on 48 hour readings. Table 4 shows the results of the *Candida spp.* with miconazole and comparator antifungal drugs and Table 5 provides a summary of the *in vitro* susceptibility tests of the ten species of *Candida* with miconazole. *C. albicans* was the most common species with a MIC ranging between 0.007 and 2.00 µg/mL. *C. krusei* showed the highest MICs.




In a study by Richter et al. (2005), 593 *Candida spp.* vaginal isolates were tested for susceptibility to miconazole and other antifungal agents using the broth microdilution recommended by the CLSI (M27-A3). The *Candida* species tested were distributed as follows *C. albicans* (*n* = 420), *C. glabrata* (*n*=112), *C. parapsilosis* (*n* =30), *C. krusei* (*n*=12), *C. tropicalis* (*n* =8), *C. lusitaniae* (*n*=1). A majority (94.3 to 98.5%) of the yeasts were found to be susceptible to miconazole and other imidazoles at MIC ≤ 1.0 µg/mL. As in the previous studies *C. albicans* was the most prevalent species tested and ranked among the most susceptible species to miconazole with all isolates susceptible at ≤ 0.5 µg/mL of the antifungal agent. The researchers did not provide the $MIC_{50}$ and $MIC_{90}$ values. The higher azole MICs were found against non-*albicans* species with *C. krusei* showing the highest miconazole MICs. The results of the susceptibility tests are presented in Table 6.

Hamza et al., (2008) performed susceptibility tests on 296 clinical oral yeast isolates from 292 HIV oropharyngeal *Candida spp.* infected individuals in Tanzania. The methodology used was a modification of CLSI: M27-A3 method. The modification was that the results were read spectrophotometrically on a microplate reader at 405 nm after 48 hours. All tests were performed in duplicate. The MIC for the azoles, including miconazole, was defined as the lowest concentration of the antifungal agent that inhibited the growth by 50% or 90%. *C. albicans* was the most frequently tested species comprising 84 % of the isolates. Table 7 shows the susceptibility of *Candida species* to six antifungal agents and Table 8 shows the results of susceptibility tests of the same *Candida spp* isolates to miconazole.

In 1972 VanCutsem and Thienpont reported testing of 13 strains of yeast from 6 species of *Candida* for determination of the fungistatic effect of various concentrations of miconazole, 0.001, 0.01, 0.1, 1.0, 10, 100, and 1000 µg/mL. The *Candida* species tested were *C. albicans, C. krusei, C. parapsilosis, C. tropicalis, C. pseudotropicalis, C. stellatoidea.* All of the *Candida* species were completely inhibited by 10 µg/mL of miconazole except *C. tropicalis* which was inhibited by 100 µg/mL. The test was performed using sabouraud's broth with glucose. Miconazole was added to the broth to obtain the concentrations listed above. Growth was scored from 0 to 4 with 0 representing complete absence of growth and 4 maximum growth. The amount of growth in the test was determined by comparison with the growth in the drug free control. The authors did not provide the temperature or length of incubation of the cultures. Figure 7 shows the results of the experiment. There was no growth of *C. albicans* at 10 µg/ ml of miconazole. All other species of *Candida* except *C. tropicalis* were inhibited by 10 µg/ mL.

In another study by Jansen Pharmaceutical (Fothergill, A.W., 2006), *Candida* species were shown to be generally susceptible to miconazole. Table 9 shows the susceptibility of *Candida spp.* to miconazole. The $MIC_{90}$ in this study which used CLSI methodology (M27- A2) is higher than the other studies performed above while that for *C. glabrata* (CLSI) is the same. The isolates were collected from non-HIV patients.



The susceptibility of oral *Candida* isolates was assessed using seven antifungal agents (Kuriyama et. al., 2005). The 618 isolates in the study were cultured from 553 patients with a variety of oral diseases. The antifungal agents used in this study were fluconazole, itraconazole, voriconazole, ketoconazole, miconazole, amphotericin B, and nystatin. The isolates were cultured on sabouraud's dextrose agar at $37^0$C for 48 hours and identified using API 32C (BioMerieux, Basingstoke, UK) or the Auxacolor 2 (BioRad, Marnes-la-Coquette, France) system. The MIC was determined using a broth microdilution CLSI M27-A3 method. The range for testing itraconazole, voriconazole, ketoconazole, miconazole, amphotericin B and nystatin was 0.015–16 µg/mL, for fluconazole concentrations tested was 0.06–64 µg/mL.

The authors used the following resistance breakpoints (CLSI or values set from previous investigations by Blignaut, et. al, 1995, Davey, et. al., 1998, Espinel-Ingroff et. al., Kronvall, et. al., 2001):

- fluconazole : resistant, ≥ 64 µg/ml; susceptible dose dependent, 16–32µg/ml; susceptible, ≤ 8 µg/ml;
- itraconazole: resistant, ≥ 1µg/ml; susceptible dose dependent; 0.25–0.5 µg/ml; susceptible ≤ 0.125 µg/ml;
- voriconazole: resistant, ≥ 8 µg/ml; susceptible dose dependent, 2–4 lg/ml; susceptible, ≤1 µg/ml;
- ketoconazole: resistant, ≥ 4 µg/ml;
- miconazole: resistant, ≥ 8 µg/ml;
- amphotericin B: resistant, ≥ 2 µg/ml;
- nystatin: resistant,≥ 16 µg/mL.

Table 10 shows the *in vitro* susceptibility profiles to the seven antifungal agents (Kuriyama et. al., 2005). The most common fungus tested was *C. albicans.* Only 0.3% of the 521 isolates of *C. albicans* were resistant to fluconazole and voriconazole; none were resistance to miconazole. The only *Candida spp.* that showed resistance to miconazole was *C. krusei.* One percent of *C. albicans* was resistant to itraconazole.

Overall, the results show miconazole $MIC_{90}$ to be < 1.0 µg/mL against most of the *Candida* species except *C. krusei, C. guilliermondii,* and *C. tropicalis.* The $MIC_{50}$ and $MIC_{90}$ of *C. albicans,* were 0.03 µg/mL and 0.56 µg/mL, respectively. The $MIC_{90}$ for *C. krusei* was the highest of all the *Candida* species isolated. For some species the number of isolates tested was small and this precluded determination of $MIC_{90}$ values.

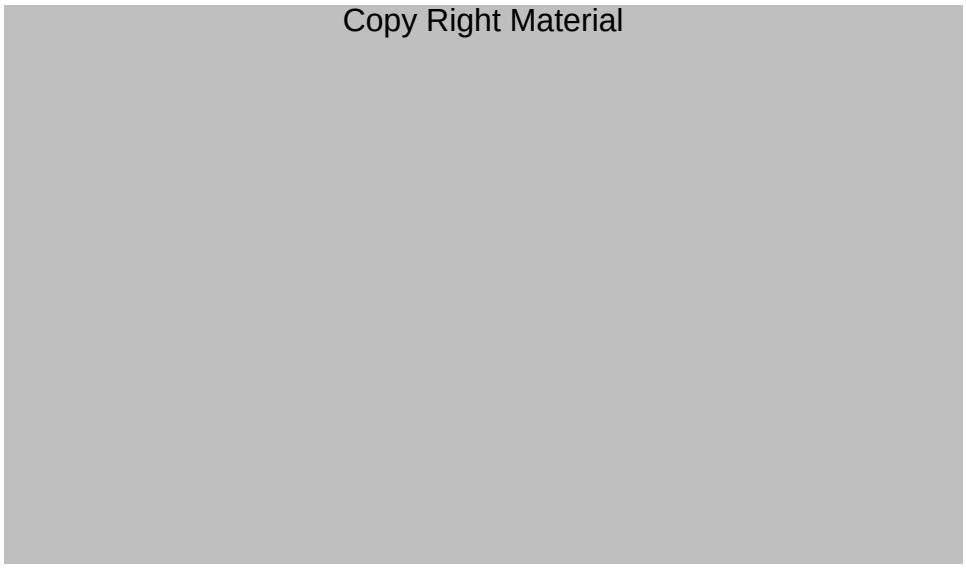
### 3.3. Activity *in vivo* (ANIMAL STUDIES)

Adult albino guinea pigs weighing approximately 700 g were used to study the effect of miconazole and other imidazoles in cutaneous *Candida* animal model (VanCutsem and Thienpont, 1972). The animals were first given 200 mg/kg of alloxan intramuscularly. The back of each animal was electrically clipped and 0.25 mL of a 48 hour culture of *C. albicans* in sabouraud's broth was applied to the intact skin. Miconazole was mixed with 60% and 40% of polyethylene glycol to give a concentration of 2% and 0.5% of the antifungal agent. In one group of animals, about 1 gm of this ointment was applied each day to the skin for 14 days. For topically treated animals, treatment was initiated one day after the application of the yeast on the skin. Another group of animals infected in the same way as the group of guinea pigs treated topically was also treated orally for 14 days with a dose of 160, 40 or 10 mg/kg body weight of the antifungal agent suspended in polyethylene glycol 200. For orally treated animals miconazole was started two days prior to the application of *C. albicans* to the skin for prophylactic purposes. The authors state that miconazole treatment using the same topical or oral formulations where relevant as described above began at day 3 after the infection of the skin with *C. albicans.* However, the study design appeared to be for evaluation of prophylactic activity. The total duration of the study was 42 days but the infections were assessed on a weekly basis.

Assessments were made using a 4 point scoring system for infectious lesions. The scoring system was compared to the lesions formed on the control untreated animal. The numerical codes were as follows:

0 indicated no infection,
1 represented lesions that were 1/4 of those of the control
2 represented lesions that were 1/2 of those of the control
3 represented lesions that were 3/4 of those of the control animal.

Infection was also assessed by microscopic examination and by culture. Table 11 shows the results of treatment on day 15, of *Candida spp.* infected animals and controls, with different concentrations of miconazole. For the topical treatment with 2% miconazole by day 15, 1 of the 20 animals was cured and 11 had score 1 lesions. The author states that 9 of the 10 guinea pigs were completely cured at the end of the experiment and the remaining ten had very small lesions (score1). Miconazole administered orally was effective against the *C. albicans* induced lesions. Ten of the 12 animals that received 160 mg/kg of miconazole were cured after nine days and all of the animals had recovered by 21 days. Nine of the 14 that received 40 mg/kg of miconazole were cured by day 15 and the authors state that 13 of 14 had recovered by day 28. Only slight to marginal effects were observed in the animals treated orally with 10 mg/kg of miconazole.




### 3.4. Drug Resistance

Fothergill (2006) stated that cross resistance mechanisms found in various classes of fungi did not appear to exist with miconazole. Details of experimental method and results were not available for review. The authors state that a *C. albicans* isolate from a vulvovaginal specimen was tested for *in vitro* susceptibility to miconazole. The isolate had resistance to fluconazole (MIC > 64 µg/mL) and itraconazole (MIC >16 µg/mL) but the MIC for miconazole remained at 0.125 µg/mL. Attempts to induce an increase in MIC for miconazole by culture on media with consistently higher concentrations of miconazole has not been successful. It was felt that this could be due to an additional mechanism by which miconazole exhibits it antifungal effect i.e., the inhibition of peroxidases by miconazole nitrate, compared to other azoles.

## 4. CLINICAL MICROBIOLOGY

### 4.1. Description of studies

The applicant conducted three clinical studies (BA2004/01/04, BA2002/01/02, and BA2002/01/03) to support the efficacy of Oravig.

#### 4.1.1. Study No. BA/2004/01/04

The pivotal study BA2004/01/04 was a multicenter, randomized, placebo-controlled, double-blind, double-dummy study in HIV patients with OPC that compared the clinical efficacy of a daily dose of miconazole BT (50 mg for 14 days) with 10 mg troches of clotrimazole administered 5 times a day for 14 days. The study was carried out in 30 sites in the U. S.A., Canada and South Africa. Twenty-eight of the sites randomized the patients. Patients were randomly assigned to each of the two treatment groups so that the groups were approximately equally distributed. Each patient made 5 scheduled visits to the clinic. Patients were screened on Visit 1 (screening) and treatment commenced at Visit 2 (day 1). The patients were evaluated at Day 7 (days 6-8, visit 3), days 14 – 15 (visit 4), days 17-22 (visit 5; TOC visit) and followed for 21 days after the last dose (visit 6). The primary endpoint was clinical cure at the end of treatment. Efficacy was defined as the complete or partial disappearance of oropharyngeal candidiasis lesions. Mycological eradication was a secondary endpoint and was tested after 14 days of treatment.

The inclusion criteria include:

- Patients with a clinical manifestation of OPC as indicated by creamy white curd-like patches on the oral mucosal surface that can be removed by scraping or a typical erythematous lesion on the oral mucosa.
- Confirmation of diagnosis by potassium hydroxide preparation and culture. If, the culture was negative even though the KOH preparation was positive, the patient was excluded from the analysis.
- Documented HIV sero-positivity prior to enrollment.
- Patients be ≥ 18 years and sign a consent form.

The exclusion criteria include:

- Upper full or partial dentures with an acrylic border in the canine fossa.
- Signs or symptoms of systemic candidiasis (candidemia or invasive infection).
- Patients who (i) had taken a systemic antifungal agent within the past 15 days or a local antifungal agent within the past 7 days, (ii) are being treated simultaneously with agents that are likely to interfere with pharmacokinetics of miconazole such as antiarrhythmics, anticoagulants (antivitamin K type, acenocoumarol, and warfarin), astemizole, cisapride, and phenytoin, (iii) participated in another therapeutic trial that evaluated new drugs, or (iv) had taken drugs which could interfere with the evaluation of the drug in this study within the preceding 30 days such as undergoing antibiotic treatment at inclusion, except prophylactic antibiotics in the management and care of HIV (such as trimethoprim-sulfamethoxazole) and anti-tuberculosis treatments for stable tuberculosis.
- Patients allergic to milk or who have shown hypersensitivity to one of the ingredients of the products into which these antibiotics are incorporated or have a hereditary problems of galactose intolerance, lactase enzyme deficiency, or glucose galactose malabsorption, hepatocellular deficiency defined by international normalized ratio (INR) >1.7.
- Procedural factors such as patients who were unable to complete the diary or to adhere to protocol procedures, and patients who had a life expectancy of under 45 days or a clinical condition that may not allow them to complete the study.

**Removal of Patients from analysis**

Termination prior to the test of cure (TOC) on visit 5 (early termination) could occur under a number of conditions. Of relevance to microbiology

- Either voluntarily by the patient or by investigator primarily for the well being of the patient or violation of the protocol, or loss of contact. Patients who withdrew from the trial or were terminated by the investigator were allowed to continue to receive treatment.
- Patients with samples that resulted in a baseline KOH preparation positive but were culture negative.

**Definitions**
**Clinical Response:**
The lesions and sign and symptoms were scored as follows:

Clinical cure: if there were no symptoms of OPC defined by a score of 0 for the extent of lesion in combination with a score for the signs and symptoms of 0 at the TOC visit (Days 17-22).

Non-responders: a patient whose lesions had progressed to a more visible lesion with a higher score, or a patient whose lesion had not reduced at the TOC (Days 17 – 22).

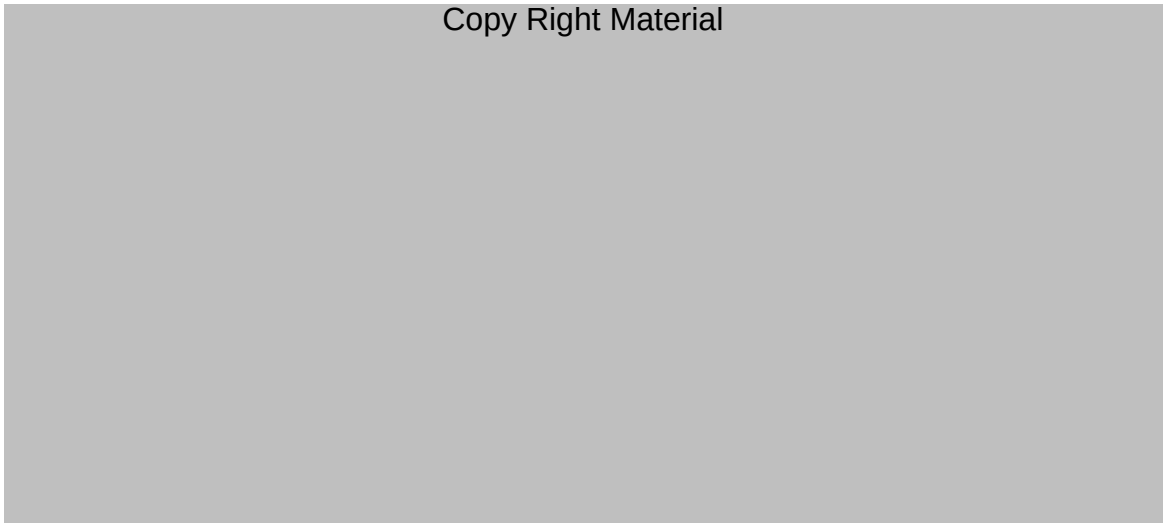
Clinical improvement: a complete resolution of visible extent of lesion score to 0 and minimal symptoms.

Clinical failure: a patient who failed to be completely cured by Day 17 to 22.

Relapse: was defined as the state of a patient who responded to treatment by clinical cure or improvement at TOC but subsequently had an increase in the extent of the lesion or symptoms by post-therapy follow up.

Global assessment was based on a change in total signs and symptoms from those seen at baseline.

**Mycological measurements and response:**
The fungi were cultured on CHROMagar *Candid*a (Becton Dickinson) medium. CHROMagar is a selective and differential medium used for the isolation and differentiation of some species of *Candida* by colonial morphology e.g. *C. albicans, C. krusei* and *C. tropicalis.* On CHROMagar *C. albicans* produces light to medium green colonies, *C. krusei* produces flat, mauve colonies and *C. tropicalis* blue colonies. *C. glabrata* also produces mauve colonies. Inoculated plates are incubated at $37^0$C for 48 hours. The colonies are counted after 48 hours. Negative cultures were re-incubated and read after 3 days. Growth was initially identified by colonial color on CHROMagar and then API *Candida* (Becton Dickinson). Figure 8 shows a variety of *Candida* spp. colonies on CHROMagar. Susceptibility tests are performed by use of the CLSI protocol (M27-A3) broth microdilution method.




Identification of the yeasts was done at the local laboratories and susceptibility testing of yeast isolates was performed at a central laboratory (b) (4) (b) (4) by the CLSI method (M27 A3). *In vitro* susceptibility to miconazole and clotrimazole was determined for all baseline isolates, any isolates obtained at the TOC visit (visit 5), and isolates from patients with late relapse at day 35-38 (visit 6). Two swabs were collected from each subject. One of the swabs was used for direct microscopic examination and the other for fungal culture.

At the local laboratory, testing comprised of microscopic examination of scraping of an oral lesion by a potassium hydroxide (KOH) smear test. Microscopy was used to provide semi-quantitative results. Fungal elements were visualized microscopically by the use of KOH preparations. Each preparation was examined using either the 40X or 100X magnification. The results were quantitated using the 10X magnification. The results were reported semi- quantitatively < 10, 10 - 50, or > 50 cells per low power field. If filamentous forms were seen their presence was reported. Samples that indicated the presence of a *Candida spp.* were confirmed by culture.

Fungal cultures were performed routinely at Day 0 (before first treatment), and at the TOC visit (Days 17-22) in all patients. Cultures were also performed at Day 7 (days 6-8) in cases of clinical cure or clinical improvement, and during the follow-up period at Day 35 (Days 35-38) in cases of relapse. Mycological cure was evaluated at the TOC visit (Days 17-22, visit 5). The combination of mycological cure associated with clinical response was considered as overall response and also evaluated at the TOC visit (Days 17-22, visit 5). The mycological responses were defined as follows:

- Negative culture: no fungal growth on the culture plates or
- Mycologic cure: the isolation of less than 10 colonies of *Candida spp.* from the culture of a swab of the oral cavity. The criteria used for establishing such a threshold for mycological cure are unclear. Additionally, when more than one species was present at baseline, mycological success required absence (eradication) of all of those species at the time of evaluation.
- Persistent pathogen: baseline pathogen isolated at the time of test of cure (TOC) visit.
- New infection: the presence at the TOC visit of a *Candida spp* that was not present at baseline or the same species present at baseline but with a different susceptibility pattern.
- No result: patients who did not have a test at TOC visit.
- Overall responder: a patient who was both clinically and mycologically cured.

**Results:**

The primary population for this study was the intent-to-treat (ITT) population (n=564) comprising of 283 patients in the miconazole arm and 281 in the clotrimazole arm; the per protocol (PP) population (n=476) comprised of 240 subjects in the miconazole arm and 236 in the clotrimazole arm) who harbored pathogens identified at baseline. A single *Candida* species was isolated at baseline in a majority of the patients, 2 pathogens were isolated in 4 patients in the miconazole arm and 2 in the clotrimazole treatment arm. A non-*Candida* species *(Tricosporon mucoides)* was isolated in one of the patients in the clotrimazole arm and was excluded from analysis (Table 12).

Table 12. PP patient population and number of baseline pathogens isolated

| | Treatment Group | |
|---|---|---|
| | **Miconazole Lauriad® 50 mg MBT N (%)** | **Clotrimazole 10 mg N (%)** |
| **PP Population (Subjects) *** | **N = 240** | **N = 236** |
| Single Baseline Pathogen | 236 (98%) | 234 (99%) |
| Two Different Baseline Pathogens | 4 (2%) | 2* (1%) |
| **Total Baseline Pathogens:** | **244** | **238** |

* one of the dually infected subjects was infected with *C. tropicalis* and *Tricosporon mucoides* therefore only the *C. tropicalis* was counted

Table 13 lists the *Candida* species isolated in the PP population and the clinical and mycological response by baseline pathogen at visit 5. At visit 5, the results in Table 14 show that for patients who had a single *Candida* species at baseline and clinical cure in the miconazole arm was 67% (161/240**)** and mycological cure 31 % (74/240). In the clotrimazole arm the clinical and mycological responses were 73% (173/236) and 31 % (62/236), respectively. *C. albicans* was the most common species isolated. Patients with *C. tropicalis* had an 81% (17/21) clinical cure rate compared to 43 % mycological cure rate for miconazole and a 50% clinical as well as mycological cure rate for clotrimazole.

Overall the clinical cure rate was at least twice as high as the mycological cure rate, except for *C. tropicalis* in the clotrimazole arm at TOC visit where both the clinical and mycological cure rates were the same (50%). The disparity between the clinical cure and the mycological cure rates might be related to the fact that many infecting *Candida* species are normal flora or commensals in the body and may become pathogenic only in immuno-compromised hosts therefore normally these species are found in numbers that exceed the upper limit for mycological cure. The fungal burden increases during an infectious process but even though a reduction occurs after treatment the yeasts are not necessarily totally eradicated. None of the subjects with multiple baseline pathogens was mycologically cured.

For patients with mixed infections, a favorable clinical response for each subject required that both of the baseline pathogens be cured. The response to treatment by subjects from whom more than one baseline pathogen was isolated differed for each pathogen in the pair. None of the patients with multiple infections received a mycological cure by treatment either with miconazole (n=4) or clotrimazole (n=1). However, 4/5 subjects infected with multiple species were clinically cured. One subject with a baseline infection of *C. albicans* and *C. krusei* and treated with miconazole was neither clinically nor mycologically cured.

Table 13. Clinical and mycological response, at visit 5, for PP patients in BA2004/01/04 study in the Miconazole and Clotrimazole arms

| ***Candida spp.*** | **Clinical Response (n= 240)** | | **Mycologic Eradication** (n= 240) | **Clinical Response (n=236** | | **Mycologic Eradication** (n=236)* |
|---|---|---|---|---|---|---|
| | **Miconazole** | | | **Clotrimazole** | | |
| *C. albicans* | F | 133/205(65%) | 60/205 (29%) | F | 156/210 (74%) | 52/210 (25% |
| | UF | 72/205 (35%) | 145/205(71%) | UF | 54/210 (26%) | 158/210 (75%) |
| *C. dubliniensis* | F | 1/2 | 1/2 | F | 0/2 | 0/2 (0%) |
| | UF | 1/2 | 1/2 | UF | 2/2 | 2/2 |
| *C. guilliermondii* | F | 2/2 | 1/ 2 | F | 1/2 (50%) | 0/2 (0%) |
| | UF | 0/2 | 1/2 | UF | 1/2 (50%) | 2/2 |
| *C. lusitaniae* | F | 0 | 0 | F | 1/1 | 0/1 |
| | UF | 0 | 0 | UF | 0/1 | 1/1 |
| *C.parapsilosis* | F | 5/6 (83%) | 3/6 (50%) | F | 8/8 (100%) | 4/8 (50%) |
| | UF | 1/6 (17 %) | 3/6 (50%) | UF | 0/8 | 4/8 (50%) |
| *C. tropicalis* | F | 17/21 (81%) | 9/21 (43%) | F | 6/12 (50%) | 6/12 (50%) |
| | UF | 4/21 (19%) | 12/21 ( 57. %) | UF | 6/12 (50%) | 6/12 (50%) |
| **Total** | **F** | **158/236 (67%)** | **74/236 (31%)** | **F** | **172/235 (73%)** | **62 /235 (26%)** |
| | **UF** | **78/236 (33%)** | **162/236 (69%)** | **UF** | **63/235 (27%)** | **173/235 (74%)** |
| *C. albicans* + *C. dublineinsis* | F | 0 | | F | 1/1 | 0/1 |
| | UF | 0 | | UF | 0/1 | 1/1 |
| *C. albicans* + *C. guilliermondii* | F | 1/1 | 0/1 | F | | |
| | UF | 0/1 | 1/1 | UF | | |
| *C. albicans* + *C..krusei* | F | 1/2 | 0 /2 | F | | |
| | UF | 1/2 | 2/2 | UF | | |
| *C. albicans* + *C. tropicalis* | F | 1/1 | 0/1 | F | | |
| | UF | 0/1 | 1/1 | UF | | |
| **Total** | **F** | **161/240 (67%)** | **74/240 (31%)** | **F** | **173/236 (73%)** | **62/236(26%)** |
| | **UF** | **78/240 (33%)** | **166/240 (69%)** | | **63/236 (27%)** | **174/236 (74%)** |

F = favorable response. UF = unfavorable response * *T. mucoides* excluded.

Mycological relapse occurred only in subjects that demonstrated baseline infection with *C. albicans* (23% in the subjects treated with either miconazole or clotrimazole). By visit 6 new infections had occurred with *C. albicans, C. glabrata, C. guilliermondii, C. parapsilosis* and *C. tropicalis;* there were 12 new infections in the miconazole treatment arm and 8 in the clotrimazole treatment arm. The relapse and new infection rates by species are shown in Table 14.

Table 15 shows the time of first isolation of *C. glabrata*, the *in vitro* susceptibility of each isolate to miconazole and the susceptibility patterns of the isolates. At visit 5 there were three isolates and at visit 6 there were two isolates of *C. glabrata.*

Table 14. Relapse or new infection by pathogen treated with Miconazole 50 mg MBT and 10 mg and Clotrimazole in the PP population.

| ***Candida species*** | **Relapse or New Infection** | | | |
|---|---|---|---|---|
| | **Miconazole** | | **Clotrimazole** | |
| | **Relapse** | **New infection** | **Relapse** | **New Infection** |
| *C. albicans* | 31/133 (23%) | 3 | 36/156 (23%) | 4 |
| *C. guilliermondii* | - | 2 | - | - |
| *C. parapsilosis* | - | 2 | - | - |
| *C. tropicalis* | - | 3 | - | 1 |
| *C. glabrata* | - | 2 | - | 3 |
| **Mixed infections** | | | | |
| *C. albicans +C. tropicalis* | 1 | - | - | - |
| Total | 32/161 (20%) | 12/240 (5%) | 36/173 (23%) | 8/236 (3%) |

Table 15. *C. glabrata* BA2004/01/04 Time of first isolation and susceptibility test results

| Species | Subject ID # | Treatment | Visit number | Susceptibility test Result |
|---|---|---|---|---|
| *C. glabtata* | BA04-101-0003 | Clotrimazole | 5 | 0.03 |
| *C. glabtata* | BA04-105-0012 | Miconazole | 5 | 0.015 |
| *C. glabtata* | BA04-105-0017 | Clotrimazole | 5 | 0.03 |
| *C. glabtata* | BA04-101-0013 | Clotrimazole | 6 | ND |
| *C. glabtata* | BA04-101-0067 | Miconazole | 6 | ND |

Visit 5 = TOC. Visit 6 = Late post-therapy visit . ND = not done

The *in vitro* susceptibility tests were performed at the central laboratory as stated above. Susceptibility results were reported on 481 isolates for the two arms. Repeat specimen analysis was done for some subjects. The susceptibility range for *C. albicans* species for miconazole (0.002 – 4 µg/ mL) at baseline was wider than that for clotrimazole versus (0.001 – 0.5 µg/ mL; Table 16). The $MIC_{90}$ against *C. albicans* isolates collected at baseline was slightly higher at TOC visits (0.06 µg/mL and 0.05 µg/mL, respectively). The miconazole $MIC_{90}$ for the isolates collected from patients in this study are in the same range as the $MIC_{90}$ values summarized in the "activity *in vitro"* section 3.2. In the clotrimazole arm, the clotrimazole $MIC_{50}$ of *C. albicans* isolates collected at baseline and TOC visits were also similar. The susceptibility tests for *C. tropicalis* for clotrimazole were performed on only 12 isolates.

Overall, the clinical and mycological responses in subjects with oropharyngeal candidiasis due to *Candida spp.* suggests the clinical cure rate is significantly higher than the mycological cure rate and that there was no correlation between the clinical and mycological cure rates. The most common infecting species was *C. albicans* comprising 87% of the infections. This was followed by *C. tropicalis* (7%). There were 4 cases with dually mixed infections with *Candida spp.,* in all of which *C. albicans w*as one of the pairs.

Table 16. Susceptibility patterns of baseline *Candida spp.* by treatment arm and response to treatment by miconazole and clotrimazole for the PP population for study BA2004/01/04

| ***Candida spp.* isolated** | **Day -14 to 0 Miconazole Oravig (µg/mL)** | | | | **Day 17 to 22 Miconazole (µg/mL)** | | |
|---|---|---|---|---|---|---|---|
| | **PP (n=244)** | **Range** | **$MIC_{50}$** | **$MIC_{90}$** | **Range (n=178)** | **$MIC_{50}$** | **$MIC_{90}$** |
| *C. albicans* | 209 (85.7%) | 0.002 - 4 | 0.015 | 0.06 | 0.008 – 0.05 | 0.015 | 0.05 |
| *C. dublineinsis* | 2 (0.8%) | 0.002 – 0.015 | 0.008 | - | 0.002 – 0.03 | 0.008 | 0.03 |
| *C. famata* | 0 (0%) | - | - | - | - | - | - |
| *C.glabrata* | 0 (0%) | | | | 0.015 | | - |
| *C. guilliermondii* | 3 (1.2%) | 0.008 – 0.015 | - | - | 0.008 – 0.5 | | - |
| *C.krusei* | 2 (0.8%) | 0.06 – 1 | - | - | - | - | - |
| *C.lusitaniae* | 0 (0%) | - | | | - | | |
| *C.parapsilosis* | 6 (2.5%) | 0.008 – 0.5 | 0.25 | 0.25 | 0.015 – 0.5 | 0.06 | 0.25 |
| *C. tropicalis* | 21 (9.0%) | 0.008 – 1 | 0.25 | 1 | 0.015 – 0.5 | 0.03 | 0.25 |
| ***Candida spp.* isolated** | **Day 0 Clotrimazole** | | | | **Day 17 to 22 Clotrimazole** | | |
| | **PP (n=237)** | **Range** | **$MIC_{50}$** | **MIC $_{90}$** | **Range (n=186)** | **$MIC_{50}$** | **$MIC_{90}$** |
| *C. albicans* | 211 (88.7%) | 0.001 – 0.5 | 0.008 | 0.015 | 0.001 – 2 | 0.008 | 0.03 |
| *C. dublineinsis* | 3 (1.3 %) | 0.004 – 0.008 | - | - | 0.004 – 0.015 | 0.008 | 0.015 |
| *C. famata* | 0 (0%) | - | - | - | - | - | - |
| *C. glabrata* | 0 (0%) | - | - | - | - | - | - |
| *C. guilliermondii* | 2. (0-.8%) | 0.004 -0.03 | - | - | - | - | - |
| *C. krusei* | 0 ((0%) | - | - | - | 0.004 – 0.06 | - | - |
| *C.lusitaniae* | 1 (.0.4%) | - | | - | 0 | - | - |
| *C.parapsilosis* | 8 (3.4%) | 0.004 – 0-.03 | 0.008 | 0.03 | 0.008 – 0.015 | - | - |
| *C. tropicalis* | 12 (5.0%) | 0.004 – 0.5 | 0.03 | 0.12 | 0.004 – 0. 008 | 0.006 | 0.008 |

### 4.1.2. Study No. 2002/01/02

This was an open-label, randomized, 2-arm parallel-groups, multicenter phase III clinical trial comparing the efficacy and safety of miconazole Oravig® 50 mg (for 14 days) BT with that of miconazole gel. This study was undertaken for the treatment of oropharyngeal candidiasis in patients with head and neck cancer who underwent radiotherapy. The study was expected to be conducted in 57 centers in France and 11 in North Africa. Of these centers, 24 sites were in France and 9 in North Africa. In order to substitute for a blind, double dummy design which would have introduced biases, clinical efficacy was assessed blindly at each center by inclusion of a health care worker who was unaware of the treatment administered to each patient. The primary end-point was clinical cure at Day 14. Mycological cure was the secondary endpoint which was assessed by culture on Day 14.

Inclusion and exclusion criteria as well the evaluation of patients for clinical and mycological response were similar to Study BA 2004/01/04. Samples for mycological testing were taken at the pre-inclusion screening. Patients were included in the study on Day 1 without the culture results if the direct examination was positive. If, however, the direct examination was negative, a positive culture with > 100 colonies was required for patient enrollment. Table 17 shows the schedule for mycological microscopic investigations.

Both microscopic examination and fungal cultures were performed in the pre-inclusion phase Day 0 whereas only cultures were performed at Day 14 the end of treatment.

Table 17. Schedule for mycological investigations for PP in BA2002/01/02

| | | Period of treatment | | | Post-treatment follow-up | |
|---|---|---|---|---|---|---|
| | D0 (pre-inclusion) | D1 (inclusion) | D7 | D14 (end of treatment) | D30 | D60 |
| Mycological direct examination | X | - | - | - | - | - |
| Fungal cultures | X | - | X (if score 0) | X | X (if relapse or progression) | X (if relapse or progression) |

**Results:**

The total number of subjects included in the study was 213. Of these, 73 (34%) patients were recruited in France, and 140 (66%) patients in North Africa. Of the 140 patients recruited from North Africa 71 (51%) were from Tunisia, 55 (39%) from Morocco and 14 (10%) from Algeria. Table 18 shows the distribution by the country of origin of the patients included in the study.

Table 18. Distribution of BA2002/01/02 patients by country of origin

| Country | Miconazole Oravig | Miconazole gel | Total |
|---|---|---|---|
| Algeria | 7 (6.54%) | 7 (6.60%) | 14 (6.57%) |
| Morocco | 29 (27.10%) | 26 (24.53%) | 55 (25.82%) |
| Tuinisia | 34 (31.78%0 | 37 (34.91%) | 71 (33.33%) |
| Subtotal North Africa | 70 (65.42%) | 70 (66.03%) | 140 (65.73%0 |
| France | 37 (34.58%) | 36 (33.95%) | 73 (34.27%) |
| Total | 107 | 106 | 213 |

Table 19 shows the number of pathogens isolated at baseline from patients treated with either the miconazole BT and miconazole gel. *C. albicans* accounted for 72% and 61% of the isolates in the miconazole BT and the miconazole gel arms, respectively with *C. tropicalis* a distant second (8%). One of the unique features of this study is the number and variety of the mixed infections; there were 17 dually infected subjects and four subjects with triple infections of *Candida species. C. albicans* was implicated in 10 of the mixed infection. Overall, the results show that 47% of the subjects in the miconazole BT arm and 69% subjects in the miconazole gel arm were mycologically cured. Clinical cure was reported in 57% and 59% of the subjects, respectively. Like the study BA2004/01/04, mycologically cure rates were lower than the clinical cure rate in the BT arm. As in the BA2004/01/04, there appeared to be no correlation between clinical and mycological cure rates although the difference was not as great in this study.

Table 19. Clinical and mycological response for patients in BA2002/01/02study treated with Miconazole 50 mg buccal tablet and Miconazole gel 500 mg/day

| ***Candida spp.*** | **Clinical Response (n= 107)** | | **Mycologic Eradication (n=107)** | **Clinical Response (n=103)** | | **Mycologic Eradication (n=103)** |
|---|---|---|---|---|---|---|
| | **Miconazole 50mg buccal tablet** | | | **Miconazole gel 500mg/day** | | |
| *C. albicans* | F | 44/77 (57%) | 39/77 (51%) | F | 38/63 (60%) | 42/63 (67%) |
| | UF | 33/77 (43%) | 38/77 (49%) | UF | 25/63 (40%) | 21/63 (33%) |
| *C. famata* | F | 0/1 | 1/1 | F | 1/ 2 | 2/2 |
| | UF | 1/1 | 0/1 | UF | 1/2 | 0 /2 |
| *C.glabrata* | F | 0/1 | 1/1 | F | 0 | 0 |
| | UF | 1/1 | 0/1 | UF | 0 | 0 |
| *C. guilliermondii* | F | 1/1 | 0/1 | F | | |
| | UF | 0/1 | 1/1 | UF | | |
| *C. krusei* | F | 2/3 | 0/3 | F | 0/3 | 3/3 |
| | UF | 1 /3 | 3/3 | UF | 3/3 | 0/3 |
| *C. parapsilosis* | F | 1/2 | 0/2 | F | 1/1 | 0/1 |
| | UF | 1/2 | 2/2 | UF | 0/1 | 1/1 |
| *C. tropicalis* | F | 7/9 (78%) | 4/9 (44%) | F | 10/12 (83%) | 9/12 (67%) |
| | UF | 2/9 (22%) | **5/9 (56%)** | UF | 2/12 (17%) | 3/12 (33%) |
| *C. kefyr* | F | 0/1 | 1/1 | F | 1/3 | 3/3 |
| | UF | 1/1 | 0/1 | UF | 2/3 | 0/3 |
| *C. lipolytica* | F | 0 | 0 | F | 0/1 | 1/1 |
| | UF | 0 | 0 | UF | 1/1 | 0/1 |
| *C. intermedia* | F | 1/ 1 | | F | | |
| | UF | 0/1 | 1/1 | UF | | |
| *C. rugosa* | F | 0/1 | 0/1 | F | | |
| | UF | 1/1 | 1/1 | UF | | |
| *Candida spp not identified* | F | 1/3 | 1/3 | F | 1/ | 1/3 |
| | UF | 2/3 | 2/3 | UF | 2/3 | 2/3 |
| **Total** | **F** | **57/100 (57%)** | **47/100 (47%)** | **F** | **52/88 (59%)** | **61/88 (69%)** |
| | **UF** | **43/100 (43%)** | **53/100 (53%)** | **UF** | **36/88 (41%)** | **27/88 (31%)** |
| **Mixed Species** | | | | | | |
| *C. albicans + C. krusei* | F | | | F | 0/2 | 1/2 |
| | UF | | | UF | 2/2 | 1/2 |
| *C. albicans + C. tropicalis* | F | | | F | 2/4 | 3/4 |
| | UF | | | UF | 2/4 | 1/4 |
| *C. albicans + C. glabrata* | F | | | F | 1/2 | 1/2 |
| | UF | | | UF | 1/2 | 1/2 |
| *C. albicans+ C. krusei +* | F | 0/1 | 1/1 | F | | |
| *C. tropicalis* | UF | 1/1 | 0/1 | UF | | |
| *C. albicans + C. glabrata + C.* | F | | | F | 1/1 | |
| *tropicalis* | UF | | | UF | | 1/1 |
| *C. albicans + C..rhodotorula rubra* | F | 1/1 | 0/1 | F | | |
| | UF | 0/1 | 1/1 | UF | | |
| *C. albicans + C .kefyr +* | F | | | F | 0 | 1/1 |
| *C. krusei* | UF | | | UF | 1/1 | 0 |
| *C glabrata + C. krusei* | F | 1/1 | 0/1 | F | 0/1 | 0/1 |
| | UF | 0/1 | 1/1 | UF | 1/ 1( | 1/ |
| *C. glabrata + S. cerevisiae* | F | 0/1 | 0/1 | F | | |
| | UF | 1/1 | 1/1 | UF | | |
| *C. kefyr + C. krusei* | F | 0/1 | 0/1 | F | 1/1 | 0/1 |
| | UF | 1/1 | 1/1 | UF | 0/1 | 1/1 |
| *C. krusei + S. cerevisiae* | F | 1/1 | 0/1 | F | | |
| | UF | 0/1 | 1/1 | UF | | |
| *C.kefyr + C. krusei + C. rugosa* | F | 0/1 | 0/1 | F | | |
| | UF | 1/1 | 1/1 | UF | | |
| *C. kefyr + C. valida* | F | | | F | | |
| | UF | | | UF | 1/1 | 1/1 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *C. glabrata + C tropicalis* | F | | | F | | |
| | UF | | | UF | 1/1 | 1/1 |
| *C krusei + C. parapsilosis* | F | 1/1 | 1/1 | F | | |
| | UF | 0/1 | 0/1 | UF | | |
| *C krusei + C. tropicalis* | F | | | F | 0/1 | 0/1 |
| | UF | | | UF | 1/1 | 1/1 |
| **Total** | F | **61/108 (56%)** | **49/108 (46%)** | F | **57/103 (55%)** | **67/103 (65%)** |
| | UF | **47/108 (44%)** | **59/108(54%)** | UF | **46/103 (45 %)** | **36/103 (34 %)** |

F = favorable response. UF = unfavorable response

Table 20 shows the number of patients who relapsed or developed new infection after treatment with miconazole buccal tablets or miconazole gel. *C. albicans* was the major species that relapsed.

Table 20. Relapse or New Infection by pathogen in patients treated with Miconazole50 mg BT and 50 mg and Miconazole gel 500mg/day in the PP population.

| ***Candida species*** | **Relapse or New Infection** | | | |
|---|---|---|---|---|
| | **Miconazole 50mg buccal tablet** | | **Miconazole gel 500mg/day** | |
| | **Relapse** | **New infection** | **Relapse** | **New Infection** |
| *C. albicans* | 10/44 (23%) | 5 | 7/38 (18%) | |
| *C. intermedia* | 1/1 | | | |
| *C. tropicalis* | 1/7 (11%) | 5 | 1/10 (10%) | 1 |
| *C.parapsilosis* | | | | 1 |
| *Candida spp.* | 1/1 | 1 | | 1 |
| **Mixed infections** | | | | |
| *C. albicans +C. rhodotorula rubra* | 1/1 | | | |
| *C. krusei + C. tropicalis* | | | | 1 |
| *C. glabrata + S. cerevisiae* | | 1 | | |
| *C. krusei + C .glabrata* | | | | 1 |
| Total | 14/108 (13%) | 12/108 (11%) | 8/103 (8%) | 5/103 (5%) |

### 4.1.3. Study No. BA2002/01/03

The study BA2002/01/03 was an open-label, non-comparative trial of the efficacy of treatment with miconazole (50 mg BT once daily) in HIV-positive patients. The primary objective of this study was to evaluate the clinical efficacy of a 14-day treatment with miconazole 50 mg BT. Success was defined by the applicant as the complete or partial disappearance of oropharyngeal candidiasis lesions. However, for the purpose of this review success will be defined as complete clinical cure; partial clinical response was classified as failure. Mycological eradication was a secondary endpoint that required the growth of less than 10 colonies of *Candida spp.* per primary isolation plate at day 15. Samples were collected at the time of screening examination which took place from 7 days prior to treatment (Day 0) and at test of cure visit, at the end of treatment on Day 15. Relapse was evaluated at Day 45.
The study inclusion criteria were similar to those of BA2004/01/04. Apart from the criteria listed above, the inclusion criteria included HIV-positive patients with documented viral load who had been receiving stable antiretroviral treatment for at least two months and who would continue on the HIV treatment regimen during the 14 day antifungal treatment. The applicant indicated that HIV patients not receiving anti-retroviral treatment could also be included in the study.

Exclusion criteria were the same as described for study BA 2004/01/04 except that, in study BA2002/01/03, if the spread of candidiasis occurred beyond the oropharyngeal area the patient was excluded. The withdrawal conditions were the same as previously stated in Study BA 2004/01/04. Mycological cure was defined as complete eradication indicated by the isolation of < 10 fungal colonies per plate. Here again, criteria for establishing such a threshold were not specified.

The patients were recruited from 5 of 8 centers in France. Three of the eight centers had not recruited any patients by the end of the study period. No *in vitro* susceptibility testing was done.

**Results:**

Nineteen subjects were enrolled in this study. A single *Candida* species was isolated from each of 18 subjects and one subject was dually infected with *C. albicans* and *C. glabrata.* Table 21 shows the results of the 19 HIV-positive patients treated with miconazole 50 mg BT after 14 days of treatment and the relapse rate after 45 days. Clinical success was obtained in 17 patients infected with *C. albicans* and one patient with mixed infection. Of the clinically cured patients, 8 (44%) relapsed by day 45. Six (33%) of the patients with *C. albicans* showed mycological eradication at Day 15. Three patients including the one with mixed isolates, *C. albicans and C. glabrata,* did not have a culture at the end of treatment.

Table 21. Response of baseline pathogens PP for HIV-positive patients treated with miconazole 50 mg tablets by patient study BA2000/01/03

| ***Candida species*** | **N (%)** | **Clinical Response** | | | **Mycological Response** | | |
|---|---|---|---|---|---|---|---|
| | | | **Cure Rate (Day 15)** | **Relapse (Day 45)** | | **Cure Rate (Day 15)** | **Relapse (Day 45)** |
| *C. albicans* | 18/19 (95%) | F | 17/18 (94%) | 8/17 (47%) | F | 6/18 (33%) | 3/6 (50%) |
| | | UF | 1/18 (6%) | - | UF | 12/18 (67%) [1] | - |
| *C .albicans + C. glabrata* | 1/19 (5%) | F | 1/1 | 0 | F | 0 | - |
| | | UF | 0 | - | UF | 1/1 | |
| | 19 | F | 18/19 (94.7%) | 8/18 (44.4%) | - | 7/19 (36.8%) | 4/7 |

1 (2patients withdrew)

In summary, the mycological response for *C. albicans* (33%) at day 15 was lower than the clinical cure response (94%) of HIV-positive patients to miconazole Oravig® BT. The mean CD4 count of the PP population was 164 cells/mm$^3$ with a range of 3 – 669 cells/ mm$^3$. Because *Candida spp*. are generally commensal pathogens it will be difficult to completely eradicate these yeasts from the immuno-compromised patients. Although there was little correlation between the mycological and clinical cure rate all of the patients who had been mycologically cured were also clinically cured.

Overall, the 3 studies show that miconazole BT is effective in the treatment of patients with OPC. The results in tables 22 and 23 show that 36% of the *Candida spp.* isolated were successfully eradicated but a larger percentage of the population remained infected with *Candida spp.* The results also suggest that it was easier to achieve a clinical cure of candidiasis than to mycologically eradicate the fungus.

Table 22. Summary of clinical and mycological responses by pathogen from the 3 clinical studies (BA2004/01/04, BA2002/01/02, and BA2002/01/03) who were treated with miconazole buccal tablet.

| ***Candida spp.*** | **Clinical Response** | **Mycologic Eradication** |
|---|---|---|
| *C. albicans* | 194/300 (65%) | 105/300 (35%) |
| *C. dublineinsis* | 1/2 | 1/2 |
| *C. famata* | 0/1 | 1.1 |
| *C. glabrata* | 0/1 | 1/1 |
| *C. guilliermondii* | 3/3 | 1/3 |
| *C. intermedia* | 1/1 | 0/1 |
| *C. kefyr* | 0/1 | 1/1 |
| *C. krusei* | 2/3 | 0/3 |
| *C. lipolytica* | 0/1 | 1/1 |
| *C. parapsilosis* | 6/8 (75%) | 3/8 (38%) |
| *C. rugosa* | 0/1 | 0/1 |
| *C. tropicalis* | 24/30 (80%) | 13/30 (43%) |
| *Candida spp (unidentified)* | 1/3 | 1/3 |
| **Total** | **232/355 (65%)** | **128/355(36%)** |
| *C. albicans* + *C. glabrata* | 1//1 | 0/1 |
| *C. albicans* + *C. guilliermondii* | 1/1 | 0/1 |
| *C. albicans* + *C..krusei* | 1/2 | 0/2 |
| *C. albicans* + *C. tropicalis* | 1/1 | 0/1 |
| *C. albicans* + *C. rhodotorula rubra* | 1/1 | 0/1 |
| *C. glabrata* + *C. krusei* | 1/1 | 0/1 |
| *C. glabrata* + *S. cerevisiae* | 0/1 | 0/1 |
| *C. kefyr* + *C. krusei* | 0/1 | 0/1 |
| *C .krusei* + *C. parapsilosis* | 1/1 | 1/1 |
| *C..krusei* + *S. cerevisiae* | 1/1 | 0/1 |
| ***Total*** | **240/366 (66%)** | **129/366 (35%)** |
| *C. albicans* + *C..krusei* + *C. tropicalis* | 0/1 | 1/1 |
| *C. albicans* + *C. kefyr* + *C..krusei* | 0/1 | 1/1 |
| *C. kefyr* + *C..krusei* + *C. rugosa* | 0/1 | 0/1 |
| **Total** | **240/369 (65%)** | **131/369 (36%)** |

Table 23. Summary of clinical and mycological responses by pathogen from the 3 clinical studies irrespective of single or mixed infection

| ***Candida spp.*** | **Clinical Cure** | **Mycologic Eradication** |
|---|---|---|
| *C. albicans* | 199/305 (65%) | 105/305 (35%) |
| *C. dublineinsis* | 1/3 | 2/3 |
| *C. famata* | 0/1 | 1/1 |
| *C. glabrata* | 2/4 | 1/4 |
| *C. guilliermondii* | 4/4 | 1/4 |
| *C. intermedia* | 1/1 | 0/1 |
| *C. kefyr* | 0/4 | 2/4 |
| *C. krusei* | 5/10 (50%) | 3/10 (30%) |
| *C. lipolytica* | 0/1 | 1/1 |
| *C. parapsilosis* | 7/9 (78%) | 4//9 (44%) |
| *C. rhodotorula rubra* | 1/1 | 0/1 |
| *C. rugosa* | 0/2 | 0/2 |
| *C. tropicalis* | 25/32 (78%) | 14/32 (44%) |
| *C. valida* | | |
| **Total** | **245/377 (65%)** | **134/377 (36%)** |

### 4.2. Interpretative criteria

The applicant has not requested any interpretative criteria and breakpoints nor do the studies support inclusion of interpretive criteria and breakpoints in the labeling.

## 5. DISCUSSION

Miconazole has two major mechanisms of action. The primary mechanism is the inhibition of the biosynthesis of ergosterol by inhibiting cytochrome P450, an essential component of the fungal cell membrane . This action prevents the growth of the fungal hyphae. The second mechanism is the inhibition of the synthesis of oxidative and peroxidative enzymes, thus allowing the increase of toxic reactive oxygen species within the cell leading to cell damage and eventually death.

*C. albicans* as well as many other species of *Candida* showed MIC < 0.1 µg/mL (Table 24). The miconazole $MIC_{50}$ and $MIC_{90}$ of *C. albicans* were 0.016 µg/mL and 0.06 µg/mL, respectively. The $MIC_{90}$ for *C. krusei* (4 µg/mL) was the highest of the species isolated.

Table 24. Summary of susceptibility results to miconazole of studies in this review performed by CLSI methodology

| ***Candida* species** | **Number of Studies** | **Number of Isolates** | **$MIC_{50}$ µg/mL** | | **$MIC_{90}$ µg/mL** | |
|---|---|---|---|---|---|---|
| | | | **Range** | **Median [1]** | **Range** | **Median [1]** |
| *C. albicans* | 5 | 1137 | 0.015- 0.12 | 0.03 | 0.063-1.0 | 0.56 |
| *C. dubliniensis* | 2 | 11 | -[2] | 0.03 | --[2] | 0.06 |
| *C. glabrata* | 5 | 162 | 0.06 - 0..5 | 0.25 | 0.25 -0.5--[3] | - |
| *C. guilliermondii* | 2 | 12 | 1.0 - 2.0-[3] | 1.5 | --[2] | 2.0 |
| *C. kefyr* | 3 | 14 | 0.016 – 0.12-[2] | .07 | - | 0.1-2-[1] |
| *C. krusei* | 4 | 65 | 0.5 - – 4.00[2] | 2.0 | 4.0 -[8 0] | 4.0 |
| *C. lusitaniae* | 2 | 18 | 0.06 – 0.12[3] | - | - | 0.25[2] |
| *C. parapsilosis* | 3 | 41 | 0.25[4] | 0.25[4] | - | 0.5[4] |
| *C. rugosa* | 1 | 2 | - | - | - | - |
| *C. tropicalis* | 4 | 50 | 0.5 – 2.0 | 1.1 | 0.2[4] | 2[3] |

[1] Average = median value -[2] one piece of data supplied [3] two pieces of data supplied [4] all values the same

A study in cutaneous infection model in guinea pigs, showed that on Day 14 after infection with *C. albicans,* topical (2%) treatment, administered one day post infection was less effective than oral treatment administered 2 days prior to infection. However, topical treatment with miconazole was more effective than placebo.

A study by Fothergill (2006) showed absence of cross resistance as the miconazole MIC (0.125 µg/mL) was low. Please note that this was based on testing of one *C. albicans* isolate, with high fluconazole and itraconazole MICs. There are multiple mechanisms of resistance to azole antifungal agents by *C. albicans*. These are classified into four areas:

- Efflux mechanisms: This mechanism is mediated by (*Candida* drug resistance) CDR1 and CDR2 transporter genes and multidrug resistance 1 (MDR1) genes. It involves an energy requiring efflux pump similar to the ATP-binding cassette (ABC) multidrug transporter super family. When there is an increase of CDR1 mRNA there is a concomitant change in the composition of the cell wall of the yeast and the drug fails to accumulate in the cell. This mechanism is common primarily in fluconazole resistance. Additionally, there can be a reduced level of drug uptake and an increased level of efflux of the drug (Fothergill, 2006, Fera, et. al., 2009, Ghannoum et al., 1999).
- Alteration in *ERGII.* One of the causes for azole antifungal resistance is single or multiple mutations in the ERGII gene. The changes in the gene sequences cause amino acid changes and reduction in binding of the antifungal agent to the amino acid target. This mechanism is purported to be responsible for the intrinsic resistance of *C. krusei* to azole drugs and resistance of some strains of *C. albicans* (Sanglard and Odds, 2002).
- Upregulation of *ERGII. ERGII* can be upregulated and has been the cause of resistance of some strains of *C. albicans* and *C glabrata.* However, this mechanism seems not to affect many strains or species of *Candida* (Fera et al., 2009).
- Alteration in sterol composition. Accumulation of ergosta-7,22-dienol-3β-ol due to loss of function of Erg3 alleles is one of the causes of azole resistance by some strains such as the Darlington strains of *C. albicans* (Sanglard and Odds, 2002).

The authors state that the multiplicity of these mechanisms of resistance can lead to a stepwise development in resistance to azole drugs. Evidence of generalized resistance to miconazole by *Candida spp*. was not found in the literature.

Overall, the clinical cure rate in study BA2004/01/04 in HIV subjects with oropharyngeal candidiasis, was at least twice as high as the mycological cure rate. The mycological eradication rate for *C. albicans* in the clotrimazole arm was slightly lower than that of the miconazole (25% compared to 29%) while for *C. tropicalis* the mycological eradication rate was higher in the clotrimazole arm than in the miconazole arm (50% compared to 43%). *C. albicans* was the most prevalent species isolated. All of the other *Candida* species isolated in this study *C. dubliniensis, C. guilliermondii, C. lusitaniae* and *C. parapsilosis* recorded less than 10 isolates. None of the subjects with multiple baseline pathogens was mycologically cured. The response might have differed for each pathogen isolated from patients who reported more than one baseline pathogen. A favorable clinical response for each patient meant that both of the baseline pathogens had been cured. There was no correlation between the clinical cure and mycological eradication in either the miconazole or the clotrimazole arm.

In study BA2002/01/02 undertaken to demonstrate that the miconazole 50 mg BT was as effective as the miconazole gel in the treatment of subjects with oropharyngeal candidiasis in head and neck cancer. The difference between the mycological eradication rates was less than 20% for both treatment arms, 46% in the miconazole BT and 65% for the miconazole gel indicating that the tablets were not inferior to the gel in the treatment of oropharyngeal candidiasis in patients with head and neck cancers. A majority (67%) of the subjects was infected with *C. albicans*. In the BT arm *C. albicans* accounted for 72% of the infections while there were a few with *C. tropicalis* (8%). There were 3 isolates for *C. krusei,* 2 for *C. parapsilosis*, and one each for C. *famata, C. glabrata* , *C. guilliermondii*, *C. kefyr* and *C. rugos*a. In this study, *C. albicans* had a 51% eradication rate whereas *C. tropicalis* had a 44% eradication rate.

In study BA2002/01/03 the mycological eradication rate was very low (33%) for *C. albicans* as compared to 94% clinical cure rate for the same species. *Candida species* usually reside in the body as commensals but can become opportunistic infections in the immuno-compromised host. The mean CD4 count for the subject was 166 cells /$mm^3$ making it even more difficult to eradicate the yeasts. Complete eradication is therefore not likely to occur. The clinical and mycological relapse rates were 47% and 50%, respectively. In this study as in the previous studies, there was also no correlation between the clinical and mycological cures.

Table 23summarizes the clinical cure and mycological response results for *Candida* isolates by species for patients enrolled in all three studies. *C. albicans* was the most prevalent species isolated with clinical cure rate of 65% (199/305) followed by *C. tropicalis* with 78% (25/32) cure rate and *C. parapsilosis* with 78% (7/9). The mycological eradication rate for *C. albicans* was 35%, and for both *C. tropicalis* and *C. parapsilosis* 44%. The number of patients with other *Candida* species such as *C. dubliniensis, C. guilliermondii* and *C. glabrata* was low (3%); the combined clinical cure and mycological eradication rate of these three species were 6% and 4%, respectively. Also, the sponsor has based mycological cure artes based on < 10 cfu per plate. Basis for such a threshold were not specified. None of the subjects with multiple baseline pathogens was mycologically cured. Overall, the results from the three clinical studies support miconazole Oravig susceptibility activity against *C. albicans, C. tropicalis,* and *C. parapsilosis.*

**6. REFERENCES**

1. 1.Blignaut[1], E., S. Messer, R.J. Hollis, M.A. Pfaller. Antifungal susceptibility of South African oral yeast isolates from HIV/AIDS patients and healthy individuals. Diagnostic Microbiology and Infectious Disease. 2002. 44: 169–174.

2. Borgers, M., S.De Nollin, F.Thone **and** H.Van Belle. Cytochemical localization of NADH Cxidase in *Candida Albicans.* The Journal of Histochemistry and Cytochemistry. 1977. 25 ( 3): 193-199,.

3. Davey, K., A. D. Holmes, E. M. Johnson, A. Szekely, and D. W. Warnock. Comparative Evaluation of FUNGITEST and Broth Microdilution Methods for Antifungal Drug Susceptibility Testing of *ndida* Species and *Cryptococcus neoformans.* J. of Clin. Microbiol. 1998. 926-930.

4. Ellepola, A., and L.P. Samaranayake. Antimycotic agents in Oral Candidosis: An overview: treatment of Oral Candidosis. Oral Mecdicine. 2000, 165 – 174.

5. Espinel-Ingroff A,T. White, M.A Pfaller.Antifungal agents and susceptibility test. In:Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, eds.Anual of Clinical Microbiology, 7th edn. Washington, DC: ASM Press, 1999. 1640–1662.

6. Fera, M.T., La Camera, E., De Sarro, A. New triazoles and echinocandinas : mode of action, *in vitro* activity and mechanisms of resistance. Expert Rev. Anti.Infect. Ther. 2009. 7 (8) : 981-998.

7. Fothergill, A. Miconazole: A historic perspective. Expert Rev Anti-Infect. Ther. 2006. 4(2): 171-175.

8. Gibbons, G.F.and K. A. Mitropoulos. The Role of Cytochrome P-450 in Cholesterol Biosynthesis. Eur. J. Biochem.1973. 40: 267-273

9. Gibbons, G.F., C R. Pullinger, K. A. Mitropoulos. Studies on the mechanism of lanosterol 14 alpha-demethylation a requirement for two distinct types of mixed-function-oxidase Biochem. J. 1979. 183: 309-315

10. Hamza, O. J. M, I.N. M. Matee, J. M. Mainen, E. N. M. Simon, F. Mugusi, F. H.M. Miko[5], W. H van P. Helderman, A.J.M.M. Rijs, , A. J.A.M. Van der Ven, P. E Verweij. Species distribution and *in vitro* antifungal susceptibility of oral yeast isolates from Tanzanian HIV-infected patients with primary and recurrent oropharyngeal candidiasis. BMC Microbiology 2008, 35(10): 2180-2188.

11. Kikuchi, K., T. Nagatomo, H. Abe, K. Kawakami, H. J. Duff, J. C. Makielski, C. T. January, Y.N. Kobayashi, D., K. Kondo, U. Nobuyuki, O. Seiko Naoki Tsuji, Y. Atsuhito, N. Watanabe., Endogenous Reactive Oxygen Species Is an Important Mediator of Miconazole Antifungal Effect. Antimicrobial Agents and Chemotherapy, 2002, 3113–3117.

12. Kronvall G, I. Karlsson. Fluconazole and voriconazole multidisk testing of *Candida* species for disk test calibration and MIC estimation. J Clin Microbiol 2001. 39:1422–1428.

**13.** KuriyamaT., D.W. Williams, J. Bagg, W.A. Coulter, D. Ready, M.A.Q. Lewis. In vitro susceptibility of Oral *Candida* to seven antifungal agents. Oral Microbiol and Immumol. 2005. 349 – 353.

14. McGinnis, M.R. and Rinaldi, M.G. Antifuingal Drugs: mechanisms of Action, Drug resistance, Susceptibility Testing and Assays of Activity in Biological Fluids. 1996 .

15. Moore R.D., J.C. Keruly and R.E Chaisson. Decline in CMV and other opportunistic disease with combination antiretroviral therapy. in 5th Conference on Retroviruses and Opportunistic Infections. 1998. Chicago, February 1-5.

16. Nozawa Y. and T. Morita. Molecular mechanisms of Antifungal agents associated with membrane ergosterol, dysfunction of membrane ergosterol and inhibition of ergosterol biosynthesis. Elsevier. 1986.

17. Pemberton, M.N, P. Sloan , S. Ariyaratnam, N.S. Thakker, M.H. Thornhill. Derangement of *warfarin anticoagulation* by miconazole oral gel. Br Dent J *1998*.184: 68-69.

18. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi: Approved Standard M38-A2. Clinical and Laboratory Standards Institute, Wayne, PA, U.S.A.

19. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts: Approved Standard M27-A3. Clinical and Laboratory Standards Institute, Wayne, PA, U.S.A.

20. Richter, S., R. P. Galask,,S..A. Messer, R. J. Hollis, D. J. Diekema, and M. A. Pfaller. Antifungal Susceptibilities of *Candida* Species Causing Vulvovaginitis and Epidemiology of Recurrent Cases. J of Clin. Microbiol. 2005. 2155–2162

21. Sanglard, D. , K. , F. Kuchler, .J. L. Pagani, M. Monod, and J. Bille. Mechanisms of Resistance to Azole Antifungal Agents in *Candida albicans* Isolates from AIDS Patients Involve Specific Multidrug Transporters Antimicrobial Agents and Chemotherapy, 1995. 2378–2386.

22. Van Cutsem, J.M. and D. Thienpont. Miconazole, a Broad-spectrum Antimycotic agent with Antibacterial Activity. Chemotherapy. 1972. 17:392-404.
23. Vanden Bossche. Biochemical effects of miconazole on fungi-I. effects on the uptake and/or utilization of purines, pyrimidines, nucleosides, amino acids and glucose by *Candida albicans.* Biochemical Pharmacology, 1974. 23: 887-899.

24. Vanden Bossche, H.,G. Willemsens, W. Cools, W.F.J. Lauwers* and L. Le Jeune. Biochemical effects of miconazole on fungi. II. Inhibition of ergosterol biosynthesis in *Candida albicans. Chem.-BioL Interactions.*1978. 21:59--78.

25. Vanden Bossche, H.Wim Lauwers, Gustaaf Willemsens, Patrick Marichal, Frans Cornelissen and Willy Cools. Molecular Basis for the Antimycotic and Antibacterial Activity of N-Substituted Imidazoles and Triazoles: the Inhibition of Isoprenoid Biosynthesis Pesiic. Sci. 1984.15:188- 198

26. Vanden Bossche, H. Biochemical Targets for Antifungal Azole Derivatives hypothesis on Mode of Action. 1985.

27. Walsh, R.C. and H.D. Sisler. A sterol C-14 demethylase deficient mutant of Ustilago maydis. Phytopathology. 1982. 72: 711.

28. Wilkins, C.F., K. Hetnarski. Imidazole derivatives-A new class of microsomal enzyme inhibitors Biochemical Pharmacology, 1972. 21:3187-3192.

(b) (4)

**7.2. Comments**

1. The applicant has stated that (b) (4) The studies evaluating the fungistatic and fungicidal effect of miconazole were limited to 2 concentration of the drug and examination by electron microscopy. Comparison of MICs and MFC was not done. Time kill studies were also not done. Therefore, statements referring to fungistatic and fungicidal effects will be misleading and should be deleted. However, description of resistance mechanisms for miconazole against *Candida* species is appropriate.

2. The mechanism of action paragraph was reworded to avoid repetition and provide clarity.

3. The applicant proposes to present $MIC_{90}$ values of selected *Candida* species in the mechanism of action section and also list the *Candida* species under the subheading "activity *in vitro* and *in vivo*". It is FDA's policy not to present $MIC_{90}$ values in the absence of established breakpoints as proposed by the applicant. All *Candida* species for which clinical relevance was shown in clinical trials and in which adequate numbers of isolates were tested for susceptibility *in vitro* should be listed under the subheading "activity *in vitro* and *in vivo"*

4. The applicant proposes to state that (b) (4) However, no studies were available for our review supporting such a statement.

5. In the clinical study section of the labeling the distribution of *Candida* species should be stated.

**7.3. FDA's version of the label**

(b) (4)

(b) (4)

[redacted]

8. **RECOMMENDATIONS**

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

*Lynette Berkeley*
Lynette Y. Berkeley, PhD., M.T. (ASCP)
Microbiologist, DSPTP

**CONCURRENCES:**
DSPTP /Microbiology Team Leader *Shukal Bala* Signature 2/18/10 Date
CC:
DSPTP/Original NDA
DSPTP/PM/MilsteinJ

| Application Type/Number | Submission Type/Number | Submitter Name | Product Name |
|---|---|---|---|
| NDA-22404 | ORIG-1 | BIOALLIANCE PHARMA | Lauriad (miconazole [redacted] (b) (4) tablet) |

---

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

---

/s/

---

LYNETTE Y BERKELEY
02/22/2010

SHUKAL BALA
02/22/2010

# Product Quality Microbiology Review

**4 DECEMBER 2009**

**NDA:** 22-404

**Drug Product Name**
**Proprietary:** Lauriad [redacted] (b) (4) Buccal tablets
**Non-proprietary:** miconazole

**Review Number:** 1

**Dates of Submission(s) Covered by this Review**

| Submit | Received | Review Request | Assigned to Reviewer |
|---|---|---|---|
| 15 June 2009 | 16 June 2009 | 11 August 2009 | 13 August 2009 |
| 5 February 2009 | 6 February 2009 | N/A | N/A |

**Submission History (for amendments only):** N/A

**Applicant/Sponsor**
**Name:** BioAlliance Pharma
**Address:** 49 Boulevard du General Martial Valin, 75015 Paris, France
**Representative:** Lavonne M. Patton, Ph.D. (Authorized US Agent)
**Telephone:** 913-451-3955

**Name of Reviewer:** Bryan S. Riley, Ph.D.

**Conclusion:** Approvable pending resolution of product quality microbiology deficiency (please see List of Microbiology Deficiencies on page 6)

# Product Quality Microbiology Data Sheet

**A. 1. TYPE OF SUBMISSION:** 505(b)(2)

**2. SUBMISSION PROVIDES FOR:** New Drug Product

**3. MANUFACTURING SITE:** [redacted] (b) (4)

**4. DOSAGE FORM, ROUTE OF ADMINISTRATION AND STRENGTH/POTENCY:** Non-sterile [redacted] (b) (4) tablet for buccal administration, 50 mg/tablet.

**5. METHOD(S) OF STERILIZATION:** N/A

**6. PHARMACOLOGICAL CATEGORY:** anti-fungal for treatment of oropharyngeal candidiasis

**B. SUPPORTING/RELATED DOCUMENTS:** N/A

**C. REMARKS:** This was an eCTD submission.

**filename:** N022404R1.doc

# Executive Summary

## I. Recommendations

**A. Recommendation on Approvability –** This submission is approvable pending resolution of a product quality microbiology deficiency (please see List of Microbiology Deficiencies on page 6).

**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 –** The drug product is a non-sterile tablet for buccal administration.

**B. Brief Description of Microbiology Deficiencies –** The drug product should be tested for microbial limits at release for each batch until more manufacturing experience has been attained.

**C. Assessment of Risk Due to Microbiology Deficiencies –** There is a moderate risk to the drug product and the patient if the microbial bioburden of the product is excessive.

## III. Administrative

**A. Reviewer's Signature _____**
Bryan S. Riley, Ph.D.

**B. Endorsement Block _____**
James L. McVey, NDMS Team Leader

**C. CC Block**
N/A

3 pages have been withheld in full as B(4) CCI/TS immediately following this page

| Application Type/Number | Submission Type/Number | Submitter Name | Product Name |
|---|---|---|---|
| NDA-22404 | ORIG-1 | BIOALLIANCE PHARMA | Lauriad (miconazole [redacted] (b) (4) tablet) |

---

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

---

/s/

---

BRYAN S RILEY
12/07/2009

JAMES L MCVEY
12/07/2009
I concur.

# MICROBIOLOGY FILING CHECKLIST FOR NDA or Supplement

**NDA Number:** 22-404 **Applicant:** BioAlliance Pharma **Stamp Date:** 06/15/2009

**Drug Name:** Miconazole **NDA Type:** Original

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

| | Content Parameter | Yes | No | Comments |
|---|---|---|---|---|
| 1 | Is the microbiology information (preclinical/nonclinical and clinical) described in different sections of the NDA organized in a manner to allow substantive review to begin? | √ | | |
| 2 | Is the microbiology information (preclinical/nonclinical and clinical) indexed, paginated and/or linked in a manner to allow substantive review to begin? | √ | | |
| 3 | Is the microbiology information (preclinical/nonclinical and clinical) legible so that substantive review can begin? | √ | | |
| 4 | On its face, has the applicant submitted *in vitro* data in necessary quantity, using necessary clinical and non-clinical strains/isolates, and using necessary numbers of approved current divisional standard of approvability of the submitted draft labeling? | √ | | |
| 5 | Has the applicant submitted any required animal model studies necessary for approvability of the product based on the submitted draft labeling? | √ | | |
| 6 | Has the applicant submitted all special/critical studies/data requested by the Division during pre-submission discussions? | √ | | |
| 7 | Has the applicant submitted the clinical microbiology datasets in a format which intents to correlate baseline pathogen with clinical and microbiologic outcome? | √ | | |
| 8 | Has the applicant submitted draft/proposed interpretive criteria/breakpoint along with quality control (QC) parameters and interpretive criteria, if applicable, in a manner consistent with contemporary standards, which attempt to correlate criteria with clinical results of NDA/BLA studies, and in a manner to allow substantive review to begin? | NA | | |
| 9 | Has the applicant submitted a clinical microbiology dataset in an appropriate/standardized format which intents to determine resistance development by correlating changes in the phenotype (such as *in vitro* susceptibility) and/or genotype (such as mutations) of the baseline pathogen with clinical and microbiologic outcome? | NA | | |
| 10 | Has the applicant used standardized or nonstandardized methods for measuring microbiologic outcome? If nonstandardized methods were used, has the applicant | √ | | Only standardized methods used |

# MICROBIOLOGY FILING CHECKLIST FOR NDA or Supplement

| | Content Parameter | Yes | No | Comments |
|---|---|---|---|---|
| | included complete details of the method, the name of the laboratory where actual testing was done and performance characteristics of the assay in the laboratory where the actual testing was done? | | | |
| 11 | Has the applicant submitted draft labeling consistent with current regulation, divisional and Center policy, and the design of the development package? | √ | | |
| 12 | Has the applicant submitted annotated microbiology draft labeling consistent with current divisional policy, and the design of the development package? | √ | | |
| 13 | Have all the study reports, published articles, and other references been included and cross-referenced in the annotated draft labeling or summary section of the submission? | √ | | |
| 14 | Are any study reports or published articles in a foreign language? If yes, has the translated version been included in the submission for review? | | √ | |

**IS THE MICROBIOLOGY SECTION OF THE APPLICATION FILEABLE?**
**Yes**

If the NDA is not fileable from the microbiology perspective, state the reasons and provide comments to be sent to the Applicant.

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

*Lynette Y. Berkeley* *07/28/09*

Lynette Y. Berkeley, Ph.D., M.T.(ASCP)
Reviewing Microbiologist Date

*Shukal Bala* *07/28/09*

Shukal Bala, Ph.D.
Microbiology Team Leader Date

---

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

---

/s/

---

LYNETTE Y BERKELEY
07/28/2009

SHUKAL BALA
07/28/2009

# MICROBIOLOGY FILING CHECKLIST FOR NDA or Supplement

**NDA Number:** 22-404 **Applicant:** BioAlliance Pharma **Stamp Date:** 02/05/2009

**Drug Name:** Miconazole **NDA Type:** Original

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

| | Content Parameter | Yes | No | Comments |
|---|---|---|---|---|
| 1 | Is the microbiology information (preclinical/nonclinical and clinical) described in different sections of the NDA organized in a manner to allow substantive review to begin? | √ | | |
| 2 | Is the microbiology information (preclinical/nonclinical and clinical) indexed, paginated and/or linked in a manner to allow substantive review to begin? | √ | | |
| 3 | Is the microbiology information (preclinical/nonclinical and clinical) legible so that substantive review can begin? | √ | | |
| 4 | On its face, has the applicant submitted *in vitro* data in necessary quantity, using necessary clinical and non-clinical strains/isolates, and using necessary numbers of approved current divisional standard of approvability of the submitted draft labeling? | √ | | |
| 5 | Has the applicant submitted any required animal model studies necessary for approvability of the product based on the submitted draft labeling? | √ | | |
| 6 | Has the applicant submitted all special/critical studies/data requested by the Division during pre-submission discussions? | √ | | |
| 7 | Has the applicant submitted the clinical microbiology datasets in a format which intents to correlate baseline pathogen with clinical and microbiologic outcome? | | √ | The applicant was requested to format the data sets according to the template discussed at the time of Pre-NDA meeting. In an e-mail dated March 23, 2009, the applicant has agreed to submit the data sets to the Division by April 30, 2009. |
| 8 | Has the applicant submitted draft/proposed interpretive criteria/breakpoint along with quality control (QC) parameters and interpretive criteria, if applicable, in a manner consistent with contemporary standards, which attempt to correlate criteria with clinical results of NDA/BLA studies, and in a manner to allow substantive review to begin? | NA | | |

# MICROBIOLOGY FILING CHECKLIST FOR NDA or Supplement

| | Content Parameter | Yes | No | Comments |
|---|---|---|---|---|
| 9 | Has the applicant submitted a clinical microbiology dataset in an appropriate/standardized format which intents to determine resistance development by correlating changes in the phenotype (such as *in vitro* susceptibility) and/or genotype (such as mutations) of the baseline pathogen with clinical and microbiologic outcome? | NA | | |
| 10 | Has the applicant used standardized or nonstandardized methods for measuring microbiologic outcome? If nonstandardized methods were used, has the applicant included complete details of the method, the name of the laboratory where actual testing was done and performance characteristics of the assay in the laboratory where the actual testing was done? | √ | | Only standardized methods used. |
| 11 | Has the applicant submitted draft labeling consistent with current regulation, divisional and Center policy, and the design of the development package? | √ | | |
| 12 | Has the applicant submitted annotated microbiology draft labeling consistent with current divisional policy, and the design of the development package? | √ | | |
| 13 | Have all the study reports, published articles, and other references been included and cross-referenced in the annotated draft labeling or summary section of the submission? | √ | | |
| 14 | Are any study reports or published articles in a foreign language? If yes, has the translated version been included in the submission for review? | | √ | |

**IS THE MICROBIOLOGY SECTION OF THE APPLICATION FILEABLE?** Yes

If the NDA is not fileable from the microbiology perspective, state the reasons and provide comments to be sent to the Applicant.

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

Lynette Y. Berkeley, Ph.D., M.T.(ASCP)
Reviewing Microbiologist, DSPTP Date

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

---

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

---

/s/
---
Lynette Berkeley
3/27/2009 12:03:54 PM
MICROBIOLOGIST

Shukal Bala
3/27/2009 12:07:13 PM
MICROBIOLOGIST