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APPLICATION NUMBER:

207500Orig1s000 / 207501Orig1s000

MICROBIOLOGY / VIROLOGY REVIEW(S)

**Division of Anti-Infective Products
Clinical Microbiology Review**

NDA: 207500 (b) (4) and 207501 (b) (4) Original NDA

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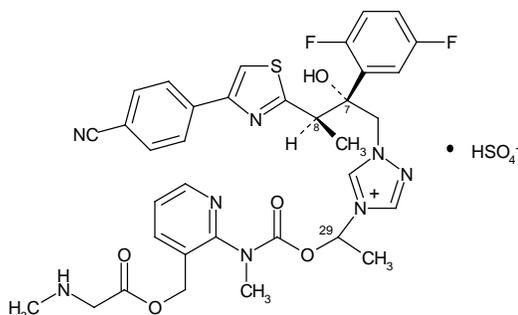
DRUG PRODUCT NAMES:

Proprietary: CRESEMBA[®]

Nonproprietary: Isavuconazonium sulfate; BAL8557; (b) (4)

Chemical Name: (b) (4)

STRUCTURAL FORMULA:



MOLECULAR FORMULA:

C₃₅H₃₅F₂N₈O₅S · HSO₄

MOLECULAR WEIGHT:

814.84

DRUG CATEGORY:

Anti-fungal

PROPOSED INDICATION:

Treatment of invasive aspergillosis and invasive mucormycosis

PROPOSED DOSAGE FORM, ROUTE OF ADMINISTRATION AND DURATION OF TREATMENT:

Sterile lyophilized powder for intravenous infusion and hard capsules for oral administration.

- Loading Dose: 200 mg every 8 hours, for 48 hours (6 total doses), via oral or IV administration.
- Maintenance Dose: 200 mg once per day via oral or IV administration starting 12 to 24 hours after the last loading dose.

Switching between the IV and oral formulations of CRESEMBA is acceptable.

Intravenous formulation is not for bolus injection, administer intravenous dose via an infusion set with an in-line filter (pore size 0.2 µM to 1.2 µM) over a minimum of 1 hour.

DISPENSED:

Rx

RELATED DOCUMENTS:

IND 72,593 and 119,307

REMARKS

The subject of this NDA is CRESEMBA[®] (isavuconazonium sulfate, a pro-drug of the active triazole - isavuconazole) for the treatment of invasive aspergillosis and invasive mucormycosis. The nonclinical and clinical microbiology studies, submitted by the applicant or obtained by an independent literature search, support the activity of isavuconazole against *Aspergillus* and *Mucor* species. A potential for development of resistance to isavuconazole exists and may be due to multiple mechanisms that include substitutions on the target *cyp51* gene, changes in sterol profile, and/or elevated efflux pump activity. The information supporting inclusion of interpretive criteria and breakpoints was limited as the minimal inhibitory concentration (MIC) data were available for a small number of baseline isolates from patients enrolled in the clinical trial. However, the epidemiological cut-off values (ECVs) are available for some of the *Aspergillus* species. The applicant should be requested to conduct surveillance studies and collect MIC data for all *Aspergillus* and Mucorales species for at least five years post-marketing.

(b) (4)

CONCLUSIONS AND RECOMMENDATIONS

From clinical microbiology perspective, this NDA submission is approvable pending an accepted version of the labeling. The changes to the proposed labeling and post-marketing request are as follows:

- **The changes proposed in the labeling:**
(Additions marked as double-underlined and deletions as struck out)

12.1 Mechanism of action

[see *Clinical Pharmacology, Microbiology (12.4)*].

(b) (4)

12.4 Microbiology

Mechanism of Action

Isavuconazole, (b) (4) inhibits (b) (4) -the synthesis of ergosterol, a key component of the fungal cell membrane, through the inhibition of cytochrome P-450 dependent enzyme lanosterol 14-alpha-demethylase. This enzyme is responsible for the conversion of lanosterol to ergosterol. (b) (4) An accumulation of methylated sterol precursors and a depletion of ergosterol within the fungal cell membrane (b) (4) weakens (b) (4) the membrane structure and function. Mammalian cell demethylation is less sensitive to isavuconazole inhibition.

Activity in vitro and (b) (4) -in Clinical Infections:

Isavuconazole has (b) (4) -activity against most strains of the following microorganisms, both *in vitro* and in clinical infections: *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, and Mucorales such as (b) (4) ; *Rhizopus oryzae*, and Mucormycetes species- (b) (4)

[see Clinical Studies (14)].

Drug Resistance

There is a potential for development of resistance to isavuconazole.

The (b) (4) mechanism of resistance to isavuconazole, like other (b) (4) is likely due to multiple mechanisms that include (b) (4) substitutions (b) (4) in the target (b) (4) gene *cyp51*. Changes in sterol profile and elevated efflux pump activity were observed.

In vitro and animal studies suggest (b) (4) -cross-resistance between isavuconazole and other azoles (b) (4). The relevance of cross-resistance to clinical outcome has not been fully characterized. However, patients failing prior azole therapy (b) (4) may require alternative antifungal therapy.

The following information should be added to Section 14 Clinical studies:

14.1 Treatment of Invasive Aspergillosis

At least one *Aspergillus* species was identified in 30% of the subjects; *A. fumigatus* and *A. flavus* were the most common pathogens identified. There were (b) (4) patients with other *Aspergillus* species (*A. niger*, *A. sydowi*, *A. terreus*, and *A. westerdijkiae*).

14.2 Treatment of Invasive Mucormycosis

There were (b) (4) patients with other Mucorales (b) (4) *Lichtheimia corymbifera*, *Mucor amphibiorum*, *Mucor circinelloides*, *Rhizomucor pusillus*, *Rhizopus azygosporus*, and *Rhizopus microsporus*.

(b) (4)

- **Post marketing request:**

Conduct surveillance studies for five years from the date of marketing CRESEMBA[®] to determine (b) (4) in organisms relevant to the indication in the package insert for invasive aspergillosis and mucormycosis.

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1. EXECUTIVE SUMMARY

The subject of this NDA is CRESAMBA[®] (isavuconazonium sulfate) for the treatment of invasive aspergillosis and invasive mucormycosis. Isavuconazonium sulfate (BAL8557) is a water soluble pro-drug of the active triazole, isavuconazole (BAL4815).

Mechanism of action

The mechanism of action of isavuconazole is similar to other azoles and involves the inhibition of cytochrome P450 sterol 14 α -demethylase (P450_{14DM}) enzyme, which has a role in the synthesis of ergosterol, a component of fungal cell membrane (encoded by the genes *cyp51A* and *cyp51B*), and is present in all fungi. Depletion of ergosterol is known to disrupt the structure and functions of the fungal membrane, including accumulation of toxic methylated sterol intermediates, leading to inhibition of fungal growth. Isavuconazole inhibits P450_{14DM} enzyme activity in *Candida albicans* at about 100 fold lower concentration than that of mammalian rat liver cells.

In vitro activity

Activity of isavuconazole alone

The isavuconazole *in vitro* susceptibility testing was standardized using a panel of quality control (QC) strains recommended by Clinical Laboratory Standards Institute (CLSI) as well as European Committee on Antimicrobial Susceptibility Testing (EUCAST). The QC strains selected (to confirm that antifungal agents are present at correct concentrations and to monitor antifungal stability) were appropriate. Based on the intra- and inter-laboratory standardization of the *in vitro* susceptibility testing of isavuconazole, the QC strains and the minimum inhibitory concentration (MIC) range selected for *in vitro* susceptibility testing against filamentous fungi by the CLSI method were appropriate and are as follows:

- *Paecilomyces variotii* MYA 3630 at a range of 0.06 – 0.5 $\mu\text{g/ml}$, and
- *Aspergillus flavus* ATCC 204304 at a range of 0.5 – 4 $\mu\text{g/ml}$.

One of the limitations of QC testing was a single source (b) (4) of micro titer plates included for testing. CLSI also recommended that “more Tier 3 data could be gathered over time (from different laboratories using different lots of plates and ideally not manufactured by same vendor).” For testing of microtiter plates from other sources, (b) (4) microtiter plates should be included as a comparator.

The activity of isavuconazole was measured *in vitro* against the different species of *Aspergillus* and Mucorales. There were three main sources of information for evaluating the *in vitro* activity of isavuconazole:

- Surveillance studies.
- Database of published and unpublished studies compiled by the applicant.
- Isolates collected from clinical trials.

Among the *Aspergillus* species, the isavuconazole MIC₉₀ values were lower against *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus nidulans*, and *Aspergillus terreus* compared to *Aspergillus niger* (Table 1). There did not appear to be any effect of the test region. Isavuconazole MIC₉₀ values were similar to those of itraconazole and voriconazole. Posaconazole was approximately 4-fold more active. The MIC₉₀ values of echinocandins were low with the exception of caspofungin.

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Species	MIC ₉₀ µg/mL (n)					
	Surveillance study		Database**	Other published studies	Clinical trial isolates	Espinel-Ingroff <i>et al.</i> (2013)/ CLSI ECV ¹
	2011	2012				
<i>Aspergillus</i> species						
<i>A. flavus</i>	1 (10)	2 (11)	4 (145)		2 (23)	1 (444) / 1
<i>A. fumigatus</i>	1 (71)	2 (90)	1 (875)	0.39 (12)	1 (54)	1 (855) / 1
<i>A. nidulans</i>	-	-	1 (85)	1 (63)	-	1 (106) -
<i>A. niger</i>	4 (11)	4 (11)	2 (101)	1.56 (1)*	8 (11)	2 (207) / 4
<i>A. oryzae</i>	-	-	-	0.39 (1)*	-	-
<i>A. terreus</i>	-	-	1 (432)	0.125 - 1(135)*	0.25-2 (8)*	0.5 (384) / 1
<i>A. lentulus</i>	-	-	-	0.25 (15)	-	-
<i>A. westerdijkiae</i>	-	-	-	-	2 (1)	-
Other <i>Aspergillus</i> species	2 (12) ¹	2 (18) ¹	-	-	-	-
<i>Aspergillus</i> species	2 (104)	2 (130)	2 (1717)	0.125 - 2 (227)*	2 (96)	-
Mucorales						
<i>Lichtheimia</i> (<i>Absidia</i>) species (Total)¹	-	-	8 (67)	-	-	-
<i>L. corymbifera</i>	-	-	8 (44)	8 (17)	8-16 (4)*	-
<i>Lichtheimia</i> species NOS	-	-	32 (23)	-	-	-
<i>Cunninghamella</i> species (Total) ¹	-	-	32 (13)	-	-	-
<i>Mucor</i> species (Total)¹	-	-	16 (68)	-	-	-
<i>M. circinelloides</i>	-	>8 (1)*	8 (18)	8 (16)	32 (1)*	-
<i>Rhizomucor</i> species (All) ¹	-	-	-	2 - >8 (9)*	-	-
<i>R. pusillus</i>	4 (1)*	-	-	-	8-32 (3)*	-
<i>Rhizopus</i> species (Total)¹	-	-	8 (134)	-	-	-
<i>R. arrhizus</i>	-	-	4 (28)	4 (27)	-	-
<i>R. microsporus</i>	1-2 (2)*	1-4 (2)*	2 (41)	-	16 (1)*	-
<i>R. oryzae</i>	-	>8 (1)*	4 (11)	-	0.5 - 32 (9)	-
<i>R. azygosporus</i>	-	-	-	-	1 (1)*	-
<i>Rhizopus</i> species NOS	-	-	16 (52)	-	32 (11)	-
<i>Apophysomyces elegans</i>	-	-	-	4 (18)	-	-
<i>Actinomucor elegans</i>	-	-	-	-	0.25 (1)	-

* represent range. If number of isolates less than 10, MIC₉₀ value was not calculated or not available.
 **Database results include surveillance study results of 2011. NOS = Not otherwise speciated
¹Include *A. terreus*, *A. nidulans*, *A. foetidus*, and *A. sydowii*.
¹Epidemiological cut-offs (ECVs) based on a study by Espinel-Ingroff *et al.* (2013); ECVs approved by the CLSI.

The MICs were higher against the hyphae compared to conidia of *Aspergillus* species by the CLSI method but not by the EUCAST method. Please note that this information is based on testing of small number of isolates.

Against isolates of the **Mucorales** [*Lichtheimia* (*Absidia*), *Cunninghamella*, *Mucor*, *Rhizomucor*, and *Rhizopus* species], the activity of isavuconazole was variable (Table 1). Isavuconazole appears to more active than voriconazole but less active than posaconazole or amphotericin B.

Overall, the MIC₉₀ values were lower against *Aspergillus* species (range 1-4 µg/mL) compared to Mucorales (1-32 µg/mL).

Activity of isavuconazole in combination with other anti-fungal drugs

A combination of isavuconazole and micafungin, *in vitro*, showed no antagonism against the strains of *A. fumigatus*, *A. flavus*, and *A. terreus* tested; the activity of the combination of isavuconazole and amphotericin B could be antagonistic depending on *Aspergillus* species and/or drug concentrations.

Activity of the combination of isavuconazole and micafungin showed indifferent or additive activity against the strains of *Cunninghamella bertholletiae*, *Rhizopus oryzae*, *Rhizopus microsporus*, and *Mucor circinelloides* tested; however, against *Lichtheimia corymbifera* strain the activity was shown to vary from synergy to antagonism depending on the drug concentrations. A combination of isavuconazole and amphotericin B could be antagonistic depending on *Mucorales* species and/or drug concentrations.

The clinical relevance of these findings is not known.

Activity in animal models

Aspergillosis

The activity of isavuconazole was reported in

- mice with disseminated aspergillosis. Animals were infected with *A. flavus* (neutropenic mice), *A. fumigatus* (neutropenic and non-neutropenic mice), or *A. terreus* (neutropenic mice) as well as
- immunocompromised mice, guinea pigs and rabbits with pulmonary aspergillosis. Animals were infected with *A. fumigatus* strains.

In all animal models, except for the guinea pig pulmonary aspergillosis model, isavuconazole was effective in improving survival and/or reducing fungal burden in animals infected with *A. flavus* or *A. fumigatus*. Such an effect was dose-dependent. The activity may decrease with a delay in time of initiation of treatment. In *A. terreus* infected mice, isavuconazole was not effective under the experimental conditions tested.

The pharmacokinetic (PK) parameters were measured in some of the studies. In the *A. flavus* neutropenic murine model of disseminated aspergillosis, a ratio of 24 hour AUC/MIC in excess of 1 were obtained after treatment with ≥ 15 mg/kg/dose isavuconazole. The authors stated that although not yet clear, it is likely that trough levels maintained above the MIC will predict a good outcome.

In the *A. fumigatus* neutropenic and non-neutropenic murine models, AUC/MIC was considered to be the driver of efficacy. In one study of non-neutropenic model of disseminated aspergillosis, the target attainment based on AUC/MIC using a pharmacodynamic (PD) marker of 14-day survival was 50.48. However, in an immunocompromised pulmonary aspergillosis model, the target attainment based on AUC/MIC using a PD marker of a one-log reduction in fungal burden on Day 7, by PCR, to achieve static effect was 503. In an immunocompromised rabbit model of pulmonary aspergillosis, isavuconazole doses equivalent to 40 or 60 mg/kg/day (which corresponds to exposure levels of 141.4×10^3 and 197.4×10^3 ng.h/mL, respectively), were associated with prolonged survival, lower pulmonary fungal burdens and reduced lung injury compared with untreated controls.

In one study in neutropenic mice infected with *A. fumigatus*, $T > MIC$, rather than AUC, was thought to be the parameter that best described the *in vivo* antifungal activity of isavuconazole.

Mucormycosis

The activity of isavuconazole was measured in neutropenic and diabetic ketoacidotic (DKA) ICR mice infected by the inhalational (pulmonary infection model of mucormycosis) or intravenous (hematogenously disseminated infection model of mucormycosis) route with a strain of *Rhizopus oryzae*. In the **pulmonary infection model**, isavuconazole at the highest dose (215 mg/kg) tested, administered 8 hours post-exposure with 4.1×10^3 spores, was effective in improving survival of neutropenic infected mice but not of DKA infected mice; untreated mice died within 3 days. However, in another experiment in DKA mice challenged with a lower inoculum concentration (2.4×10^3 spores), a trend towards increased survival was observed after treatment with isavuconazonium at a dose of 110 mg/kg three times daily compared with placebo-group of mice. Isavuconazole was as effective as high dose liposomal amphotericin B in protecting neutropenic mice.

Isavuconazonium treatment was not effective in the **hematogenously disseminated** DKA and neutropenic models suggesting that the effectiveness may vary with the severity of infection and immune status of the host. Activity against Mucorales other than *R. oryzae* was not measured.

Drug resistance

There is a potential for development or resistance to isavuconazole. An increase in isavuconazole MICs, like other triazoles, is likely due to multiple mechanisms involving substitutions in the target gene *cyp51*, alterations in sterol profile, and/or efflux pump activity. For *Rhizopus* isolates, changes in sterol profile were associated with increased MIC and there was no change in activity of efflux pumps. However, for *Aspergillus* and *Fusarium* isolates increased MICs were associated with elevated activity of efflux pumps. These observations are based on testing of a small number of isolates. The clinical relevance of this finding is not known.

Cross resistance

Isavuconazole MICs were higher against strains of *A. fumigatus* with reduced susceptibility to other azoles suggesting cross resistance. Such changes tended to mirror changes in voriconazole susceptibility. The increased MICs were against isolates with L98, TR34/L98H, M220, or G138/Y431/G434/G448 mutations but not G54 mutation (affected by itraconazole and posaconazole). The clinical relevance of such findings is not known.

Clinical Microbiology

The applicant included results of two clinical studies (Study ISN 9766-CL-0104 and Study ISN 9766-CL-0103) to support efficacy of isavuconazole for the treatment of invasive aspergillosis and invasive mucormycosis. The studies suggest that isavuconazole was effective in reducing all-cause mortality, and improving clinical and microbiological responses in patients with invasive aspergillosis and invasive mucormycosis (Table 2). The number of isolates of *Aspergillus* species other than *A. fumigatus*, *A. flavus*, and *A. niger* identified at baseline were small (Table 2) for analysis. For pathogens associated with Mucormycosis, the number of isolates of Mucorales other than *Rhizopus oryzae* and Mucormycetes were small for analysis (Table 2). There was no correlation between the MICs of the baseline pathogen and clinical or microbiological response; this could be due to the small number of isolates tested from patients prior to initiation of treatment.

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Table 2: Study ISN 9766-CL-0104 + Study ISN 9766-CL-0103 – All-cause mortality, clinical response and mycological response in mITT population with baseline <i>Aspergillus</i> or Mucorales infection at baseline irrespective of single or mixed infections						
Baseline Pathogen	Day 42			Day 84		
	All-cause mortality n/N (%)	Clinical Response n/N (%)	Mycological Response n/N (%)	All-cause mortality n/N (%)	Clinical Response n/N (%)	Mycological Response n/N (%)
<i>Aspergillus</i> species						
<i>A. flavus</i>	3/19 (15.8)	14/19 (73.7)	9/19 (47.4)	6/19 (66.7)	11/19 (57.9)	7/19 (36.8)
<i>A. fumigatus</i>	5/45 (11.1)	29/45 (64.4)	16/45 (35.6)	10/45 (22.2)	22/45 (48.9)	13/45 (28.9)
<i>A. niger</i>	2/11 (18.2)	9/11 (81.8)	6/11 (54.5)	4/11 (36.4)	6/11 (54.5)	3/11 (27.3)
<i>A. sydowi</i>	0/1	1/1	1/1	1/1	0/1	0/1
<i>A. terreus</i>	2/7	4/7	2/7	2/7	3/7	0/7
<i>A. versicolor</i>	0/1	1/1	1/1	0/1	0/1	0/1
<i>A. westerdijkiae</i>	0/1	0/1	0/1	0/1	0/1	0/1
<i>Aspergillus</i> NOS	1/4	0/4	0/4	1/4	0/4	0/4
<i>Aspergillus</i> species (Total)	13/89 (15.6)	58/89 (65.2)	35/89 (39.3)	24/89 (27.0)	42/89 (47.2)	23/89 (25.8)
Mucorales						
<i>Lichtheimia corymbifera</i>	2/4	2/4	0/4	3/4	0/4	0/4
<i>Mucor amphibiorum</i>	0/1	0/1	0/1	0/1	0/1	0/1
<i>Mucor circinelloides</i>	1/1	0/1	0/1	1/1	0/1	0/1
<i>Mucor</i> NOS	1/5	4/5	0/5	1/5	4/5	0/5
Mucormycetes NOS	3/15 (20.0)	7/15 (46.7)	1/15 (6.7)	4/15 (26.7)	5/15 (33.3)	2/15 (13.3)
<i>Rhizomucor pusillus</i>	3/4	1/4	0/4	3/4	0/4	0/4
<i>Rhizomucor</i>	1/1	0/1	0/1	1/1	0/1	0/1
<i>R. azygosporus</i>	0/1	0/1	0/1	0/1	0/1	1/1
<i>R. microsporus</i>	0/1	1/1	0/1	0/1	1/1	1/1
<i>R. oryzae</i>	6/10 (60.0)	3/10 (30.0)	1/10 (10.0)	6/10 (60.0)	3/10 (30.0)	3/10 (30.0)
<i>Rhizopus</i> NOS	1/2	1/2	1/2	1/2	1/2	1/2
Mucorales (Total)	18/45 (40.0)	19/45 (42.2)	3/45 (6.7)	20/45 (44.4)	14/45 (31.1)	6/45 (13.3)
NOS=not otherwise specified						

Interpretive criteria/breakpoints

The applicant proposed interpretive criteria/breakpoints for *A. fumigatus* (Table 3); this is based on the PK-PD target attainment in nonclinical models of aspergillosis and exposure-response analysis of patients enrolled in the clinical trial.

Table 3: Susceptibility interpretive criteria for isavuconazole			
Broth Microdilution at 48 hours (MIC in µg/mL)			
Pathogen	Susceptible (S)	Intermediate (I)	Resistant (R)
<i>Aspergillus fumigatus</i>	≤1	2	≥4

The nonclinical studies included an *in vitro* dynamic model, a non-neutropenic murine model of disseminated aspergillosis and an immunocompromised murine model of pulmonary aspergillosis; based on simulations, the target attainment was 11.2 (PD 90% effective index based on galactomannan reduction), 50.48 (PD 50% effective index based on 14 day survival rate) and 503 (PD net stasis effective index based on reduction in fungal burden on Day 6), respectively. The clinical relevance of this approach is predicated on the assumption that the target of the new antifungal agent is the same in both the experimental model and humans. It is unclear which should be the relevant model and target for predicting response in humans. The findings in murine models are limited by the short half-life of isavuconazole in mice compared to humans.

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There was no correlation between exposure and response in patients enrolled in the clinical trials.
However, this analysis was limited by small number of patients in the clinical trial [REDACTED] (b) (4)

2. INTRODUCTION AND BACKGROUND

The subject of this NDA submission is isavuconazium sulfate (CRESAMBA[®]) for the treatment of invasive aspergillosis (IA) and invasive mucormycosis (IM). In the United States, voriconazole, itraconazole, caspofungin, and amphotericin B including liposomal formulations of amphotericin B such as AmBisome are approved for the treatment of IA. There are no FDA approved therapies for mucormycosis.

The applicant was granted a priority review of this application.

2.1. Isavuconazole

Following oral or intravenous administration, isavuconazonium sulfate (the pro-drug) is rapidly cleaved by plasma esterases into the active moiety: isavuconazole (BAL4815), and an inactive moiety (BAL8728). The mean half-life is 135 hours, maximum (or peak) concentration (C_{max}) was $\sim 7 \mu\text{g/mL}$, and area under the curve (AUC) about 3 to 4 $\mu\text{g}\cdot\text{h/mL}$ in healthy subjects as well as patients with invasive fungal infection (for details see section 4 of this review and clinical pharmacology review).

2.2. Biology of invasive aspergillosis

Invasive aspergillosis is a life-threatening opportunistic fungal infection and in certain immunocompromised hosts is associated with a high mortality rate that can vary with the patient population and the study center. The prevalence in neutropenic patients (< 500 neutrophils/ μL for at least 10 days) with hematological malignancies and hematopoietic stem cell transplant (HSCT) recipients is about 5 – 24% and these patients are particularly at risk; 60-75% of all fungal infections in these high risk patients are due to IA and associated with a mortality rate ranging from 30% to 50% (Klont *et al.*, 2004¹).

The lung is the most common site of infection and vascular invasion by *Aspergillus* species is a common histopathological feature of invasive pulmonary aspergillosis. The infection may extend to mediastinal and chest-wall structures and hematogenous dissemination that can involve virtually any organ including the brain. Clinical signs and symptoms of IA are not sensitive or specific and include persistent fever, cough, pleuritic chest pain, and hemoptysis. Radiological findings on chest X-ray are also not sensitive or specific. Chest computerized tomography (CT) findings considered characteristic of *Aspergillus* infection include nodular lesions, at times with a halo sign or crescent sign or cavitation. Although CT is more sensitive than chest X-ray, CT findings are not pathognomonic for IA and can be seen in a variety of other mould infections.

Diagnosis of IA remains challenging because clinical signs and symptoms and radiologic presentations are not specific and fungal cultures of affected tissues by standard techniques, although considered to be the “gold standard” are insensitive ($\sim 40\%$). Awaiting results of cultures are time consuming and can delay initiation of treatment. Blood and respiratory cultures are usually negative for *Aspergillus* in patients with IA, while obtaining samples of tissue is an invasive process and the underlying patients’ morbidities often prohibit obtaining tissue specimen for histological diagnosis.

¹ Klont RR, Mennink-Kersten MA, Verweij PE (2004) Utility of *Aspergillus* antigen detection in specimens other than serum specimens. *Clin Infect Dis* **39**: 1467-1474.

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A laboratory test that provides faster results and is more specific and sensitive than culture in detecting *Aspergillus* species in clinical specimens would facilitate earlier diagnosis, reatment options, and improve clinical outcomes.

The Bio-Rad Platelia™ *Aspergillus* enzyme immunoassay (EIA) for detection of galactomannan (GM; a polysaccharide component of the fungal cell wall, known to be present in *Aspergillus* and other fungi), is cleared by the FDA Center for Devices and Radiological Health (CDRH) for testing of serum samples and bronchoalveolar lavage (BAL) fluids; the package insert for this device instructs that “Platelia *Aspergillus* EIA is a test which, when used in conjunction with other diagnostic procedures such as microbiological cultures, histological examination of biopsy samples and radiological evidence can be used as an aid in the diagnosis of IA.” Based on the review of multiple published studies by the Division, the conclusion was reached that use of GM as a biomarker would increase the specificity and sensitivity of the chest computerized tomography (CT) findings. However, positive GM findings should be defined as follows:

Serum: A positive result should be based on a cut-off GM index ≥ 0.5 based on testing of two separate serum samples or a single sample with a value of ≥ 1.0 . It is important to note that the cut-off is different from the currently approved cut off ≥ 0.5 and based on testing of single specimen in the package insert cleared by the CDRH.

and/or

BAL fluid: A positive result should be based on a cut-off GM index ≥ 1.0 based on testing of two aliquots of a single BAL fluid sample. It is important to note that this cut-off ≥ 1.0 is different from the currently approved cut off ≥ 0.5 in the package insert cleared by the CDRH.

The use of different criteria for enrollment of patients in clinical trials is to increase the positive predictive value of the Bio-Rad Platelia™ *Aspergillus* EIA. For more details see <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM420248.pdf>.

Positive GM findings by the Platelia *Aspergillus* EIA, in serum and BAL fluids, as defined above will be appropriate as a standalone microbiological criteria and serve as a biomarker for diagnosis of “probable” IA in patients with hematologic malignancy and recipients of allogeneic hematopoietic stem cell transplants (HSCT) who have clinical signs/symptoms including radiologic findings suggestive of invasive fungal infection as defined by the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group (EORTC) and MSG criteria.² The detection of GM by Platelia *Aspergillus* EIA was not recommended for use as an enrollment criterion for patients with other types of immunodeficiency.

Galactomannan is present in *Aspergillus* and other fungi; infection with certain fungal pathogens, such as *Penicillium*, *Paecilomyces*, *Geotrichum*, and *Histoplasma* may lead to a positive GM

² de Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, Pappas PG, Maertens J, Lortholary O, Kauffman CA, Denning DW, Patterson TF, Maschmeyer G, Bille J, Dismukes WE, Herbrecht R, Hope WW, Kibbler CC, Kullberg BJ, Marr KA, Munoz P, Odds FC, Perfect JR, Restrepo A, Ruhnke M, Segal BH, Sobel JD, Sorrell TC, Viscoli C, Wingard JR, Zaoutis T, Bennett JE (2008) Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis* **46**: 1813-1821.

assay result due to cross-reactivity. Patients known to be infected with these pathogens should not be enrolled, and if enrolled, should be excluded from the analysis of efficacy.

The presence of GM in some of the antimicrobials (such as piperacillin/tazobactam, amoxicillin/clavulanate, amoxicillin and ampicillin) and the electrolyte solution, Plasma-Lyte, is known to yield false positive results. Therefore, the clinical trial should also exclude patients who are concomitantly receiving antibiotics known to result in a false positive GM (piperacillin/tazobactam, amoxicillin/ clavulanate), or concomitantly receiving Plasma-Lyte (physiological saline may be used as an alternative).

2.3. Biology of invasive mucormycosis

Mucormycosis, a life threatening infection, refers to invasive disease by a class of fungi, Zygomycetes in the order Mucorales that includes the species of *Mucor*, *Rhizopus*, *Rhizomucor*, *Lichtheimia (Absidia)*, *Cunninghamella*, *Saksanea*, and *Actinomucor elegans*. This disease is often characterized by hyphae growing in and around vessels. The terms "Mucormycosis" and "Zygomycosis" are sometimes used interchangeably. However, zygomycota has been identified as polyphyletic, and is not included in modern fungal classification systems. In general, these filamentous fungi are fast growing and virulent and can invade the rich vascular areas such as maxillo-facial areas.

Mucormycosis frequently involves the sinuses, brain, or lungs as the areas of infection. Oral or cerebral mucormycosis are the most common types of the disease; however, the infection can also manifest in the gastrointestinal tract, skin, and maxilla as well as in other organ systems.

Invasive mucormycosis is mostly seen in patients with diabetes mellitus, hematological malignancies undergoing chemotherapy, hematopoietic stem cell transplants (HSCT) recipients, patients with prolonged and severe neutropenia, poorly controlled diabetes mellitus with or without diabetic ketoacidosis, iron overload, major trauma, prolonged use of corticosteroids, illicit intravenous (IV) drug use, neonatal prematurity and malnourishment as well as voriconazole (known to have no activity against Zygomycetes) prophylaxis. Fungal invasion into the vasculature produces tissue infarction and necrosis.

Swabs of tissue or discharge are generally unreliable; the diagnosis of Mucormycosis is generally established by a biopsy specimen of the involved tissue.

3. NON CLINICAL MICROBIOLOGY STUDIES

3.1. Mechanism of action

Aoki *et al.*, 2000³ reported the activity of isavuconazole and other azoles against P450 lanosterol C-14 demethylase (P450_{14DM}), an enzyme important for ergosterol biosynthesis, extracted from *C. albicans* (strain 652) and rat liver. The microsomal fraction was incubated with and without isavuconazole and other azoles at 30°C for 60 minutes and reaction stopped by addition of KOH; lanosterol and 4,4-dimethyl sterols were analyzed by scanning mass chromatography. The results showed lower 50% inhibitory concentrations (IC₅₀s) for isavuconazole and other azoles (0.017–0.052 µg/mL) against *C. albicans* compared to those against rat liver P450_{14DM} enzymes (Table 4). Against rat liver P450_{14DM}, the activity of isavuconazole was similar to voriconazole and ravuconazole, less than ketoconazole and itraconazole, and more than fluconazole.

Table 4: Effect of isavuconazole and azole reference drugs on P450_{14DM} activity of rat liver and *C. albicans*

Compound	IC ₅₀ (mg/L)		Selectivity (ratio) (rat/ <i>C. albicans</i>)
	Rat liver	<i>C. albicans</i>	
Isavuconazole	4.0	0.043	93
Ketoconazole	0.39	0.030	13
Fluconazole	300	0.017	17600
Itraconazole	1.1	0.042	26
Voriconazole	4.4	0.052	85
BMS207147 (Ravuconazole)	2.8	0.020	140

P450_{14DM}=P450 lanosterol C-14 demethylase

Comments:

- *Isavuconazole inhibited ergosterol production by binding to and inhibiting the cytochrome P450 dependent lanosterol-14 alpha-demethylase in yeasts, an integral membrane protein encoded by the gene cyp51, which is present in all fungi. Depletion of ergosterol in the fungal membrane disrupts the structure and functions of the fungal membrane, including accumulation of toxic methylated intermediates, leading to inhibition of fungal growth.*
- *The activity of isavuconazole against P450_{14DM} enzyme from Aspergillus species or other filamentous fungi was not measured.*

3.2. Activity in vitro

In vitro susceptibility testing was performed in accordance with the standardized methods published by the Clinical Laboratory Standards Institute (CLSI)^{4, 5} and/or European Committee

³ Aoki Y, Kokado M, and Arisawa M. (2000) An inhibitory activity of R0-09-4815 against P450 lanosterol C-14 demethylase. Roche Research Report No. J-147*000. Internal Report, Roche, Kamakura, Japan.

⁴ Clinical and Laboratory Standards Institute (CLSI). Development of *in vitro* susceptibility testing criteria and quality control parameters; Approved Guideline – Second Edition. CLSI document M23-A2. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087, USA, 2008.

⁵ Clinical and Laboratory Standards Institute (CLSI). Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; approved standard - Second Edition CLSI document M38-A2, Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA, 2008.

on Antimicrobial Susceptibility Testing (EUCAST)⁶ against laboratory reference strains and clinical isolates of *Aspergillus*, *Rhizopus*, *Mucor*, *Rhizomucor*, *Cunninghamella*, *Lichtheimia* (*Absidia*), *Candida*, and other fungal species. This review focuses on testing of *Aspergillus* and fungal species in the Order Mucorales that are relevant to the indications under review in this NDA.

The minimum inhibitory concentration (MIC) were determined and the results presented as MIC range, modal MIC, geometric mean (GM) MIC, MIC₅₀ (drug concentration which inhibits at least 50% of isolates), and/or MIC₉₀ (drug concentration which inhibits at least 90% of isolates).

3.2.1. Development of *in vitro* susceptible testing and quality control

All testing and results are based on testing against the conidial forms of fungi unless specified otherwise.

3.2.1.1. Standardization of optimal conditions for *in vitro* susceptibility testing

The optimal conditions for *in vitro* susceptibility testing of isavuconazole against strains of *Aspergillus* species was performed under various growth conditions in accordance with the CLSI M38-A2⁵ and the EUCAST⁶ (also in RPMI medium at pH 7.0) methods and minimum inhibitory concentrations (MICs) determined were compared (Ghannoum, 2013⁷). Briefly, eight strains each of three *Aspergillus* species (*Aspergillus flavus*, *Aspergillus fumigatus*, and *Aspergillus niger*, obtained from the culture collection of the [REDACTED]^{(b) (4)}), were tested; voriconazole was included as a comparator for testing. The following growth conditions in RPMI medium were tested:

pH 5.4, pH 5.4 or pH 7 with 1% bile salts, pH 5.4 or pH 7 with 0.1% Tween 20, RPMI at pH 5.4 or pH 7 with 5% Tween 20, pH 5.4 or pH 7 with 10% pooled human serum (PHS), and pH 5.4 or pH 7 with 50% pooled PHS. MICs were also measured in CSM medium at pH 5.4 and pH 7, with and without bile salts, Tween 20 and PHS.

Isavuconazole MICs were measured at both 24 and 48 hours and at reading endpoints of 50% and 100% growth inhibition for comparison to the CLSI standard method. Voriconazole MICs were read at 48 hours and 100% growth inhibition, according to the CLSI standards. MICs within a two dilution range were considered equivalent.

The isavuconazole MIC ranges at 24 hours based on 50% inhibition and 100% inhibition were 0.125–1 µg/mL and 0.25–2 µg/mL, respectively, against all the eight strains by the CLSI method using RPMI 1640 medium; at 48 hours, the MIC ranges were 0.25–2 µg/mL and 0.5–4 µg/mL at 50% and 100% inhibition, respectively (Table 5). The voriconazole MIC range against all strains, tested by the CLSI method, was 1–4 µg/mL. The isavuconazole MIC range at 100% inhibition at 48 hours was similar to the CLSI range of voriconazole.

⁶ EUCAST. Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing. (2008) EUCAST Definitive Document EDef 9.1: method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for conidia forming moulds.

⁷ Ghannoum MA. (2013) Identify the optimal conditions for determining antifungal susceptibility testing of isavuconazole against *Aspergillus* strains. Internal report 9766-PH-0117.

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The isavuconazole MICs by the CLSI or EUCAST methods in RPMI at pH 5.4, and in RPMI with 10% PHS at pH 7 were comparable (Table 5). MICs in RPMI medium with bile at pH 7, 0.1% Tween at pH 5.4, 0.1% Tween at pH 7, 10% PHS at pH 5.4, and 50% PHS at both pH 5.4 and 7 were generally higher than those tested using CLSI methodology. There were no MIC endpoints for any strains in RPMI with 5% Tween at pH 5.4 or 7; none of the *A. fumigatus* strains were able to grow in RPMI with bile at pH 7. Precipitation was observed in all wells containing RPMI with bile at pH 5.4, and in the *A. fumigatus* strains grown in RPMI with 50% PHS at pH 5.4, which prevented reading of growth inhibition.

In CSM medium alone, and with additional 0.1% Tween or 10% PHS, MICs at pH 5.4 were comparable to the RPMI medium by the CLSI method. No growth was observed in the majority of the wells containing medium alone, Tween, or 10% PHS at pH 7, and with bile at either pH 5.4 or 7. There was no inhibition endpoint in all wells with CSM and 5% Tween at pH 5.4; the addition of 50% PHS caused precipitation that prevented the reading of growth inhibition at both pH 5.4 and 7.

Voriconazole MICs determined in the majority of the growth conditions in RPMI were comparable (within 2 dilutions) to those by the CLSI method. However, the majority of voriconazole MICs in CSM were not comparable to those by the CLSI method.

Overall, the results showed that the isavuconazole MIC range at 100% inhibition at 48 hours was comparable to the voriconazole MIC range by the CLSI or the EUCAST methods. Against the *Aspergillus* strains tested, both isavuconazole and voriconazole MICs were lower and more reproducible with CLSI M38-A2 methodology than any of the other test conditions.

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Table 5: Comparison of isavuconazole MICs obtained against *Aspergillus* strains using CLSI methodology and various growth conditions in RPMI-1640 and CSM medium

RPMI medium						CSM medium							
Species (no. of isolates)	Method or growth condition	24-hour MIC* (mg/L)		48-hour MIC* (mg/L)		Species (no. of isolates)	Method or growth condition	24-hour MIC* (mg/L)		48-hour MIC* (mg/L)			
		50%	100%	50%	100%			50%	100%	50%	100%		
<i>A. flavus</i> (8)	CLSI	0.25-0.5	1	0.5-1	1-2	<i>A. flavus</i> (8)	CLSI	0.25-0.5	1	0.5-1	1-2		
	EUCAST	within 1 dil	within 1 dil	within 1 dil	within 1 dil		pH 5.4	within 1 dil	within 1 dil	within 1 dil	within 1 dil		
	pH 5.4	0-2 dil higher	within 1 dil	within 1 dil	within 1 dil		pH 7	ng	ng	u	u		
	pH 5.4, 1% bile salts	u	u	u	u		pH 5.4, 1% bile salts	ng	ng	sd	sd		
	pH 7, 1% bile salts	2-4 dil higher	2-3 dil higher	3-4 dil higher	2-3 dil higher		pH 7, 1% bile salts	ng	ng	ng	ng		
	pH 5.4, 0.1% Tween 20	2-3 dil higher	2 dil higher	1-3 dil higher	1-3 dil higher		pH 5.4, 0.1% Tween 20	1-3 dil higher	0-2 dil higher	1-2 dil higher	1-2 dil higher		
	pH 7, 0.1% Tween 20	3-4 dil higher	2-3 dil higher	2-3 dil higher	2-3 dil higher		pH 7, 0.1% Tween 20	ng	ng	ng	ng		
	pH 5.4, 5% Tween 20	ne	ne	ne	ne		pH 5.4, 5% Tween 20	ne	ne	ne	ne		
	pH 7, 5% Tween 20	ne	ne	ne	ne		pH 7, 5% Tween 20	ng	ng	ng	ng		
	pH 5.4, 10% PHS	2-4 dil higher	1-3 dil higher	1-2 dil higher	1-2 dil higher		pH 5.4, 10% PHS	2-3 dil higher	1-2 dil higher	1-2 dil higher	1-2 dil higher		
	pH 7, 10% PHS	1-3 dil higher	0-2 dil higher	2 dil higher	2 dil higher		pH 7, 10% PHS	ng	ng	ng	ng		
	pH 5.4, 50% PHS	4-6 dil higher	3-5 dil higher	3-5 dil higher	3-5 dil higher		pH 5.4, 50% PHS	u	u	u	u		
	pH 7, 50% PHS	3-5 dil higher	3-4 dil higher	3-5 dil higher	3-4 dil higher		pH 7, 50% PHS	u	u	u	u		
	<i>A. fumigatus</i> (8)	CLSI	0.25	0.5	0.25-0.5		0.5-1	<i>A. fumigatus</i> (8)	CLSI	0.25	0.5	0.25-0.5	0.5-1
		EUCAST	within 1 dil	within 1 dil	within 1 dil		within 1 dil		pH 5.4	within 1 dil	equal	0-2 dil higher	0-2 dil higher
pH 5.4		within 1 dil	within 1 dil	within 1 dil	within 1 dil	pH 7	ng		ng	ng	ng		
pH 5.4, 1% bile salts		u	u	u	u	pH 5.4, 1% bile salts	ng		ng	ng	ng		
pH 7, 1% bile salts		ng	ng	ng	ng	pH 7, 1% bile salts	ng		ng	ng	ng		
pH 5.4, 0.1% Tween 20		2-3 dil higher	2-3 dil higher	1-2 dil higher	1-2 dil higher	pH 5.4, 0.1% Tween 20	2-3 dil higher		2 dil higher	2-3 dil higher	2-3 dil higher		
pH 7, 0.1% Tween 20		3 dil higher	3 dil higher	2-3 dil higher	2-3 dil higher	pH 7, 0.1% Tween 20	ng		ng	ng	ng		
pH 5.4, 5% Tween 20		ne	ne	ne	ne	pH 5.4, 5% Tween 20	ne		ne	ne	ne		
pH 7, 5% Tween 20		ne	ne	ne	ne	pH 7, 5% Tween 20	ng		ng	ng	ng		
pH 5.4, 10% PHS		within 1 dil	within 1 dil	0-3 dil higher	0-3 dil higher	pH 5.4, 10% PHS	1-2 dil higher		1-2 dil higher	1-2 dil higher	1-2 dil higher		
pH 7, 10% PHS		within 1 dil	within 1 dil	1-2 dil higher	1-2 dil higher	pH 7, 10% PHS	ng		ng	ng	ng		
pH 5.4, 50% PHS		u	u	u	u	pH 5.4, 50% PHS	u		u	u	u		
pH 7, 50% PHS		3-4 dil higher	3-4 dil higher	2-5 dil higher	2-4 dil higher	pH 7, 50% PHS	u		u	u	u		
<i>A. niger</i> (8)		CLSI	0.125-1	0.25-2	0.25-2	0.5-4	<i>A. niger</i> (8)		CLSI	0.125-1	0.25-2	0.25-2	0.5-4
		EUCAST	within 1 dil	within 1 dil	within 1 dil	within 1 dil			pH 5.4	sd	sd	0-2 dil higher	0-2 dil higher
	pH 5.4	1-2 dil higher	1-2 dil higher	1-2 dil higher	1-2 dil higher	pH 7		ng	ng	ng	ng		
	pH 5.4, 1% bile salts	u	u	u	u	pH 5.4, 1% bile salts		ng	ng	ng	ng		
	pH 7, 1% bile salts	1-3 dil higher	1-3 dil higher	1-3 dil higher	1-3 dil higher	pH 7, 1% bile salts		ng	ng	ng	ng		
	pH 5.4, 0.1% Tween 20	3-5 dil higher	3-5 dil higher	3-5 dil higher	3-5 dil higher	pH 5.4, 0.1% Tween 20		0-4 dil higher	0-4 dil higher	1-4 dil higher	1-4 dil higher		
	pH 7, 0.1% Tween 20	2-3 dil higher	2-3 dil higher	2-3 dil higher	1-3 dil higher	pH 7, 0.1% Tween 20		ng	ng	ng	ng		
	pH 5.4, 5% Tween 20	ne	ne	ne	ne	pH 5.4, 5% Tween 20		ne	ne	ne	ne		
	pH 7, 5% Tween 20	ne	ne	ne	ne	pH 7, 5% Tween 20		ng	ng	ng	ng		
	pH 5.4, 10% PHS	1-4 dil higher	1-4 dil higher	1-4 dil higher	1-4 dil higher	pH 5.4, 10% PHS		0-4 dil higher	1-4 dil higher	1-4 dil higher	1-4 dil higher		
	pH 7, 10% PHS	0-2 dil lower	0-2 dil lower	1-2 dil higher	1-2 dil higher	pH 7, 10% PHS		ng	ng	ng	ng		
	pH 5.4, 50% PHS	1-4 dil higher	1-3 dil higher	1-4 dil higher	1-4 dil higher	pH 5.4, 50% PHS		u	u	u	u		
	pH 7, 50% PHS	0-5 dil higher	2-5 dil higher	2-5 dil higher	2-4 dil higher	pH 7, 50% PHS		u	u	u	u		

*Only MIC values obtained using CLSI M38-A2 methodology are shown
dil = dilution(s); PHS = pooled human serum; u = unreadable due to precipitation; ng = no growth; ne = no MIC endpoint (MIC above or below range tested) for comparison

*Only MIC values obtained using CLSI M38-A2 methodology are shown
dil = dilution(s); PHS = pooled human serum; u = unreadable due to precipitation; ng = no growth in any well, including growth control; ne = no MIC endpoint (MIC above or below range tested) for comparison; sd = strain-dependent

sd: indicates that the MICs of some strains were higher than those of CLSI and others were lower, while some strains did not produce visible growth in any wells, including the growth control (no anti-fungal).

3.2.1.2. Optimal storage conditions for isavuconazole *in vitro* susceptibility testing assays

The optimal storage conditions for isavuconazole for *in vitro* susceptibility testing were determined in accordance with the CLSI M38-A2 method⁵ (Ghannoum, 2014⁸). Briefly, serial dilutions of the isavuconazole stock solutions were dispensed into 96-well plates and stored at 4° C, -20° C, and -80° C. At one month intervals up to six months, plates stored under each temperature were brought to room temperature and inoculated with two CLSI quality control (QC) strains (*A. fumigatus* MYA-3627 and *A. flavus* ATCC 204304 from the Center for Medical Mycology culture collection) to determine MICs; a divergence of greater than 3 dilutions from the initial MICs was considered significant. The results showed that the MICs against the two *Aspergillus* QC strains were stable over a period of six months when stored at 4° C, -20° C, and -80° C (Table 6).

Table 6: Isavuconazole MICs against <i>A. fumigatus</i> (strain MYA-3627) and <i>A. flavus</i> (strain ATCC 204304) at baseline and after storage at 4° C, -20° C and -80° C over a period of six months													
Baseline MICs													
Organism		24 h				48 h							
		50%		100%		50%		100%		50%		100%	
<i>A. flavus</i>		0.25		0.5		0.25		0.5		0.25		0.5	
<i>A. fumigatus</i>		0.25		0.5		0.25		0.5		0.25		0.5	
MIC values obtained using CLSI M38-A2 methodology.													
MICs after storage at 4° C, -20° C and -80° C over a period of six months													
Storage Condition		4° C				-20° C				-80° C			
Organism	Month	24 h		48 h		24 h		48 h		24 h		48 h	
		50%		100%		50%		100%		50%		100%	
<i>A. flavus</i>	1	0.5	1	0.5	1	0.5	1	0.5	1	0.5	1	0.5	2
<i>A. fumigatus</i>		0.25	0.5	0.5	1	0.25	0.5	0.25	1	0.25	1	0.5	1
<i>A. flavus</i>	2	0.5	1	0.5	1	0.5	1	0.5	1	0.5	1	0.5	1
<i>A. fumigatus</i>		0.25	1	0.25	1	0.25	1	0.5	2	0.25	1	0.5	1
<i>A. flavus</i>	3	0.25	0.5	0.25	1	0.25	0.5	0.25	1	0.25	0.5	0.25	1
<i>A. fumigatus</i>		0.25	0.5	0.25	1	0.25	0.5	0.25	1	0.25	0.5	0.25	1
<i>A. flavus</i>	4	0.5	1	0.5	1	0.5	1	0.5	1	0.5	1	0.5	1
<i>A. fumigatus</i>		0.5	1	0.5	1	0.5	1	0.5	1	0.5	1	0.5	1
<i>A. flavus</i>	5	0.25	0.5	0.25	0.5	0.25	0.5	0.25	0.5	0.25	0.5	0.25	0.5
<i>A. fumigatus</i>		0.25	0.5	0.25	0.5	0.25	0.5	0.25	0.5	0.25	0.5	0.25	0.5
<i>A. flavus</i>	6	0.25	2	0.5	1	0.25	1	0.5	1	0.25	1	0.5	1
<i>A. fumigatus</i>		0.25	2	0.5	1	0.25	1	0.5	1	0.25	1	0.5	1
Range		0.25 - 0.5		0.5 - 1		0.25 - 0.5		0.5 - 1		0.25 - 0.5		0.5 - 2	
MIC values obtained using CLSI M38-A2 methodology.													

3.2.1.3. Inter-laboratory and intra-laboratory comparison of isavuconazole MICs data

An intra-laboratory and inter-laboratory standardization of the *in vitro* susceptibility testing for isavuconazole was performed against 15 clinical isolates of *Aspergillus* species in eight laboratories to evaluate the reproducibility of susceptibility testing by the CLSI method; voriconazole was used as a control (Ghannoum, 2013⁹). The QC strains, *Paecilomyces variotii* MYA 3630 and *A. flavus* MYA 3631, were included on each day of testing. All testing was conducted in accordance with the CLSI-M38-A2 method.⁵ Each strain was tested 10 times in

⁸ Ghannoum MA. (2014) Identify the optimal storage conditions for isavuconazole in susceptibility testing assays. Internal report 9766-PH-0119.

⁹ Ghannoum MA. (2013) An inter-laboratory QC guidelines study for the testing of isavuconazole against *Aspergillus* species. Study report 9766-PH-0104.

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each RPMI medium lot by the eight laboratories. Isavuconazole MICs were recorded at both 50% and 100% growth inhibition endpoint as compared to the growth in control cultures (no drug exposure); voriconazole MICs were recorded at 100% growth inhibition endpoint. The results showed >90% inter-laboratory agreement at the 50% and 100% inhibition endpoint for all the *Aspergillus* strains (*A. fumigatus*, *A. flavus*, *A. nidulans*, *A. niger*, and *A. terreus*) tested (Table 7; Figures 1 and 2).

Table 7: MIC values of isavuconazole at the 50% and 100% end point and voriconazole at 100% end point

Isavuconazole MIC at 50% end point				Isavuconazole MIC at 100% end point			
Isolate #	Organism	% Agreement	Range (µg/ml)	Isolate #	Organism	% Agreement	Range (µg/ml)
1	<i>A. fumigatus</i>	97.90%	0.12-1	1	<i>A. fumigatus</i>	99.60%	0.25-2
2	<i>A. flavus</i>	99.60%	0.25-2	2	<i>A. flavus</i>	100%	0.5-4
3	<i>A. niger</i>	86.25%	0.12-1	3	<i>A. niger</i>	99.20%	0.5-4
4	<i>A. terreus</i>	99.20%	0.03-0.25	4	<i>A. terreus</i>	97.50%	0.12-1
5	<i>A. nidulans</i>	97.50%	0.015-0.12	5	<i>A. nidulans</i>	95.80%	0.06-0.5
6	<i>A. fumigatus</i>	95.80%	0.12-1	6	<i>A. fumigatus</i>	99.20%	0.25-2
7	<i>A. flavus</i>	100%	0.25-2	7	<i>A. flavus</i>	100%	0.5-4
8	<i>A. niger</i>	93.75%	0.12-1	8	<i>A. niger</i>	100%	0.5-4
9	<i>A. terreus</i>	97.90%	0.06-0.5	9	<i>A. terreus</i>	98.30%	0.25-2
10	<i>A. nidulans</i>	96.25%	0.015-0.12	10	<i>A. nidulans</i>	94.20%	0.06-0.5
11	<i>A. fumigatus</i>	97.90%	0.06-0.5	11	<i>A. fumigatus</i>	98.30%	0.25-2
12	<i>A. flavus</i>	100%	0.12-1	12	<i>A. flavus</i>	99.60%	0.5-4
13	<i>A. niger</i>	97.90%	0.25-2	13	<i>A. niger</i>	99.20%	0.5-4
14	<i>A. terreus</i>	99.20%	0.12-1	14	<i>A. terreus</i>	98.75%	0.25-2
15	<i>A. nidulans</i>	88.75%	0.008-0.06	15	<i>A. nidulans</i>	94.20%	0.03-0.25

Voriconazole MIC at 100% end point			
Isolate #	Organism	% Agreement	Range (µg/ml)
1	<i>A. fumigatus</i>	95.80%	0.12-1 µg/ml
2	<i>A. flavus</i>	100%	0.25->1
3	<i>A. niger</i>	100%	0.25->1
4	<i>A. terreus</i>	98.30%	0.06-0.5
5	<i>A. nidulans</i>	97.10%	0.12-1
6	<i>A. fumigatus</i>	95.40%	0.12-1
7	<i>A. flavus</i>	100%	0.25->1
8	<i>A. niger</i>	100%	0.25->1
9	<i>A. terreus</i>	99.20%	0.12-1
10	<i>A. nidulans</i>	97.50%	0.03-0.25
11	<i>A. fumigatus</i>	96.70%	0.12-1
12	<i>A. flavus</i>	100%	0.25->1
13	<i>A. niger</i>	99.50%	0.25->1
14	<i>A. terreus</i>	100%	0.25->1
15	<i>A. nidulans</i>	94.60%	0.03-0.25

Figure 1: Isavuconazole MICs for some of the *Aspergillus* strains at 50% and 100% growth inhibition endpoint using 3 lots of the drug

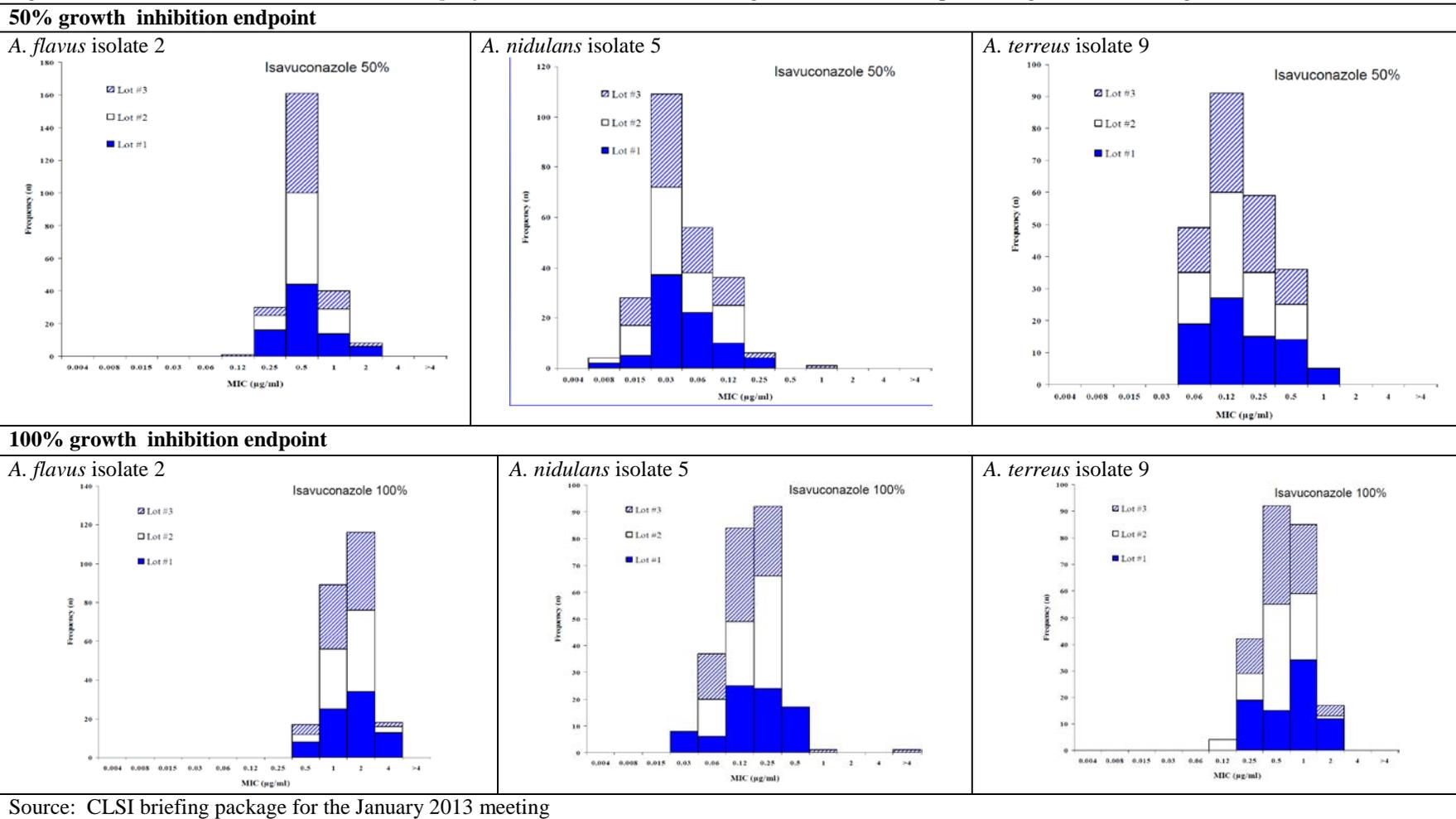
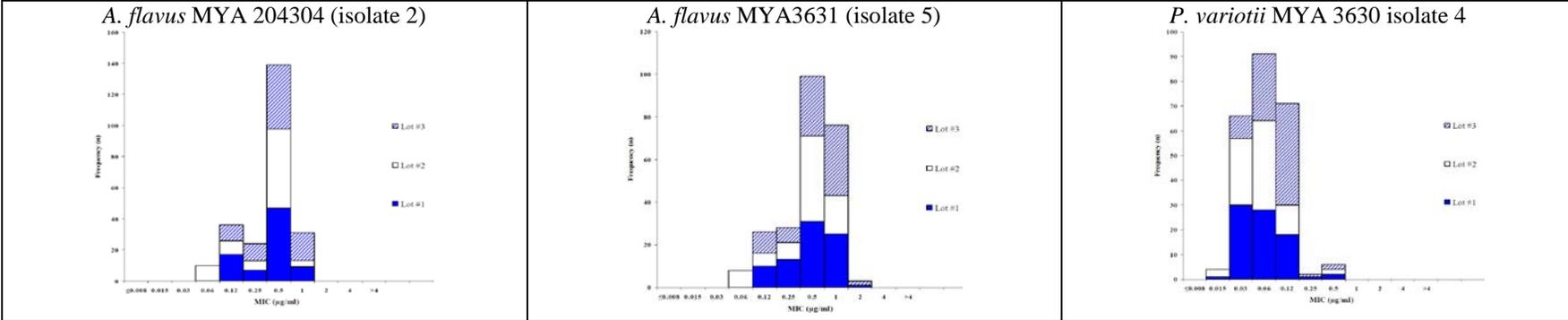
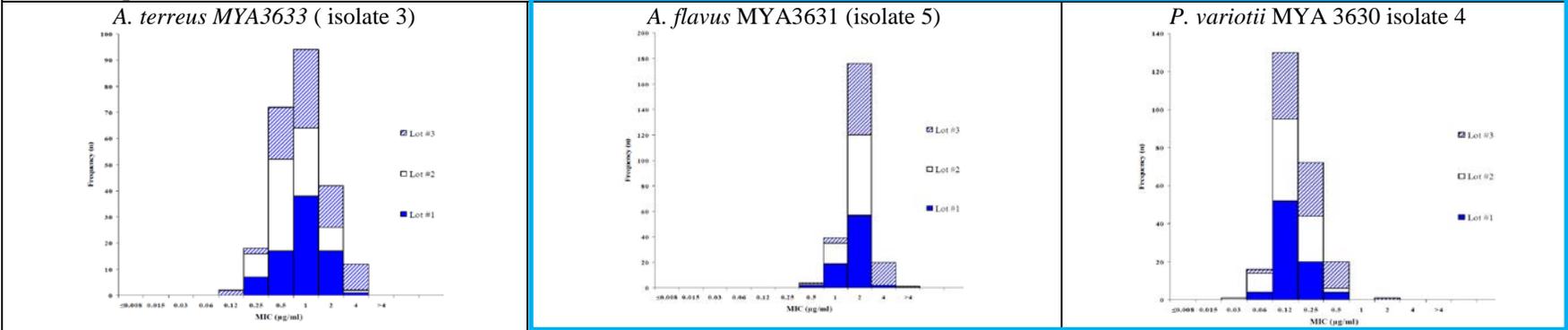


Figure 2: Isavuconazole MICs for some of the QC *Aspergillus* strains at 50% and 100% growth inhibition endpoint

50% inhibition endpoint



100% end point



Source: CLSI briefing package for the January 2013 meeting

Aspergillus Testing

Two strains were recommended as QC strains for testing isavuconazole against *Aspergillus* isolates at 48 hours (100% inhibition)

- *Paecilomyces variotii* MYA 3630 - Proposed Range: 0.06-0.5 µg/ml

- *Aspergillus flavus* ATCC 204304 - Proposed Range: 0.5-4 µg/ml

These also serve as QC strains for the most antifungals already included in the M38-A2 standard

Since there was >90% agreement inter-laboratory agreement of isavuconazole MICs against all *Aspergillus* strains, at the 100% inhibition point, the CLSI Subcommittee on Antifungal Susceptibility Testing (January 2013), recommended MICs be based on 100% inhibition endpoint at 48 hours.

3.2.1.4. Susceptibility testing of six QC strains of *Aspergillus* species to isavuconazole

An inter-laboratory QC study for the testing of isavuconazole against *Aspergillus* species was performed in eight laboratories to determine the reproducibility of MIC testing of candidate QC strains (6 ATCC strains: *A. fumigatus* ATCC MYA 3626 and ATCC MYA 3627, *A. terreus* ATCC MYA 3633, *A. flavus* ATCC 204304 and ATCC MYA 3631, as well as *Paecilomyces variotii* ATCC MYA-3630).¹⁰ All testing was performed in accordance with the CLSI guidelines (M38A2⁵ and M23-A2⁴) and MICs determined based on both 50% and 100% inhibition endpoint at 48 hours. Briefly, microtiter plates containing serial dilutions of isavuconazole were prepared with three lots of RPMI-1640 by (b) (4); voriconazole was included for testing as a control. Microdilution trays were incubated at 35° C. Each strain was tested ten times in each lot of RPMI 1640 medium by the eight laboratories. The results showed >90% inter-laboratory agreement with 4 (*A. flavus* and *P. variotii* strains) of the 6 strains (average 91.65%) at the 50% inhibition endpoint; the two *A. fumigatus* strains showed > 85% agreement (Table 8). At the 100% inhibition endpoint >90% (average 98.8%) agreement was observed for all of the 6 strains in all laboratories (Table 8). Voriconazole MIC values for the two QC strains were within the expected ranges of 0.015- 0.012 µg/mL for *P. variotii* MYA-3630 strain and 0.5-2.0 µg/mL for *A. flavus* MYA-3631 strain.

50% endpoint				100% endpoint			
Isolate #	Organism	% Agreement	Range (µg/ml)	Isolate #	Organism	% Agreement	Range (µg/ml)
1	<i>A. fumigatus</i> MYA 3626	85.8%	0.12-1	1	<i>A. fumigatus</i> MYA 3626	100%	0.5-4
2	<i>A. flavus</i> ATCC 204304	95.8%	0.12-1	2	<i>A. flavus</i> ATCC 204304	100%	0.5-4
3	<i>A. terreus</i> MYA 3633	90.8%	0.06-0.5	3	<i>A. terreus</i> MYA 3633	94.2%	0.25-2
4	<i>P. variotii</i> MYA 3630	96.7%	0.015-0.12	4	<i>P. variotii</i> MYA 3630	99.2%	0.06-0.5
5	<i>A. flavus</i> MYA 3631	95.4%	0.12-1	5	<i>A. flavus</i> MYA 3631	99.6%	0.5-4
6	<i>A. fumigatus</i> MYA 3627	85.4%	0.12-1	6	<i>A. fumigatus</i> MYA 3627	100%	0.5-4

The information summarized above was presented at the CLSI Subcommittee on Antifungal Susceptibility Testing, (January 2013); the subcommittee recommended the following QC strains for isavuconazole testing based on 100% inhibition endpoint at 48 hours.:

- *Paecilomyces variotii* MYA 3630: MIC range 0.06 – 0.5 µg/ml, and
- *Aspergillus flavus* ATCC 204304: MIC range of 0.5 – 4 µg/ml.

¹⁰ Ghannoum MA (2013) An inter and intra-laboratory study of minimum inhibitory concentration determination of isavuconazole against *Aspergillus*. Study report 9766-PH-0106.

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The applicant presented QC limits for susceptibility testing by the EUCAST method in the submission. In general, the isavuconazole MICs were one dilution higher by the EUCAST method compared to the CLSI method (Table 9).

Organism	MIC range (mg/L)
<i>A. fumigatus</i> ATCCC204305	0.25–0.5
<i>A. flavus</i> ATCC204304	1–2
<i>A. fumigatus</i> CNM-CM-237*	0.25–0.5
<i>A. fumigatus</i> CNM-CM-2159*	0.5–2
<i>A. terreus</i> CNM-CM-2524*	1–2
<i>A. flavus</i> CNM-CM-1838*	1–2
<i>S. apiospermum</i> CNM-CM-678*	>8
<i>P. variotii</i> ATCC22319	0.12–0.25
<i>C. parapsilosis</i> ATCC22019	0.015–0.03
<i>C. krusei</i> ATCC6258	0.03–0.12
<i>C. albicans</i> ATCC64548	≤0.015
<i>C. albicans</i> ATCC64550	0.25–0.5
<i>C. glabrata</i> ATCC90030	0.06–0.12
<i>S. cerevisiae</i> ATCC9763	0.03–0.06
<i>C. tropicalis</i> ATCC200956	>8
<i>C. lusitaniae</i> ATCC200956	≤0.015

*Mould Collection of the Spanish National Center for Microbiology (CNM-CM)

Comments:

- *The isavuconazole MICs were established against a panel of well-defined CLSI and EUCAST QC strains. The QC strains selected (to confirm that antifungal agents are present at correct concentrations, to monitor antifungal stability, investigate effects of medium, assist in test optimization studies, and to assess intra- and inter-laboratory variation) were appropriate.*
- *Based on the intra- and inter-laboratory standardization of the in vitro susceptibility testing of isavuconazole, the QC strains and the MIC range selected for in vitro susceptibility testing against filamentous fungi by the CLSI method were appropriate and are as follows:*
 - *Paecilomyces variotii MYA 3630 at a range of 0.06 – 0.5 µg/ml, and*
 - *Aspergillus flavus ATCC 204304 at a range of 0.5 – 4 µg/ml.*
- *One of the limitations of QC testing was a single source (b) (4) of microtiter plates included for testing. CLSI also recommended that “more Tier 3 data could be gathered over time (from different laboratories using different lots of plates and ideally not manufactured by same vendor).” For testing of microtiter plates from other sources, (b) (4) microtiter plates should be included as a comparator.*

3.2.2. Activity against Aspergillus species

The applicant compiled isavuconazole MIC data from different sources that are summarized below.

3.2.2.1. Surveillance studies

SENTRY, a global antifungal surveillance program operated by JMI Laboratories, was initiated in 2011; the applicant included study reports for 2011¹¹ and 2012¹² that summarized the activity of isavuconazole and other anti-fungal drugs against different fungal species. The identification of all organisms was confirmed at JMI Laboratories using microbiological and molecular methods. The susceptibility of *Aspergillus* and other filamentous fungal isolates to isavuconazole and comparator agents was determined using broth microdilution according to CLSI guidelines M38-A2.⁵

During 2011, SENTRY examined the activity against 1,573 clinical isolates collected from 75 medical centers located in North America (30 sites), Europe (24), Latin America (10), and the Asia-Pacific region (11). These strains were recovered consecutively from patients with bloodstream infections (n = 1,042), from normally sterile body fluids, tissues, abscesses (n = 163), from respiratory tract specimens (n = 215), and from non-specified sites (n = 153). Of the 1,573 fungal clinical isolates tested, 104 (6.4%) were *Aspergillus* species, 41 (2.6%) other moulds, 1,364 (86.7%) *Candida* species, 18 (1.1%) non-candidal yeasts, and 46 *Cryptococcus neoformans* (2.9%). The vast majority of the isolates came from either North America (46.6%) or Europe (31.6%). Of the 104 isolates of *Aspergillus* species, 71 were *A. fumigatus*, 11 were *A. niger*, 10 *A. flavus*, 6 *A. terreus* (n = 6), 3 *A. sydowii*, 1 *A. foetidus*, and 2 were unidentified *Aspergillus* species. Of the 104 total isolates, 63 were collected in North America, 25 in Europe, 11 in Latin America and five in the Asia-Pacific region.

During 2012, SENTRY examined the activity against a total of 1,670 clinical fungal isolates collected from 70 medical centers located in North America (29 sites), Europe (24), Latin America (10) and the Asia-Pacific region (7). These strains were recovered from patients with bloodstream infections (n = 1,094), from normally sterile body fluids, tissues and abscesses (n = 162), from respiratory tract specimens (n = 255), and from non-specified sites (n = 189). Of the 1,670 fungal clinical isolates tested, 130 (7.8%) were *Aspergillus* species, 38 (2.3%) other moulds, 1,421 (85.1%) *Candida* species, 31 (1.9%) non-candidal yeasts, including 11 *Trichosporon asahii*, and 50 *Cryptococcus neoformans* (3.0%). The majority of the isolates came from either North America (46.3%) or Europe (36.2%). Of the 130 isolates of *Aspergillus* species, majority were *A. fumigatus* (n = 90), 11 *A. flavus*, 11 *A. niger*, five *A. nidulans* and *A. sydowii*, 6 *A. terreus*, and one of *A. nomius* and *A. udagawae* (n = 1 for both). Of these, 87 isolates were collected in North America, 36 in Europe, and seven in Latin America.

During 2011 and 2012, isavuconazole MIC₅₀ and MIC₉₀ values were 1 µg/mL and 2 µg/mL, respectively, against all isolates of *Aspergillus* species (Table 10). Against both *A. fumigatus* and *A. flavus* isolates collected in 2011 and 2012, isavuconazole MIC₉₀ values were 1 µg/mL and 2 µg/mL, respectively (Table 10). *A. niger* MIC₉₀ values were 4 µg/mL in 2011 and 2012 (Table 10). Against unidentified *Aspergillus* species, the MIC_{90s} were 2 µg/mL. Overall, the studies showed that the isavuconazole MICs were similar to itraconazole, posaconazole, and

¹¹ Castanheira M, Pfaller MA, and Jones RN. (2012) International antifungal surveillance program for isavuconazole using the SENTRY antimicrobial surveillance program platform for 2011 (11-AST-05). Internal Report. JMI Laboratories, IA, USA.

¹² Castanheira M, Pfaller MA, and Jones RN. (2013) International antifungal surveillance program for isavuconazole using the SENTRY antimicrobial surveillance program platform for 2012 (12-AST-03). Internal Report. JMI Laboratories, IA, USA.

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voriconazole MICs. Fluconazole and flucytosine were not active. Echinocandin MICs were low $\leq 0.03 \mu\text{g/mL}$ (Table 10).

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Table 10: Activity of isavuconazole and comparators against <i>Aspergillus</i> species collected in 2011 in 2012						
2011						
<i>Aspergillus</i> species (n=104)						
Antifungal agent	MIC ₅₀		MIC (mg/L)	CLSI ^a	ECV ^b	
	MIC ₅₀	MIC ₉₀	Range		%S ^a / %R ^a	%WT ^b / %NWT ^b
Isavuconazole	1	2	0.12-4	-/-	-/-	-/-
Itraconazole	1	1	0.5-2	-/-	-/-	-/-
Posaconazole	0.5	1	0.12-1	-/-	-/-	-/-
Voriconazole	0.5	1	0.25-2	-/-	-/-	-/-
Fluconazole	>128	>128	128-128	-/-	-/-	-/-
Micafungin	0.015	0.03	$\leq 0.008-0.12$	-/-	-/-	-/-
Anidulafungin	0.015	0.03	$\leq 0.008-0.12$	-/-	-/-	-/-
Caspofungin	0.03	0.03	0.015-2	-/-	-/-	-/-
Amphotericin B	2	2	1-2	-/-	-/-	-/-
Flucytosine	>32	>32	4-32	-/-	-/-	-/-
2012						
<i>Aspergillus</i> species (n=130)						
Antifungal agent	MIC ₅₀		MIC (mg/L)	CLSI ^a	ECV ^b	
	MIC ₅₀	MIC ₉₀	Range		%S ^a / %R ^a	%WT ^b / %NWT ^b
Isavuconazole	1	2	0.25-8	-/-	-/-	-/-
Itraconazole	1	1	0.25-4	-/-	-/-	-/-
Posaconazole	0.5	1	0.12-1	-/-	-/-	-/-
Voriconazole	0.5	1	0.06-2	-/-	-/-	-/-
Fluconazole	0.015	0.015	$\leq 0.008-0.03$	-/-	-/-	-/-
Micafungin	0.015	0.015	$\leq 0.008-0.12$	-/-	-/-	-/-
Anidulafungin	0.03	0.06	$\leq 0.008-0.25$	-/-	-/-	-/-
Caspofungin	2	2	1-2	-/-	-/-	-/-
Amphotericin B	2	2	1-2	-/-	-/-	-/-
Flucytosine	>32	>32	4-32	-/-	-/-	-/-
<i>A. fumigatus</i> (n=71)						
Antifungal agent	MIC ₅₀		MIC (mg/L)	CLSI ^a	ECV ^b	
	MIC ₅₀	MIC ₉₀	Range		%S ^a / %R ^a	%WT ^b / %NWT ^b
Isavuconazole	1	1	0.25-2	-/-	-/-	-/-
Itraconazole	1	1	0.5-2	-/-	98.6 / 1.4	-/-
Posaconazole	0.5	1	0.12-1	-/-	88.7 / 11.3	-/-
Voriconazole	0.5	0.5	0.25-1	-/-	100.0 / 0.0	-/-
Fluconazole	128	128	128-128	-/-	97.8 / 2.2	-/-
Micafungin	0.015	0.03	$\leq 0.008-0.03$	-/-	-/-	-/-
Anidulafungin	0.03	0.03	$\leq 0.008-0.06$	-/-	-/-	-/-
Caspofungin	0.03	0.03	0.015-0.06	-/-	98.9 / 1.1	-/-
Amphotericin B	2	2	1-2	-/-	97.2 / 2.8	-/-
Flucytosine	>32	>32	16-32	-/-	-/-	-/-
<i>A. fumigatus</i> (n=90)						
Antifungal agent	MIC ₅₀		MIC (mg/L)	CLSI ^a	ECV ^b	
	MIC ₅₀	MIC ₉₀	Range		%S ^a / %R ^a	%WT ^b / %NWT ^b
Isavuconazole	1	2	1-4	-/-	-/-	-/-
Itraconazole	1	1	0.25-1	-/-	100.0 / 0.0	-/-
Posaconazole	0.25	0.5	0.12-1	-/-	94.4 / 5.6	-/-
Voriconazole	0.25	0.5	0.12-1	-/-	100.0 / 0.0	-/-
Fluconazole	0.015	0.015	$\leq 0.008-0.03$	-/-	-/-	-/-
Micafungin	0.015	0.015	$\leq 0.008-0.06$	-/-	-/-	-/-
Anidulafungin	0.03	0.06	$\leq 0.008-0.12$	-/-	98.9 / 1.1	-/-
Caspofungin	2	2	1-2	-/-	100.0 / 0.0	-/-
Amphotericin B	2	2	1-2	-/-	-/-	-/-
Flucytosine	>32	>32	>32	-/-	-/-	-/-
<i>A. flavus</i> (n=10)						
Antifungal agent	MIC ₅₀		MIC (mg/L)	CLSI ^a	ECV ^b	
	MIC ₅₀	MIC ₉₀	Range		%S ^a / %R ^a	%WT ^b / %NWT ^b
Isavuconazole	1	1	0.5-2	-/-	-/-	-/-
Itraconazole	1	1	1	-/-	100.0 / 0.0	-/-
Posaconazole	0.5	1	0.25-1	-/-	33.3 / 66.7	-/-
Voriconazole	1	1	0.5-1	-/-	100.0 / 0.0	-/-
Fluconazole	>128	>128	>128	-/-	-/-	-/-
Micafungin	≤ 0.008	0.03	$\leq 0.008-0.03$	-/-	-/-	-/-
Anidulafungin	≤ 0.008	0.03	$\leq 0.008-0.03$	-/-	-/-	-/-
Caspofungin	0.015	0.03	0.015-0.03	-/-	-/-	-/-
Amphotericin B	2	2	2	-/-	100.0 / 0.0	-/-
Flucytosine	>32	>32	>32	-/-	-/-	-/-
<i>A. flavus</i> (n=11)						
Antifungal agent	MIC ₅₀		MIC (mg/L)	CLSI ^a	ECV ^b	
	MIC ₅₀	MIC ₉₀	Range		%S ^a / %R ^a	%WT ^b / %NWT ^b
Isavuconazole	1	2	1-2	-/-	-/-	-/-
Itraconazole	1	1	0.5-1	-/-	100.0 / 0.0	-/-
Posaconazole	0.5	1	0.25-1	-/-	81.8 / 18.2	-/-
Voriconazole	0.5	1	0.25-1	-/-	100.0 / 0.0	-/-
Fluconazole	0.015	0.03	$\leq 0.008-0.03$	-/-	-/-	-/-
Micafungin	≤ 0.008	0.015	$\leq 0.008-0.015$	-/-	-/-	-/-
Anidulafungin	≤ 0.008	0.03	$\leq 0.008-0.03$	-/-	100.0 / 0.0	-/-
Caspofungin	0.015	0.03	0.015-0.06	-/-	100.0 / 0.0	-/-
Amphotericin B	2	2	2	-/-	100.0 / 0.0	-/-
Flucytosine	>32	>32	32-32	-/-	-/-	-/-
<i>A. niger</i> (n=11)						
Antifungal agent	MIC ₅₀		MIC (mg/L)	CLSI ^a	ECV ^b	
	MIC ₅₀	MIC ₉₀	Range		%S ^a / %R ^a	%WT ^b / %NWT ^b
Isavuconazole	2	4	0.5-4	-/-	-/-	-/-
Itraconazole	1	2	1-2	-/-	100.0 / 0.0	-/-
Posaconazole	1	1	0.5-1	-/-	45.5 / 54.5	-/-
Voriconazole	1	2	0.5-2	-/-	100.0 / 0.0	-/-
Fluconazole	>128	>128	>128	-/-	-/-	-/-
Micafungin	≤ 0.008	0.03	$\leq 0.008-0.03$	-/-	-/-	-/-
Anidulafungin	≤ 0.008	0.03	$\leq 0.008-0.03$	-/-	-/-	-/-
Caspofungin	0.015	0.03	0.015-0.06	-/-	-/-	-/-
Amphotericin B	1	1	1	-/-	100.0 / 0.0	-/-
Flucytosine	16	>32	4-32	-/-	-/-	-/-
<i>A. niger</i> (n=11)						
Antifungal agent	MIC ₅₀		MIC (mg/L)	CLSI ^a	ECV ^b	
	MIC ₅₀	MIC ₉₀	Range		%S ^a / %R ^a	%WT ^b / %NWT ^b
Isavuconazole	4	4	2-8	-/-	-/-	-/-
Itraconazole	2	2	1-4	-/-	90.9 / 9.1	-/-
Posaconazole	1	1	0.5-1	-/-	100.0 / 0.0	-/-
Voriconazole	1	1	0.5-2	-/-	100.0 / 0.0	-/-
Fluconazole	≤ 0.008	0.015	$\leq 0.008-0.03$	-/-	-/-	-/-
Micafungin	≤ 0.008	0.015	$\leq 0.008-0.015$	-/-	-/-	-/-
Anidulafungin	≤ 0.008	0.03	0.015-0.06	-/-	100.0 / 0.0	-/-
Caspofungin	0.015	0.03	0.015-0.06	-/-	100.0 / 0.0	-/-
Amphotericin B	1	1	1	-/-	100.0 / 0.0	-/-
Flucytosine	16	>32	4-32	-/-	-/-	-/-
Other <i>Aspergillus</i> species (n=12)						
Antifungal agent	MIC ₅₀		MIC (mg/L)	CLSI ^a	ECV ^b	
	MIC ₅₀	MIC ₉₀	Range		%S ^a / %R ^a	%WT ^b / %NWT ^b
Isavuconazole	0.5	2	0.12-2	-/-	-/-	-/-
Itraconazole	1	2	0.5-2	-/-	-/-	-/-
Posaconazole	0.5	1	0.25-1	-/-	-/-	-/-
Voriconazole	0.5	1	0.25-2	-/-	-/-	-/-
Fluconazole	>128	>128	>128	-/-	-/-	-/-
Micafungin	0.015	0.06	$\leq 0.008-0.12$	-/-	-/-	-/-
Anidulafungin	0.015	0.12	$\leq 0.008-0.12$	-/-	-/-	-/-
Caspofungin	0.015	0.25	0.015-2	-/-	-/-	-/-
Amphotericin B	2	2	1-2	-/-	-/-	-/-
Flucytosine	>32	>32	8-32	-/-	-/-	-/-
Other <i>Aspergillus</i> species (n=18)						
Antifungal agent	MIC ₅₀		MIC (mg/L)	CLSI ^a	ECV ^b	
	MIC ₅₀	MIC ₉₀	Range		%S ^a / %R ^a	%WT ^b / %NWT ^b
Isavuconazole	1	2	0.25-4	-/-	-/-	-/-
Itraconazole	0.5	2	0.5-2	-/-	-/-	-/-
Posaconazole	0.5	1	0.25-1	-/-	-/-	-/-
Voriconazole	0.5	1	0.06-2	-/-	-/-	-/-
Fluconazole	≤ 0.008	0.03	$\leq 0.008-0.03$	-/-	-/-	-/-
Micafungin	0.015	0.06	$\leq 0.008-0.12$	-/-	-/-	-/-
Anidulafungin	0.015	0.06	0.015-0.25	-/-	-/-	-/-
Caspofungin	2	2	1-2	-/-	-/-	-/-
Amphotericin B	2	2	1-2	-/-	-/-	-/-
Flucytosine	>32	>32	16-32	-/-	-/-	-/-

^aS = susceptible; R = resistant

^bAccording to Espinel-Ingroff *et al* (2011). ECV = epidemiological cut-off value; WT = wild-type; NWT = non wild-type

Where an epidemiological cut-off value (ECV) has been established for antifungal agents against certain fungal species, data showing the percentage of isolates that were categorized as wild-type (MIC < ECV) or non-wild-type (MIC > ECV) was also presented.

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Comments:

- *The activity of isavuconazole was similar over the 2 years of global surveillance against different Aspergillus species (Table 11). Overall, the activity of isavuconazole was comparable with that of itraconazole, posaconazole, and voriconazole under same test conditions.*

Table 11: Isavuconazole MIC₉₀s (µg/mL) from the surveillance studies in 2011 and 2012.

Pathogen	MIC ₉₀ (n)		
	2011	2012	2011 +2012
<i>Aspergillus</i> species	2 (104)	2 (130)	2 (234)
<i>A. fumigatus</i>	1 (71)	2 (90)	1-2 (161)
<i>A. flavus</i>	1 (10)	2 (11)	1-2 (21)
<i>A. niger</i>	4 (11)	4 (11)	4 (22)
Other <i>Aspergillus</i> species	2 (12)	2 (18)	2 (30)

3.3.2.2. Database

The applicant presented compiled MIC data, from several published or unpublished studies including the data from the 2011 SENTRY global antifungal surveillance program (see Appendix-1¹³), in an analyzable Excel database; compilation was undertaken by (b) (4) under Master Services Agreements with Basilea Pharmaceutica International ('Basilea') and Astellas Pharma Global Development, Inc. ('Astellas').¹⁴ Data collation was based upon original source results, i.e., MIC results for each fungal isolate. For this, several data conventions were followed:

- Where a test was replicated, the highest (i.e., most resistant) result was included for reporting.
- Where an MIC had a ">" sign, the value was doubled – i.e., moved up one halving dilution, since the true result was at least one dilution higher; < sign did not result in any change.
- In order to standardize all MIC values to the usual halving dilutions, any values falling between two levels were reported at the level above. For example 0.063 was reported as 0.12.
- MIC and MFC (minimum fungicidal concentration) was handled in the same way.

It should be noted that some of the early *in vitro* studies employed some variation of test conditions, particularly duration of incubation period, and these were delineated in the data summaries. QC checks were undertaken throughout the collation, entry and analysis stages. The country of collection was not reported for the majority of isolates. Isolates were collected from at least 67 countries worldwide. On the basis of available geographical information and location of test laboratory, the approximate percentage of isolates collected by region was assumed (Table 12). The majority of isolates were tested in laboratories in Europe and the remainder in the US. Data quality control checks were undertaken throughout the collation, entry, and analysis stages.

For *Aspergillus* species, data were based on 3,306 isolates collected worldwide; the isolates were collected and tested at centers in the US (n = 317; 9.6%) and Europe (n = 2,989; 90.4%) by

¹³ The studies may have been presented at conferences, as posters or oral presentations, published in journals or provided as study reports to Basilea or Astellas. The main requirement for including data was that the methodology was fully stated and that raw data values for MIC or similar assays were available.

(b) (4)

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either the CLSI or the EUCAST method. The isolates comprised 29 species/subspecies and 10 unspciated isolates; *A. fumigatus* (n = 1,538; 46.5%) was the most prevalent species, followed by *A. terreus* (n = 675; 20.4%), *A. flavus* (n = 392; 11.9%), *A. niger* (n = 342; 10.3%) and *A. nidulans* (n = 331; 10.0%).

Isavuconazole MICs against *Aspergillus* species by the CLSI method (M38-A2⁵; 48 hour incubation and 100% endpoint), was determined against 1,717 isolates; the MIC₅₀ and MIC₉₀ were 0.5 and 2 µg/mL, respectively, and MIC range 0.06 to 32 µg/mL (Table 12; Figure 3). There were minor differences in the MIC₅₀ and MIC₉₀ values for isolates tested in the US and Europe.

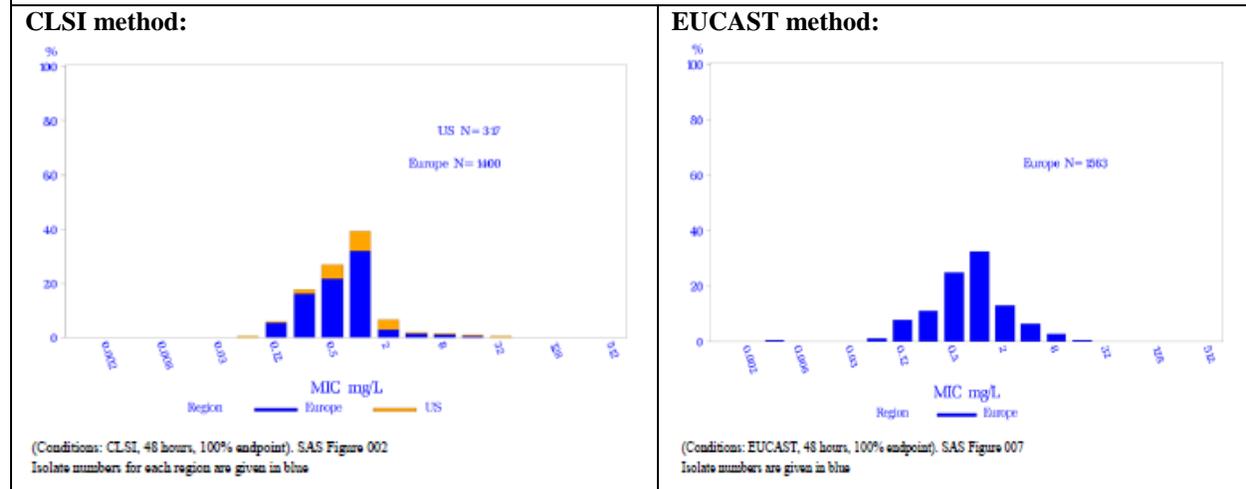
By the EUCAST method, isavuconazole MICs were determined against 1,563 isolates; the MIC₅₀ and MIC₉₀ values were 1 and 2 µg/mL, respectively (Figure 3).

Table 12: Summary of MIC distributions for isavuconazole against *Aspergillus* spp. using CLSI and EUCAST methods

Species	Method	Isolates (n)	MIC parameter (µg/L)						SAS Ref
			MIN	MAX	MIC ₅₀	MIC ₉₀	MIC ₉₅	GM	
All	CLSI	1,717	0.06	32	0.5	2	2	0.65	T1
	EUCAST	1,563	0.004	16	1	2	4	0.75	T1b
<i>A. fumigatus</i>	CLSI	875	0.12	8	1	1	2	0.79	T1.1
	EUCAST	434	0.12	16	1	1	2	0.8	T1.1b
<i>A. flavus</i>	CLSI	145	0.12	16	1	4	8	0.89	T1.2
	EUCAST	233	0.12	4	1	2	4	1.1	T1.2b
<i>A. niger</i>	CLSI	101	0.12	32	1	2	4	0.97	T1.3
	EUCAST	222	0.25	16	2	4	8	2.24	T1.3b
<i>A. terreus</i>	CLSI	432	0.06	32	0.25	1	2	0.30	T1.4
	EUCAST	431	0.06	8	0.5	4	4	0.65	T1.4b
<i>A. nidulans</i>	CLSI	85	0.12	16	0.5	1	1	0.30	T1.5
	EUCAST	206	0.004	8	0.12	0.5	1	0.19	T1.5a

Isolates collected world wide

Figure 3: MIC distribution of isavuconazole against *Aspergillus* species from the US and Europe using CLSI or EUCAST method



Isavuconazole MICs, by the CLSI method, against 5 species of *Aspergillus* isolates, collected from the US or Europe, were similar; one dilution differences were reported (Table 13)

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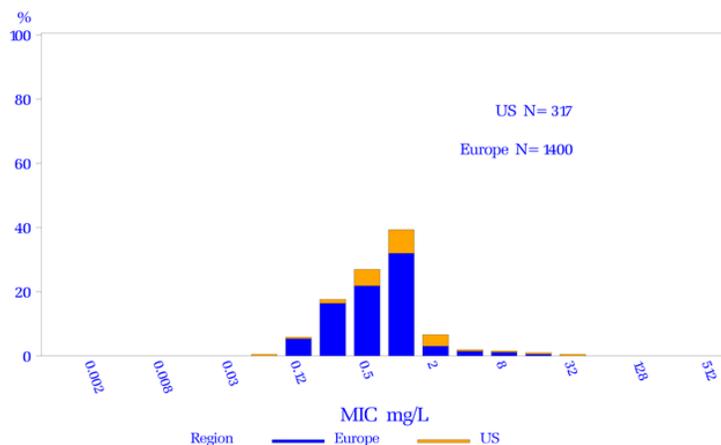
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suggesting no marked effect of region upon MIC distributions. No data were available for this comparison by the EUCAST method.

Table 13: Effect of region upon MIC distributions for isavuconazole against *Aspergillus* species tested by the CLSI method

Species	Region	Isolates (n)	MIC parameter (mg/L)					SAS Ref	
			MIN	MAX	MFC ₅₀	MFC ₉₀	MFC ₉₅		GM
All	Europe	1,400	0.12	16	0.5	1	2	0.6	T1
	US	317	0.06	32	1	2	2	0.9	T1
<i>A. fumigatus</i>	Europe	728	0.12	8	1	1	1	0.78	T1.1
	US	147	0.25	4	1	2	2	0.86	T1.1
<i>A. flavus</i>	Europe	87	0.12	16	0.5	4	8	0.83	T1.2
	US	58	0.12	16	1	2	16	0.99	T1.2
<i>A. niger</i>	Europe	64	0.12	8	1	2	4	0.97	T1.3
	US	37	0.25	32	1	2	4	0.96	T1.3
<i>A. terreus</i>	Europe	406	0.12	16	0.25	1	2	0.38	T1.4
	US	27	0.06	32	0.5	2	2	0.52	T1.4
<i>A. nidulans</i>	Europe	73	0.12	16	0.25	1	1	0.36	T1.5
	US	12	0.12	2	0.5	1	2	0.59	T1.5



The minimum fungicidal concentrations (MFCs) were determined against 740 *Aspergillus* species isolates according to CLSI guidelines. Both MFC₅₀ and MFC₉₀ values were 1 µg/mL. MFC₅₀ and MFC₉₀ values for *A. fumigatus* were both 1 µg/mL, based on 609 isolates tested by CLSI method (Table 14). The other three species displayed higher MFC₉₀ values of 4 µg/mL.

Table 14: MFC distributions for isavuconazole against *Aspergillus* spp. by the CLSI method

Species	Region	Isolates (n)	MFC parameter (mg/L)					SAS Ref	
			MIN	MAX	MFC ₅₀	MFC ₉₀	MFC ₉₅		GM
All	Europe	740	0.12	16	1	1	4	0.94	T1h
<i>A. fumigatus</i>	Europe	609	0.12	8	1	1	1	0.87	T1.1h
<i>A. flavus</i>	Europe	43	0.5	8	1	4	8	1.17	T1.2h
<i>A. niger</i>	Europe	34	0.25	8	1	4	8	1.36	T1.3h
<i>A. terreus</i>	Europe	41	0.25	8	1	4	4	1.43	T1.4h

The isavuconazole MICs were similar to itraconazole and voriconazole MICs and higher than posaconazole MICs (Table 15). The echinocandin MICs were generally low compared to triazoles.

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Table 15: Summary MIC parameters of isavuconazole and comparators against *Aspergillus* spp. by the CLSI method

Aspergillus species

Antifungal agent	Isolates (n)	MIC parameter (mg/L)					
		MIN	MAX	MIC ₅₀	MIC ₉₀	MIC ₉₅	GM
Isavuconazole	1717	0.06	32	0.5	2	2	0.65
Itraconazole	695	0.008	16	0.25	1	1	0.27
Posaconazole	822	0.008	2	0.12	0.5	1	0.18
Voriconazole	1648	0.06	8	0.5	1	1	0.44
Micafungin	546	0.008	16	0.015	0.06	0.12	0.02
Caspofungin	952	0.015	32	0.25	4	16	0.31
Anidulafungin	546	0.008	16	0.015	0.03	0.06	0.02
Amphotericin B	930	0.06	16	2	4	8	1.49
Flucytosine	317	1	128	64	128	128	53.03

A. fumigatus

Antifungal agent	Isolates (n)	MIC parameter (mg/L)					
		MIN	MAX	MIC ₅₀	MIC ₉₀	MIC ₉₅	GM
Isavuconazole	875	0.12	8	1	1	2	0.79
Itraconazole	152	0.06	16	1	1	16	0.5
Posaconazole	172	0.03	1	0.25	0.5	1	0.21
Voriconazole	854	0.06	2	0.5	1	1	0.41
Micafungin	148	0.008	16	0.015	16	16	0.04
Caspofungin	258	0.015	32	0.25	32	32	0.33
Anidulafungin	148	0.008	16	0.03	4	16	0.04
Amphotericin B	244	0.06	4	1	2	2	0.95
Flucytosine	147	8	128	64	128	128	73.72

A. flavus

Antifungal agent	Isolates (n)	MIC parameter (mg/L)					
		MIN	MAX	MIC ₅₀	MIC ₉₀	MIC ₉₅	GM
Isavuconazole	145	0.12	16	1	4	8	0.89
Itraconazole	34	0.03	0.5	0.12	0.25	0.5	0.12
Posaconazole	81	0.03	0.5	0.12	0.5	0.5	0.14
Voriconazole	131	0.12	4	0.5	1	1	0.47
Micafungin	60	0.008	16	0.015	0.03	0.06	0.02
Caspofungin	97	0.015	32	0.06	1	32	0.12
Anidulafungin	60	0.008	16	0.015	0.015	0.015	0.02
Amphotericin B	95	0.5	4	1	2	4	1.25
Flucytosine	58	2	128	128	128	128	81.28

A. niger

Antifungal agent	Isolates (n)	MIC parameter (mg/L)					
		MIN	MAX	MIC ₅₀	MIC ₉₀	MIC ₉₅	GM
Isavuconazole	101	0.12	32	1	2	4	0.97
Itraconazole	23	0.06	2	0.25	0.5	1	0.25
Posaconazole	44	0.06	2	0.25	1	1	0.27
Voriconazole	96	0.06	4	0.5	1	2	0.53
Micafungin	37	0.015	0.03	0.015	0.015	0.03	0.02
Caspofungin	66	0.015	32	0.12	0.5	32	0.17
Anidulafungin	37	0.015	0.03	0.015	0.015	0.015	0.02
Amphotericin B	64	0.12	2	0.5	1	2	0.56
Flucytosine	37	1	128	8	128	128	9.83

A. terreus

Antifungal agent	Isolates (n)	MIC parameter (mg/L)					
		MIN	MAX	MIC ₅₀	MIC ₉₀	MIC ₉₅	GM
Isavuconazole	432	0.06	32	0.25	1	2	0.39
Itraconazole	355	0.008	1	0.12	1	1	0.19
Posaconazole	375	0.008	0.5	0.12	0.5	0.5	0.14
Voriconazole	409	0.06	4	0.5	1	1	0.49
Micafungin	217	0.008	16	0.008	0.06	0.12	0.02
Caspofungin	381	0.015	32	0.5	2	4	0.5
Anidulafungin	217	0.008	8	0.008	0.015	0.015	0.01
Amphotericin B	380	0.25	16	2	8	8	2.26
Flucytosine	27	4	128	128	128	128	82.73

A. nidulans

Antifungal agent	Isolates (n)	MIC parameter (mg/L)					
		MIN	MAX	MIC ₅₀	MIC ₉₀	MIC ₉₅	GM
Isavuconazole	85	0.12	16	0.5	1	1	0.39
Itraconazole	65	0.06	2	1	1	2	0.71
Posaconazole	75	0.12	1	0.5	1	1	0.45
Voriconazole	82	0.06	8	0.5	2	2	0.4
Micafungin	12	0.015	16	0.015	0.03	16	0.03
Caspofungin	75	0.03	16	0.12	0.25	2	0.15
Anidulafungin	12	0.015	16	0.015	0.03	16	0.03
Amphotericin B	75	1	8	2	4	8	2.64
Flucytosine	12	2	128	8	128	128	10.08

• **Activity against the hyphae**

The isavuconazole MICs (MIC₅₀ and MIC₉₀ values of 2 and 8 µg/mL, respectively) against 40 *Aspergillus* species isolates in hyphal form, determined by the CLSI method, were higher than previous studies with conidia.

3.2.2.3. Other studies

There were few other published studies (Yamazaki *et al.*, 2010;¹⁵ Pelaez *et al.*, 2009;¹⁶ Rudramurthy *et al.*, 2011;¹⁷ Datta *et al.*, 2013¹⁸) included that were not listed in Appendix-1; therefore, it is assumed that the data from these studies was not a part of the database and are summarized in Table 16 for completeness. Overall, the results from these studies support the activity of isavuconazole against *Aspergillus* species.

Table 16: Isavuconazole MICs by the CLSI method (unless specified otherwise) from published studies				
Species	Reference	Country (number of isolates)	MIC (µg/mL)	
			Range	MIC ₉₀
<i>A. fumigatus</i>	Yamazaki <i>et al.</i> , 2010 ¹⁵	Japan (12)	0.1-0.39	0.39
<i>A. flavus</i>	Pelaez <i>et al.</i> , 2009 ¹⁶	-		
	Rudramurthy <i>et al.</i> , 2011 ^{17*}	India and Netherlands (187)	0.125-2	Not available
	Yamazaki <i>et al.</i> , 2010 ^{15,15}	Japan (1)	0.78	-
<i>A. niger</i>	Yamazaki <i>et al.</i> , 2010 ¹⁵	Japan (1)	1.56	
<i>A. terreus</i>	Pelaez <i>et al.</i> , 2009 ¹⁶	Madrid (132)	0.125-1	0.5
	Yamazaki <i>et al.</i> , 2010 ¹⁵	Japan (3)	0.2-0.39	0.39
<i>A. nidulans</i>	Pelaez <i>et al.</i> , 2009 ¹⁶	Madrid (63)	0.06-1	1
<i>A. oryzae</i>	Yamazaki <i>et al.</i> , 2010 ¹⁵	Japan (1)	0.39	
<i>A. lentulus</i>	Datta <i>et al.</i> , 2013 ¹⁸	US (15)	0.063–0.5	0.25

Only MIC range shown and not MIC₉₀ when n<10 isolates
*Testing by EUCAST method. The authors stated there was “essential agreement between the EUCAST and CLSI methods determined as percentage of results within ±1 dilution was 89.8% for isavuconazole, better than observed for the other azole antifungal agents (range 15.5% to 80.2%)”

¹⁵ Yamazaki T, Inagaki Y, Fujii T, Ohwada J, Tsukazaki M, Umeda I, Kobayashi K, Shimma N, Page MG, and Arisawa M. (2010) In vitro activity of isavuconazole against 140 reference fungal strains and 165 clinically isolated yeasts from Japan. *Int J Antimicrob Agents*. **36** (4):324-331.

¹⁶ Peláez T, Gama B, Guinea J, Sanchez-Cambronero L, Martin-Rabadan P, Flores R, Alcalá L, Muñoz P, and Bouza E. (2009) Assessment of the antifungal susceptibility of *Aspergillus terreus* over an 18-year period in a general hospital. 49th Interscience Conference on Antimicrobial Agents and Chemotherapy, Poster M-1714, San Francisco, USA.

¹⁷ Rudramurthy SM, Chakrabarti A, Geertsen E, Mouton JW, and Meis JF. (2011) *In vitro* activity of isavuconazole against 208 *Aspergillus flavus* isolates in comparison with 7 other antifungal agents: assessment according to the methodology of the European Committee on Antimicrobial Susceptibility Testing. *Diagn Microbiol Infect Dis*. **71** (4):370-377.

¹⁸ Datta K, Rhee P, Byrnes E 3rd, Garcia-Effron G, Perlin DS, Staab JF, and Marr KA. (2013) Isavuconazole activity against *Aspergillus lentulus*, *Neosartorya udagawae*, and *Cryptococcus gattii*, emerging fungal pathogens with reduced azole susceptibility. *J Clin Microbiol*. **51**(9):3090-3093.

- **Activity against the hyphae**

Perkhofer *et al.* (2009)¹⁹ reported the *in vitro* activity of isavuconazole against 96 isolates of *Aspergillus* species by the EUCAST method. The MICs and MFCs of isavuconazole, voriconazole and posaconazole were determined against the hyphal and conidial forms of *Aspergillus* species. The isolates were recovered at the Innsbruck Medical University over a period of 10 years. Isavuconazole was active against a wide range of *Aspergillus* conidia, including *A. terreus* (Table 17). Isavuconazole MICs against the hyphal and conidial forms were similar.

Table 17: *In vitro* susceptibility of the conidial and hyphal forms of *Aspergillus* species and zygomycetes to isavuconazole and other azoles, according to EUCAST methodology

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3.2.2.4. Clinical trial isolates

Clinical fungal isolates (n=164) from patients (n=127: 102 treated with isavuconazole and 25 treated with voriconazole) in the two phase 3 clinical trials (Studies 9766-CL-0104 and 9766-CL-0103) with positive culture were tested for antifungal susceptibility at a central laboratory. Three central laboratories were utilized for these studies (b) (4)

¹⁹ Perkhofer S, Lechner V, Lass-Flörl C; European Committee on Antimicrobial Susceptibility Testing. (2009) *In vitro* activity of isavuconazole against *Aspergillus* species and Zygomycetes according to the methodology of the European Committee on Antimicrobial Susceptibility Testing. *Antimicrob Agents Chemother.* **53** (4):1645-1647.

²⁰ Ghannoum, M. (2013) Genotyping of fungal isolates including rare moulds obtained from Phase 3 clinical trials (003 and 004). Astellas Internal Report no. 9766-PH-0126.

²¹ Wiederhold, NA. (2014) Identification of Fungal Isolates Obtained from Phase 3 Clinical Trial 9766-CL-0103 (WSA-CS-003) Using morphologic features and molecular sequencing methods. Astellas Internal Report no. 9766-PH-0129.

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All isolates from both studies were shipped to [REDACTED] (b) (4)

[REDACTED] and processed for morphological identification of the fungus and subcultured. These isolates were then shipped to the secondary central laboratory for *in vitro* susceptibility testing and molecular identification [REDACTED] (b) (4) for 9766-CL-0104 and 9766-CL-0103). A small set of isolates from Study 9766-CL-0103, designated as Class A organisms required the use of biosafety level 3 laboratory, were shipped to [REDACTED] (b) (4) certified to receive and test Class A organisms) for *in vitro* susceptibility testing and molecular identification. *In vitro* susceptibility testing was performed in accordance with the CLSI M38-A2⁵ and EUCAST⁶ methods for filamentous fungi.

PCR and Sequencing. At the [REDACTED] (b) (4) genotyping was performed by PCR and sequencing. Initial amplification and sequencing of the 5.8 S rRNA was performed using the universal fungal primers ITS1 and ITS4. All species negative for ITS amplification underwent further MLST testing using three loci; ITS2, β -tubulin, and CPS. Most *A. fumigatus* species were genotyped based on an amplified fragment of a gene encoding for the putative cell surface protein (CSP). The molecular identification for all isolates was retrieved by performing a local alignment against fungal UNITE and NCBI database using the BLASTn algorithm. PCR products were then sequenced using the ITS1 and ITS4 primers as well as NL1 and NL4 primers at the [REDACTED] (b) (4). Sequences were assembled and analyzed using DNASTAR software (DNASTAR, Inc., Madison, WI) and queried in Genbank using the BLASTn algorithm at the NCBI site (<http://www.ncbi.nlm.nih.gov>). Sequences were also compared to those available in the CBS-KNAW Fungal Biodiversity Centre database (<http://www.cbs.knaw.nl>).

Overall, the results showed $\geq 98\%$ concordance in fungal species identification by the culture and molecular methods (for details see Appendix-2 and Appendix-3). Of the 164 isolates, 71 were identified as *Aspergillus* species. The majority of the isolates came from the US, Belgium, and Israel. The remaining isolates came from Argentina, Brazil, Egypt, France, Germany, India, Malaysia, Russia, and Thailand. The isavuconazole MIC₉₀ against *Aspergillus* species was 2 $\mu\text{g/mL}$ and one dilution lower than voriconazole (4 $\mu\text{g/mL}$) and amphotericin B (4 $\mu\text{g/mL}$) but higher than posaconazole (1 $\mu\text{g/mL}$) and caspofungin (0.25 $\mu\text{g/mL}$) (Table 18).

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Table 18: MIC₉₀ (Range) of baseline clinical isolates from patients enrolled in Study 9766-CL-0104 and Study 9766-CL-0103 by the CLSI⁵ and EUCAST⁶ methods

Organism	MIC ₉₀ (Range) of baseline clinical isolates (µg/mL)				
	Isavuconazole	Voriconazole	Posaconazole	Caspofungin [#]	Amphotericin B
	CLSI method				
<i>Actinomyces elegans</i> (n=1)	(0.25)	(8)	(0.25)	(128)	(0.5)
<i>Aspergillus</i> species (n=96) [*]	4 (0.25, 32)	4 (0.03, 32)	1 (0.03, 32)	1 (0.25, 2)	4 (0.5, 32)
<i>A. flavus</i> (n=23)	2 (0.25, 4)	2 (0.5, 4)	1 (0.25, 2)	0.5 (0.25, 1)	4 (0.5, 4)
<i>A. fumigatus</i> (n=54) ^{&}	2 (0.25, 32)	2 (0.12, 32)	0.5 (0.06, 2)	0.5 (0.25, 2)	4 (0.5, 8)
<i>A. niger</i> (n=11)	8 (0.25, 8)	4 (0.03, 8)	1 (0.03, 32)	0.25 (0.25, 0.5)	1 (0.5, 4)
<i>A. terreus</i> (n=7)	(0.25, 4)	(0.25, 16)	(0.12, 0.5)	(0.25, 2)	(1, 8)
<i>A. westerdijkiae</i> (n=1)	(2)	(32)	(1)	(2)	(32)
<i>Candida albicans</i> (n=1)	(0.004)	(0.12)	(0.06)	(1)	(0.5)
<i>Chaetomium brasiliense</i> (n=1)	(0.12)	(0.25)	(0.5)	(0.5)	(1)
<i>Coccidioides immitis</i> (n=6)	(0.06, 0.12)	(0.06)	(0.06, 0.25)	(0.06, 0.25)	(0.5)
<i>Cryptococcus</i> species (n=11) ^{***}	0.12 (0.008, 0.12)	0.06 (0.03, 0.25)	0.12 (0.03, 0.25)	16 (4, 16)	1 (0.06, 1)
<i>C. gatti</i> (n=6)	(0.008, 0.12)	(0.03)	(0.03, 0.06)	(8, 16)	(0.5, 1)
<i>C. neoformans</i> (n=5) ^{&&}	(0.008, 0.12)	(0.03, 0.25)	(0.03, 0.25)	(4, 16)	(0.06, 1)
<i>Curvularia lunata</i> (n=1)	(32)	(0.25)	(32)	(0.5)	(0.5)
<i>Exserohilum rostratum</i> (n=1)	(32)	(1)	(0.5)	(0.25)	(0.5)
<i>Fonsecaea monophora</i> (n=1)	(0.25)	(2)	(8)	(1)	(2)
<i>Fusarium</i> species (n=7) [‡]	(32)	(2, 64)	(0.25, 32)	(64, 128)	(1, 4)
<i>F. fujikuroi</i> (n=1)	(32)	(4)	(0.25)	(128)	(1)
<i>F. oxysporum</i> (n=1)	(32)	(4)	(8)	(64)	(1)
<i>F. solani</i> (n=4) [‡]	(32)	(2, 64)	(1, 16)	(64, 128)	(1, 2)
<i>F. subglutinans</i> (n=1)	(32)	(8)	(32)	(64)	(4)
<i>Histoplasma capsulatum</i> (n=2) [¶]	(0.03)	(0.25)	(0.12)	(0.25, 1)	(0.12)
<i>Lichtheimia (Absidia) corymbifera</i> (n=4)	(8, 16)	(32, 64)	(0.5, 1)	(16, 128)	(0.5, 1)
<i>Mucor circinelloides</i> (n=1)	(32)	(16)	(32)	(128)	(0.5)
<i>Paecilomyces parvisporus</i> (n=1)	(1)	(2)	(0.25)	(0.5)	(2)
<i>Paracoccidioides brasiliensis</i> (n=1)	(0.001)	(0.03)	(0.03)	(4)	(0.25)
<i>Penicillium</i> species (n=2)	(1, 16)	(1, 8)	(0.5, 4)	(1, 128)	(0.5, 2)
<i>Penicillium piceum</i> (n=1)	(16)	(8)	(4)	(1)	(2)
<i>Penicillium sizovae</i> (n=1)	(1)	(1)	(0.5)	(128)	(0.5)
<i>Pseudallescheria boydii</i> (n=1)	(32)	(0.5)	(2)	(16)	(32)
<i>Rhizomucor pusillus</i> (n=3)	(8, 32)	(32, 64)	(0.5, 1)	(64)	(0.25, 8)
<i>Rhizopus</i> species (n=11)	32 (0.5, 32)	32 (4, 32)	32 (0.25, 32)	128 (0.12, 128)	4 (0.5, 4)
<i>Rhizopus azygosporus</i> (n=1)	(1)	(4)	(1)	(0.12)	(1)
<i>Rhizopus microspores</i> (n=1)	(16)	(32)	(32)	(128)	(4)
<i>Rhizopus oryzae</i> (n=9)	(0.5, 32)	(4, 32)	(0.25, 32)	(32, 128)	(0.5, 4)
<i>Scedosporium prolificans</i> (n=1)	(32)	(32)	(32)	(4)	(32)
<i>Schizophyllum commune</i> (n=1)	(0.12)	(0.06)	(0.5)	(16)	(0.5)
<i>Scopulariopsis chartarum</i> (n=1)	(16)	(8)	(32)	(4)	(32)
<i>Trichosporon asahii</i> (n=7) [€]	(0.03, 0.06)	(0.03, 0.06)	(0.12, 1)	(8, 16)	(0.5, 1)
<i>Verticillium tricorpus</i> (n=1)	(32)	(32)	(32)	(16)	(4)

AMB: amphotericin B; CAS: caspofungin; CLSI: Clinical and Laboratory Standards Institute; ISA: isavuconazole; PSA: posaconazole; VRC: voriconazole

MEC: minimum effective concentration; MIC90: MIC level at which 90% of the isolates are inhibited at or below that MIC value;

#Caspofungin results represent MEC values for filamentous and dimorphic fungi, *Mucorales* order, *Scedosporium* or *Pseudallescheria* species;

*Only 95 *Aspergillus* spp. isolates tested to ISA and CAS under the same conditions (inhibitory level or timepoint);

&Only 53 *A. fumigatus* isolates tested to ISA and CAS under the same conditions (inhibitory level or timepoint);

*** Only 10 *Cryptococcus* spp. isolates tested to AMB and VRC under the same conditions (inhibitory level or timepoint);

&& Only 4 *C. neoformans*. isolates tested to AMB under the same conditions (inhibitory level or timepoint);

‡ Only 6 *Fusarium* spp. isolates tested to ISA and CAS under the same conditions (inhibitory level or timepoint);

‡ Only 3 *F. solani* isolates tested to ISA and CAS under the same conditions (inhibitory level or timepoint);

¶ Only 1 *H. capsulatum* isolates tested to ISA, VRC, and PSA under the same conditions (inhibitory level or timepoint);

€Only 6 *Trichosporon asahii* isolates tested to AMB under the same conditions (inhibitory level or timepoint)

Comments:

- The activity of isavuconazole was measured *in vitro* against the different species of *Aspergillus*. There were three main sources of information for evaluating the *in vitro* activity of isavuconazole:
 - Surveillance studies
 - Database of published and unpublished studies
 - Isolates collected from clinical trials

Overall, the results showed isavuconazole to be active against *Aspergillus* species (Table 19). The isavuconazole MIC_{90s} appear to be lower against *A. fumigatus*, *A. flavus*, *A. nidulans*, and *A. terreus* compared to *A. niger*. There was no marked effect of test region upon MICs. Isavuconazole MIC₉₀ values were similar to those of itraconazole and voriconazole. Posaconazole was approximately 4-fold more active. The MIC₉₀ values of echinocandins were low (0.03 - 0.06 µg/mL), with the exception of caspofungin (4 µg/mL).

Species	MIC ₉₀ µg/mL (n)				
	Surveillance study		Database**	Other published studies	Clinical trial isolates
	2011	2012			
<i>A. flavus</i>	1 (10)	2 (11)	4 (145)		2 (23)
<i>A. fumigatus</i>	1 (71)	2 (90)	1 (875)	0.39 (12)	1 (54)
<i>A. nidulans</i>	-	-	1 (85)	1 (63)	-
<i>A. niger</i>	4 (11)	4 (11)	2 (101)	1.56 (1)*	8 (11)
<i>A. oryzae</i>	-	-	-	0.39 (1)*	-
<i>A. terreus</i>	-	-	1 (432)	0.125 - 1(135)*	0.25-2 (8)*
<i>A. lentulus</i>	-	-	-	0.25 (15)	-
<i>A. westerdijkiae</i>	-	-	-	-	4 (1)
Other <i>Aspergillus</i> species	2 (12) [†]	2 (18) [†]	-	-	-
<i>Aspergillus</i> species	2 (104)	2 (130)	2 (1717)	0.125 – 2 (227)*	2 (96)

* represent range. If number of isolates less than 10, MIC₉₀ value was not calculated or not available
 **Database results include surveillance study results of 2011
 †Include *A. terreus*, *A. nidulans*, *A. foetidus*, and *A. sydowii*

- The MICs were higher against the hyphae compared to conidia of *Aspergillus* species by the CLSI method but not by the EUCAST method. Please note that this information is based on testing of small number of isolates.

3.2.3. Activity against Mucorales

3.2.3.1. Surveillance studies

As a part of the SENTRY global antifungal surveillance program, operated by JMI Laboratories (for details see section 3.2.2. above), there were three isolates of Mucorales tested in 2011 and four in 2012 (Castanheira *et al.*, 2012¹¹ and 2013¹²). Isavuconazole MICs against these isolates were 1–>8 µg/mL for both years (Table 20). Posaconazole MICs ranged from 0.5–1 µg/mL, voriconazole MICs ranged from 4 – 8 µg/mL and micafungin MICs were >16 µg/mL (Table 20).

Table 20: Activity of isavuconazole and comparators against three isolates of Mucorales collected in 2011 and four isolates of Mucorales collected in 2012

A: 2011				
Species	MIC (mg/L)			
	Isavuconazole	Posaconazole	Voriconazole	Micafungin
<i>Rhizomucor pusillus</i>	4	1	>8	>16
<i>Rhizopus microsporus</i> group	1	1	8	>16
<i>Rhizopus microsporus</i> group	2	1	8	>16
B: 2012				
Species	MIC (mg/L)			
	Isavuconazole	Posaconazole	Voriconazole	Micafungin
<i>Mucor circenelloides</i>	>8	1	>8	>16
<i>Rhizopus microsporus</i> group	1	0.5	4	>16
<i>Rhizopus microsporus</i> group	4	0.5	8	>16
<i>Rhizopus oryzae</i> species complex	>8	1	>8	>16

3.2.3.2. Database

The applicant compiled all MIC data for 374 isolates of Mucorales worldwide, from several published or unpublished studies including the data from the 2011 SENTRY global antifungal surveillance program (see Appendix-1¹³). Of the 374 isolates, 119 (31.8%) were collected and tested at centers in the US and 255 (68.2%) in Europe. The isolates comprised a total of five genera: *Lichtheimia* (*Absidia*) species (n = 82; 21.9%), *Cunninghamella* species (n = 22; 5.9%), *Mucor* species (n = 83; 22.2%), *Rhizomucor* species (n = 32; 8.6%), and *Rhizopus* species (n = 155; 41.4%); 182 (48.7%) of these isolates could be further identified to at least nine species/subspecies, and the other 192 (51.3%) isolates were non-specified.

The isavuconazole MICs were determined by the CLSI method using 100% endpoint and readings read at 48 hours (please note that readings at 48 hours tend to result in higher MICs than when read at 24 hours.). The MIC₉₀ values ranged from 2–32 µg/mL against the five genera of Mucorales (Table 21). Only nine isolates of *Rhizomucor* spp. were tested by the EUCAST method; the MIC range was 2 to 16 µg/mL, compared with 0.12–8 µg/mL by the CLSI method.

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Table 21: Summary of MIC distributions for isavuconazole against Mucorales by CLSI method

Genus	Species	Isolates (n)	MIN	MAX	MIC Parameter (mg/L)		MIC ₉₅	GM
					MIC ₅₀	MIC ₉₀		
<i>Absidia</i> spp.	All	67	0.12	32	1	8	8	1.57
	<i>A. corymbifera</i>	44	0.12	8	1	8	8	1.37
	<i>Absidia</i> sp.	23	0.5	32	1	32	32	2.06
<i>Cunninghamella</i> spp.	All	13	0.25	32	4	32	32	4.45
<i>Mucor</i> spp.	All	68	0.12	32	4	16	32	3.65
	<i>M. circinelloides</i>	18	2	8	4	8	8	3.7
<i>Rhizomucor</i> spp.	All	18	0.12	8	1	4	8	1.08
	EUCAST	9	2	16	16	16	16	10.08
<i>Rhizopus</i> spp.	All	134	0.12	32	1	8	16	1.48
	<i>R. arrhizus</i>	28	0.12	8	2	4	8	2.15
	<i>R. microsporus</i>	41	0.5	32	1	2	2	1.05
	<i>R. oryzae</i>	11	0.5	4	1	4	4	1.46
	<i>Rhizopus</i> sp.	52	0.12	32	1	16	32	1.53
	MFC <i>Rhizopus</i> spp.	14	1	32	8	32	32	7.61

Overall, posaconazole and amphotericin B were more active than other antifungal drugs tested, including isavuconazole; voriconazole appears to be least active (Table 22).

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Table 22: Summary MIC parameters of isavuconazole and comparators against different Mucorales species by the CLSI method.

<i>Lichtheimia (Absidia)</i> species							
Agent	Isolates (n)	MIN	MAX	MIC Parameter (mg/L)			GM
				MIC ₅₀	MIC ₉₀	MIC ₉₅	
Isavuconazole	67	0.12	32	1	8	8	1.57
Itraconazole	54	0.06	16	0.5	2	16	0.47
Posaconazole	60	0.03	16	0.5	1	2	0.41
Voriconazole	60	0.25	32	16	32	32	13.77
Ravuconazole	37	0.12	8	2	8	8	1.34
Micafungin							
Caspofungin	17	0.5	64	64	64	64	40.87
Anidulafungin							
Amphotericin B	57	0.12	2	0.25	1	1	0.32
Flucytosine							

<i>Cunninghamella</i> species							
Antifungal agent	Isolates (n)	MIC parameter (mg/L)					
		MIN	MAX	MIC ₅₀	MIC ₉₀	MIC ₉₅	GM
Isavuconazole	13	0.25	32	4	32	32	4.45
Itraconazole	2	0.12	0.25	-	-	-	0.17
Posaconazole	9	0.12	1	-	-	-	0.68
Voriconazole	6	16	32	-	-	-	25.4
Ravuconazole	2	0.12	1	-	-	-	0.35
Amphotericin B	6	0.12	32	-	-	-	2.23

<i>Mucor</i> species							
Agent	Isolates (n)	MIN	MAX	MIC Parameter (mg/L)			GM
				MIC ₅₀	MIC ₉₀	MIC ₉₅	
Isavuconazole	68	0.12	32	4	16	32	3.65
Itraconazole	32	0.12	32	1	16	32	1.16
Posaconazole	45	0.06	32	0.5	2	2	0.66
Voriconazole	53	4	32	16	32	32	17.08
Ravuconazole	19	2	8	4	8	8	4.46
Micafungin							
Caspofungin	13	32	64	64	64	64	57.53
Anidulafungin							
Amphotericin B	37	0.06	1	0.25	1	1	0.28
Flucytosine							

<i>Rhizomucor</i> species							
Antifungal agent	Isolates (n)	MIC parameter (mg/L)					
		MIN	MAX	MIC ₅₀	MIC ₉₀	MIC ₉₅	GM
Isavuconazole	18	0.12	8	1	4	8	1.08
Itraconazole	12	0.03	1	0.12	1	1	0.19
Posaconazole	19	0.03	1	0.25	1	1	0.29
Voriconazole	12	4	32	16	16	32	11.99
Ravuconazole	5	0.12	1	-	-	-	0.38
Caspofungin	7	32	64	-	-	-	52.5
Amphotericin B	13	0.06	2	0.12	0.5	2	0.19

<i>Rhizopus</i> species							
Antifungal agent	Isolates (n)	MIC parameter (mg/L)					
		MIN	MAX	MIC ₅₀	MIC ₉₀	MIC ₉₅	GM
Isavuconazole	134	0.12	32	1	8	16	1.48
Itraconazole	94	0.03	32	0.5	8	8	0.96
Posaconazole	122	0.03	32	0.5	2	2	0.49
Voriconazole	109	1	32	8	32	32	8.21
Ravuconazole	30	0.06	8	2	4	8	1.99
Micafungin	5	16	32	-	-	-	21.11
Caspofungin	67	16	64	64	64	64	56.53
Anidulafungin	5	16	32	-	-	-	21.11
Amphotericin B	104	0.06	2	0.5	1	1	0.42
Flucytosine	5	64	128	-	-	-	97.01

MIC₅₀, MIC₉₀ and MIC₉₅ are not reported if the number of isolates is less than 10.

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One study reported MICs and MFCs by the EUCAST method; based on 14 isolates of *Rhizopus* species; the MIC₉₀ and MFC₉₀ values were 16 µg/mL and 32 µg/mL, respectively.

3.2.3.3. Other studies

There were few other published studies (Verweij *et al.*, 2009;²² González, 2009;²³ Yamazaki *et al.*, 2010;¹⁵ Chakrabarti *et al.*, 2010²⁴) included that were not listed in Appendix-1; therefore, it is assumed that the data from these studies was not a part of the database and are summarized in Table 23 for completeness. Overall, the results from these studies support the activity of isavuconazole against *Mucorales* species.

Table 23: Isavuconazole MICs by the CLSI method (unless specified otherwise) from published studies				
Species	Reference	Country (number of isolates)	MIC (µg/mL)	
			Range	MIC ₉₀
<i>Rhizomucor</i>	Verweij <i>et al.</i> , 2009 ²²	Europe, USA, and Mexico (9)	2->8	-
<i>R. arrhizus</i>	González, 2009 ²³	Mexico (27)	1-8	4
<i>M. circinelloides</i>	González, 2009 ²³	Mexico (16)	2-8	8
<i>A. corymbifera</i>	González, 2009 ²³	Mexico (17)	2-8	8
<i>Lichtheimia (Absidia) corymbifera</i>	Yamazaki <i>et al.</i> , 2010 ¹⁵	Japan (4)	0.78-3.1	-
<i>A. hyalopsora</i> IFO8084	Yamazaki <i>et al.</i> , 2010 ¹⁵	Japan (1)	0.78	-
<i>Cunninghamella bertholletiae</i>	Yamazaki <i>et al.</i> , 2010 ¹⁵	Japan (2)	>100	-
<i>Mucor circinelloides</i> IFO4554	Yamazaki <i>et al.</i> , 2010 ¹⁵	Japan (1)	3.1	-
<i>M. rouxianus</i> IFO5773	Yamazaki <i>et al.</i> , 2010 ¹⁵	Japan (1)	>200	-
<i>M. ramosissimus</i> ATCC 28933	Yamazaki <i>et al.</i> , 2010 ¹⁵	Japan (1)	6.25	-
<i>Rhizomucor pusillus</i>	Yamazaki <i>et al.</i> , 2010 ¹⁵	Japan (3)	1.56-6.25	-
<i>R. oryzae</i>	Yamazaki <i>et al.</i> , 2010 ¹⁵	Japan (4)	1.56-12.5	-
<i>R. microsporus</i>	Yamazaki <i>et al.</i> , 2010 ¹⁵	Japan (3)	1.56-3.13	-
<i>Apophysomyces elegans</i>	Chakrabarti <i>et al.</i> , 2010 ²⁴	India and Netherlands (18) [‡]	1-4	4
Only MIC range shown and not MIC ₉₀ when n<10 isolates *Testing by EUCAST method. The authors stated there was “essential agreement between the EUCAST and CLSI methods determined as percentage of results within ±1 dilution was 89.8% for isavuconazole, better than observed for the other azole antifungal agents (range 15.5% to 80.2%)” ‡Of the 18 isolates, 2 were environmental isolates of <i>Apophysomyces elegans</i>				

²² Verweij PE, González GM, Wiederhold NP, Lass-Flörl C, Warn P, Heep M, Ghannoum MA, and Guinea J. (2009) *In vitro* antifungal activity of isavuconazole against 345 mucorales isolates collected at study centers in eight countries. *J Chemother.* **21** (3):272-281.

²³ González GM. (2009) *In vitro* activities of isavuconazole against opportunistic filamentous and dimorphic fungi. *Med Mycol.* **47** (1):71-76.

²⁴ Chakrabarti A, Shivaprakash MR, Curfs-Breuker I, Baghela A, Klaassen CH, and Meis JF. (2010) *Apophysomyces elegans*: epidemiology, amplified fragment length polymorphism typing, and *in vitro* antifungal susceptibility pattern. *J Clin Microbiol.* **48** (12):4580-4585.

3.2.3.4. Clinical trial isolates

Clinical fungal isolates (n=30) from patients treated with isavuconazole in the phase 3 clinical trials (Study 9766-CL-0103 and Study 9766-CL-0104) with positive culture were tested for antifungal susceptibility by the CLSI method⁵ in a central laboratory. Of the 30 isolates, the most common species was *Rhizopus* species (n=25) of which 9 isolates were *R. oryzae* (Table 18). Isavuconazole MICs ranged from 0.25 to 32 µg/mL (Table 18).

Comments:

The activity of isavuconazole in vitro against isolates of the Mucorales order [(Lichtheimia (Absidia), Cunninghamella, Mucor, Rhizomucor, and Rhizopus species)] was variable based on the small number of isolates/strains tested (Table 24). Isavuconazole appears to be more active than voriconazole but less active than posaconazole or amphotericin B.

Table 24: Summary of <i>in vitro</i> activity of isavuconazole against <i>Mucorales</i> by the CLSI method					
Species	MIC ₉₀ µg/mL (n)				
	Surveillance study		Database**	Other published studies	Clinical trial isolates
	2011	2012			
<i>Lichtheimia (Absidia) species (Total)</i>[†]	-	-	8 (67)	-	-
<i>A. corymbifera</i>	-	-	8 (44)	8 (17)	8-16 (4)*
<i>Absidia</i> species NOS	-	-	32 (23)	-	-
<i>Cunninghamella</i> species (All) [†]	-	-	32 (13)	-	-
<i>Mucor</i> species (Total)[†]	-	-	16 (68)	-	-
<i>M. circinelloides</i>	-	>8 (1)*	8 (18)	8 (16)	32 (1)*
<i>Rhizomucor</i> species (Total)[†]	-	-	-	2 - >8 (9)*	-
<i>R. pusillus</i>	4 (1)*	-	-	-	8-32 (3)*
<i>Rhizopus</i> species (Total)[†]	-	-	8 (134)	-	-
<i>R. arrhizus</i>	-	-	4 (28)	4 (27)	-
<i>R. microsporus</i>	1-2 (2)*	1-4 (2)*	2 (41)	-	16 (1)*
<i>R. oryzae</i>	-	>8 (1)*	4 (11)	-	0.5 - 32 (9)
<i>R. azygosporus</i>	-	-	-	-	1 (1)*
<i>Rhizopus</i> species	-	-	16 (52)	-	32 (11)
<i>Apophysomyces elegans</i>	-	-	-	4 (18)	-
<i>Actinomucor elegans</i>	-	-	-	-	0.25 (1)
* represent range. If number of isolates less than 10, MIC ₉₀ value was not calculated or not available					
**Database results include surveillance study results of 2011					
† include unidentified species					
Note: MICs published by Yamazaki <i>et al.</i> , 2010 ¹⁵ not included in the Table due to different dilution range tested					

3.2.4. Epidemiological cut-off values

Epidemiological cut-off value (ECVs), also known as the wild-type (WT) cut-off value, is defined as the highest MIC that represent WT population/strains with no detectable acquired resistance mechanisms; the ECV categorizes an isolate as WT or non-WT and are considered a practical tool for monitoring the emergence of azole resistance.

A microorganism is defined as WT for a species by the absence of acquired and mutational mechanisms of resistance to the agent. ECVs are set by evaluating a large MIC dataset from multiple laboratories using a standard susceptibility test methodology and can assist in

identifying isolates with raised MICs and/or a greater risk of the presence of a mechanism of resistance. ECVs are often used until clinical breakpoints are established and therefore, are an important component in the development of clinical breakpoints once more data are available including pharmacodynamics (PD) and clinical trial data.

3.2.4.1. *Aspergillus* species

Espinel-Ingroff *et al.* (2013)²⁵ reported ECVs based on MIC data for individual *Aspergillus* species [*A. fumigatus* (n = 855), *A. flavus* (n = 444), *A. terreus* (n = 384), *A. niger* (n = 207), *A. nidulans* (n = 106), *A. versicolor* (n = 75), and 29 isolates of *Aspergillus* section *Usti* comprising of *A. calidoustus* (n = 17), *A. pseudodeflectus* and *A. ustus* (n = 2 for each) and *A. insuetus* (n = 1)]. Testing was performed by up to eight centers among Europe (Austria, Spain and The Netherlands), India, Mexico, and the United States (Iowa and Virginia) by the CLSI M38-A2 method.⁵ Isavuconazole showed *in vitro* activity against the clinical isolates of *Aspergillus* species.

Highly-skewed distributions and distributions which had a modal MIC at the lowest concentration tested were excluded. The WT distributions were obtained from the overall MIC distributions from all centers collectively. ECVs were defined as the WT MIC that accounted for $\geq 95\%$ or $\geq 97.5\%$ of the aggregated MIC distribution. ECVs were only defined for species with at least 100 isolates tested or where data were available from three or more laboratories.

The modal isavuconazole MIC was lowest against *A. nidulans* (0.125 $\mu\text{g/mL}$), followed by *A. terreus* and *A. versicolor* (0.25 $\mu\text{g/mL}$ for both), *A. fumigatus* and *A. flavus* (0.5 $\mu\text{g/mL}$ for both), and *A. niger* and *A. section Usti* (1 $\mu\text{g/mL}$ for both) (Table 25). MICs against the *A. nidulans* showed a skewed WT distribution, which may have been due to a lower number of centers (3) and isolates (106). The authors proposed isavuconazole ECVs against *A. fumigatus*, *A. flavus*, and *A. terreus* as 1 $\mu\text{g/mL}$, 4 $\mu\text{g/mL}$ against *A. niger*, 0.25 $\mu\text{g/mL}$ against *A. nidulans*, and 1 $\mu\text{g/mL}$ against *A. versicolor* (Table 25). An ECV was not proposed for *Aspergillus* section *Usti* due to the low number of isolates.

²⁵ Espinel-Ingroff A, Chowdhary A, González GM, Lass-Flörl C, Martin-Mazuelos E, Meis J, Pelaez T, Pfaller MA, and Turnidge J. (2013) Multicenter study of isavuconazole MIC distributions and epidemiological cut-off values for *Aspergillus* spp. for the CLSI M38-A2 broth microdilution method. *Antimicrob Agents Chemother.* **57** (8):3823-3828.

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Table 25: Pooled MIC distributions of isavuconazole for *Aspergillus* spp. from two to eight laboratories and isavuconazole ECVs based on MICs by the CLSI method⁵

A: Pooled MIC distributions of isavuconazole for *Aspergillus* spp. from two to eight laboratories

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Note: Although the column heading states “No. of isolates/no. of laboratories, it is unclear whether that No. of isolates represent number of isolates tested in each laboratory or MIC data points based on testing in different laboratories.

B: Isavuconazole ECVs based on MICs

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Source: Espinel-Ingroff *et al.*, 2013²⁵

The same data were presented at the CLSI anti-fungal subcommittee meeting in January, 2013 and January, 2014. In January 2014, the subcommittee accepted the ECVs for *A. fumigatus*, *A. flavus*, *A. niger*, and *A. terreus* (**Votes approved: 7-0**); the ECVs for all *Aspergillus* species except *A. nidulans* proposed by Espinel-Ingroff *et al.* (2013)²⁵ are same as approved by the CLSI (Table 26). For *A. nidulans*, the subcommittee noted that the distribution of the MICs was skewed and can only be listed as tentative. The subcommittee suggested that more data should be collected and analyzed.

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Table 26: Isavuconazole epidemiological cutoff values (ECVs) from three to eight laboratories as determined by the CLSI M38-A2 broth microdilution method⁵

Species Complex ^a	No. Isolates/ No. of labs	Cutoff values ^b				
		Range	Mode ^c	Statistical ECV $\geq 95\%$	Statistical ECV $\geq 97.5\%$	Statistical ECV $\geq 99\%$
<i>A. fumigatus</i>	855/8	0.06-8	0.5	1	1	1
<i>A. flavus</i>	444/7	0.06-2	0.5	1	1	2
<i>A. nidulans</i> ^d	106/3	0.06-1	0.12	0.25	0.25	0.25
<i>A. niger</i>	207/6	0.06->8	1	4	4	4
<i>A. terreus</i>	386/5	0.06-2	0.25	1	1	1

^a*A. fumigatus*, *A. flavus*, *A. nidulans*, *A. niger*, *A. terreus*, and *A. versicolor* are listed as species complexes as per non-molecular identification

^bCalculated ECVs comprising >95 , >97.5 or $>99\%$ of the statistically modeled population.

^c Mode, most frequent MIC

^dTentative value until more data are obtained.

Source: CLSI January 2014 meeting package

3.2.4.2. Mucorales

No ECVs were established or proposed for fungal species in the order Mucorales.

Comments:

- The ECVs proposed by the applicant for *A. fumigatus*, *A. flavus*, *A. niger* and *A. terreus* are appropriate (Table 27). For *A. nidulans*, the data was skewed and insufficient to establish ECVs.

Table 27: ECV Values of Isavuconazole Using Either CLSI or EUCAST Methodology

Organism	No. of Isolates	ECV (mg/L) (CLSI methodology) †	No. of Isolates	ECV (mg/L) (EUCAST methodology) ‡
<i>Aspergillus fumigatus</i>	855	1	401	2
<i>Aspergillus flavus</i>	444	1	215	2
<i>Aspergillus niger</i>	207	4	209	4
<i>Aspergillus terreus</i>	386	1	206	2
<i>Aspergillus nidulans</i>	106	0.125	206	0.25

ECV: epidemiological cut-off value; ND: not determined;

† Calculated ECVs comprising $\geq 95\%$ and $\geq 97.5\%$ of the statistically modeled MIC population as per [Espinel-Ingroff et al, 2013]

‡ ECVs determined using a standard non-statistical approach (eye-ball method) as per [Howard et al, 2013]

The applicant included MIC data based on testing by the EUCAST method (this was not available in the publication by Espinel-Ingroff et al. (2013)).²⁵ With the exception of *A. niger*, the EUCAST ECVs were one dilution higher than the CLSI values.

The ECVs may aid in detecting non-WT isolates with reduced susceptibility to isavuconazole as well as to distinguish non-WT from WT isolates. The authors stated that these tentative ECV may change when more laboratories and MICs become available.

- *No ECVs are proposed for Mucorales, which is appropriate.*

3.3. Activity in a dynamic *in vitro* model

3.3.1. Pulmonary aspergillosis

Box *et al.* (2014)²⁶ reported the activity of isavuconazole in a dynamic *in vitro* model representing invasive pulmonary aspergillosis to generate the pharmacokinetics/pharmacodynamics (PK-PD) profile of isavuconazole against two WT and two mutant strains of *A. fumigatus*. The human alveolus was used to simulate isavuconazole pharmacokinetics (PK) across the alveolar wall. The model comprised of a cellular bilayer consisting of an epithelial cell layer (human alveolar epithelial cells, A549 cells) and an endothelial cell layer (human pulmonary arterial endothelial cells, HPAECs) both grown on opposite sides of a transwell membrane insert; the membrane insert with monolayers of the two cell types was housed in a bioreactor that was connected to a circuit which perfused cell culture medium and isavuconazole through the system. Cell culture inserts were transferred to a 12 well plate containing EBM-2 media supplemented with 2% FBS. A conidial suspension (400 μ L of $1-3 \times 10^4$ /mL) was incubated at 37°C for 20 minutes and then pipetted into the top compartment of each cell culture insert and incubated at 37°C, 5% CO₂ for 6 hours. Isavuconazole (0 mg, 0.1 mg, 0.3 mg, 0.5 mg, and 1.5 mg) was added directly into the central compartment as a 100 μ L bolus which was followed by 1 mL of medium. The PK and PD of isavuconazole against each isolate of *A. fumigatus* were determined in four individual circuits; different dosages of isavuconazole were added to each circuit. A fifth untreated circuit was used as a control. Samples for both PK and PD were collected every 6 hours between 6-54 hours post-inoculation. After sampling, media taken out of the system during sampling was replaced with an equal volume of complete DMEM. The applicant stated that removal of the isavuconazole-containing medium and replacement of isavuconazole-free medium generated first-order PK. Pumps were adjusted to allow the concentration time profile of isavuconazole to approximate the concentration time profile of humans. Isavuconazole concentrations in media were measured using high performance liquid chromatography (HPLC).

Galactomannan was used as a quantitative biomarker to evaluate the antifungal activity of isavuconazole. Galactomannan levels were measured by the Bio-Rad Platelia *Aspergillus* EIA.

Mathematical modelling: A population methodology was used to fit the mathematical model to the data from each strain. The “big” version program nonparametric adaptive grid (BIG NPAG) was used. The structural mathematical model consisted of the following two inhomogeneous ordinary differential equations:

$$dX_1/dt = B(1)-(SCL/V_c)*X_1$$

Equation 1

$$dN/dt = Kgmax*(1-(N/POPMAX))*N$$
$$*(1-(X_1/V_c)^{Hg}/(X_1/V_c)^{Hg}+C_{50g}^{Hg}))$$

Equation 2a

Equation 2b

²⁶ Box H, Gregson L, Livermore J, Felton TW, Howard SJ, Whalley S, Goodwin J, and Hope WW. (2014) Pharmacodynamics of isavuconazole in a dynamic *in vitro* model of invasive pulmonary aspergillosis. Internal report no. 9766-PH-0121.

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Where: $B(1)$ is the bolus input of isavuconazole, SCL is the clearance of isavuconazole from the circuit, V_c is the volume of the circuit, N is the GM concentration, K_{gmax} is the maximal rate of growth; POP_{MAX} is the theoretical maximal density within the circuit; H_g is the slope function for the suppression of growth; and, C_{50g} is the concentration of isavuconazole in the circuit where there is half-maximal suppression of growth.

Equation 1 describes the rate of change of isavuconazole concentrations in the circuit.

Equation 2 describes the rate of change of GM in the circuit and contains terms that describe fungal growth in the absence of isavuconazole (Equation 2a) and the isavuconazole induced suppression of growth (Equation 2b).

The model was fitted to the dataset obtained for each strain (i.e., the data from each of 5 circuits consisting of a single control and 4 drug treated circuits). In this process, each circuit was treated as an “individual”. A mathematical model using differential equations determined the rate of change of isavuconazole concentrations and rate of change of GM concentrations in the circuit. The model was fitted to the dataset obtained for each strain and Bayesian posterior estimates for each of the parameters in the model defined the concentration-time profile of isavuconazole and the resultant effect on GM concentrations. These parameter estimates were used to estimate the AUC that developed in each circuit, and consequently the AUC/MIC ratio for the four strains of *A. fumigatus*.

Pharmacodynamic target associated with maximal antifungal activity: The relationship between the AUC (in this case the total AUC that developed throughout the experimental period) and the final GM concentration was explored. A GM reading of < 1 and < 0.5 was used as an endpoint. Logistic regression (using the statistical program SYSTAT version 11) was used to link the AUC:MIC value for each strain and the GM concentration quantified as a dichotomous outcome (i.e., “success” defined as a GM as < 0.5 or 1.0 , depending on the specific analysis being performed). The drug exposure (AUC:MIC) associated with a 90% probability of a suppressed GM was defined as the drug exposure target associated with maximal antifungal activity.

The MICs for isavuconazole against the isolates were determined in accordance with CLSI⁵ and EUCAST⁶ methods and the geometric mean was used in the PD analysis (AUC/MIC value). Isavuconazole MICs were higher against the *cyp51A* mutated strains than the WT strains (Table 28).

Table 28: Minimal inhibitory concentrations of isavuconazole against <i>A. fumigatus</i> isolates by the CLSI and EUCAST method				
CLSI method				
Isolate	Amino acid substitution	Mode (mg/L)	Range	Geometric mean
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MICs were determined in 10 experiments using CLSI method				
EUCAST method				
<i>A. fumigatus</i> Isolates	Amino acid substitution	Mode (mg/L)	Range	Geometric mean
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MICs were determined in 10 experiments using EUCAST methodology.				

The authors stated that the dynamic *in vitro* model of the human alveolus generated human-like concentration-time profiles for isavuconazole. There was a lack of translocation of 1% dextran blue after 48 hours within the circuit demonstrating the integrity of the cellular bilayer throughout the experimental period.

Galactomannan kinetics: The kinetics of GM released into the circulating medium in untreated (control) circuits differed between isolates. Concentrations of GM in all untreated circuits started to increase at around 18-24 hours (Figures 4 to 7). A maximum GM concentration was observed at approximately 24 hours for the GFP, F/11628, and NIH 4215 strains (Figures 4 to 6); the strain F/16216 appeared to grow more slowly and maximal GM concentrations were reached at around 36 hours post-inoculation (Figure 6). The maximum GM concentrations for the four strains ranged from an index of approximately 6-9.

Exposure-response relationship: There were differences in exposure-response relationships for isavuconazole against the four strains. A trough concentration of approximately 0.2-0.5 µg/mL resulted in near maximal suppression of GM release from the GFP and NIH 4215 WT strains. However, the exposure-response relationships for the two non-WT isolates were different; for F/16216 strain, only the highest concentrations of isavuconazole resulted in suppression of GM and for the F/11628 strain none of the isavuconazole concentrations suppressed the release of GM. The results suggest that the MIC (and genotype) appeared to account for at least some differences in the observed drug exposure-response relationships.

Mathematical modeling: The authors stated that the fit of the mathematical model to the data by Bayesian posterior estimates for each circuit suggested the fit to be acceptable (Figures 4 to 7; represented by the solid continuous line).

Figure 4: Pharmacokinetics and pharmacodynamics of isavuconazole against the *A. fumigatus* GFP transformant

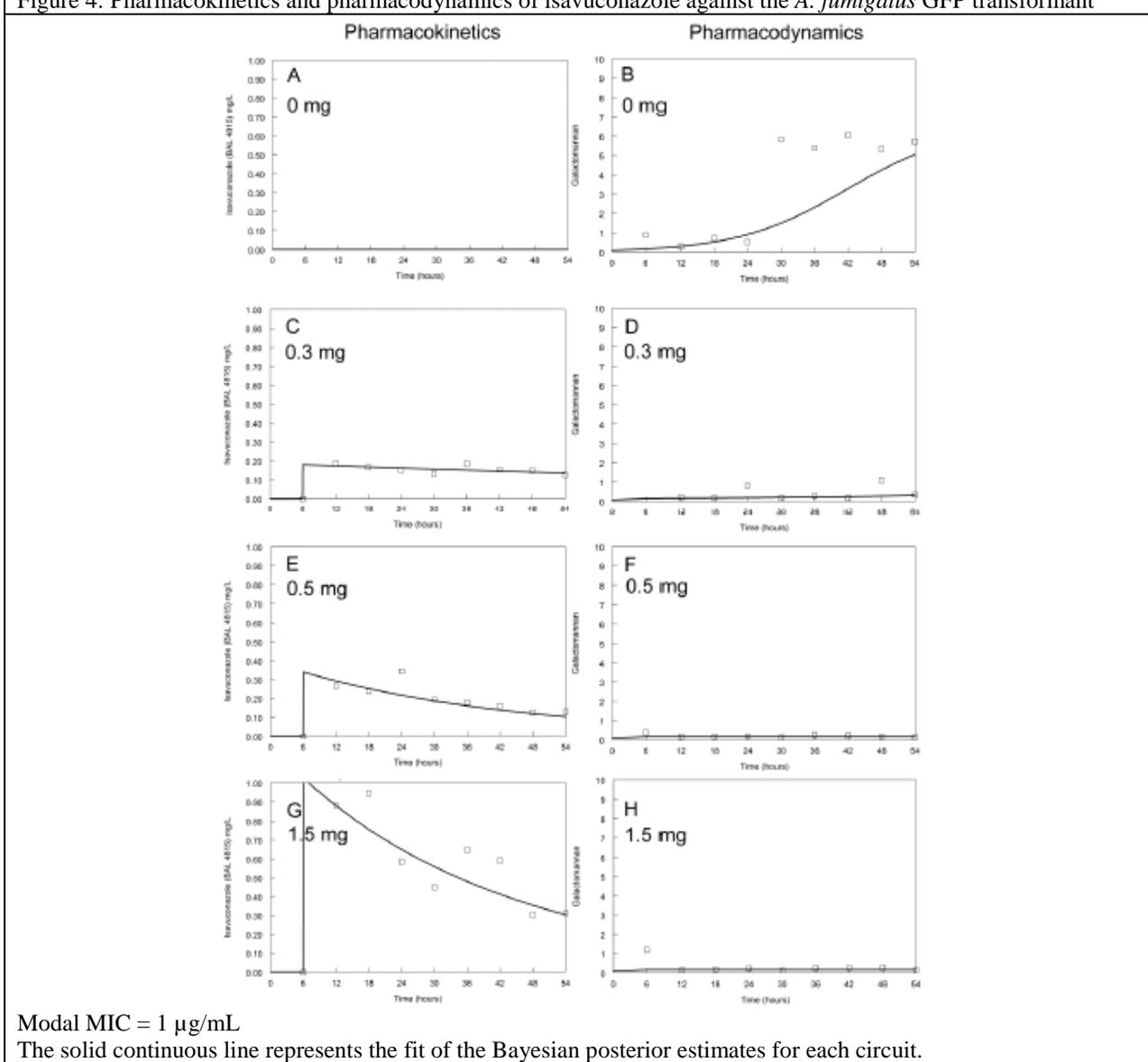
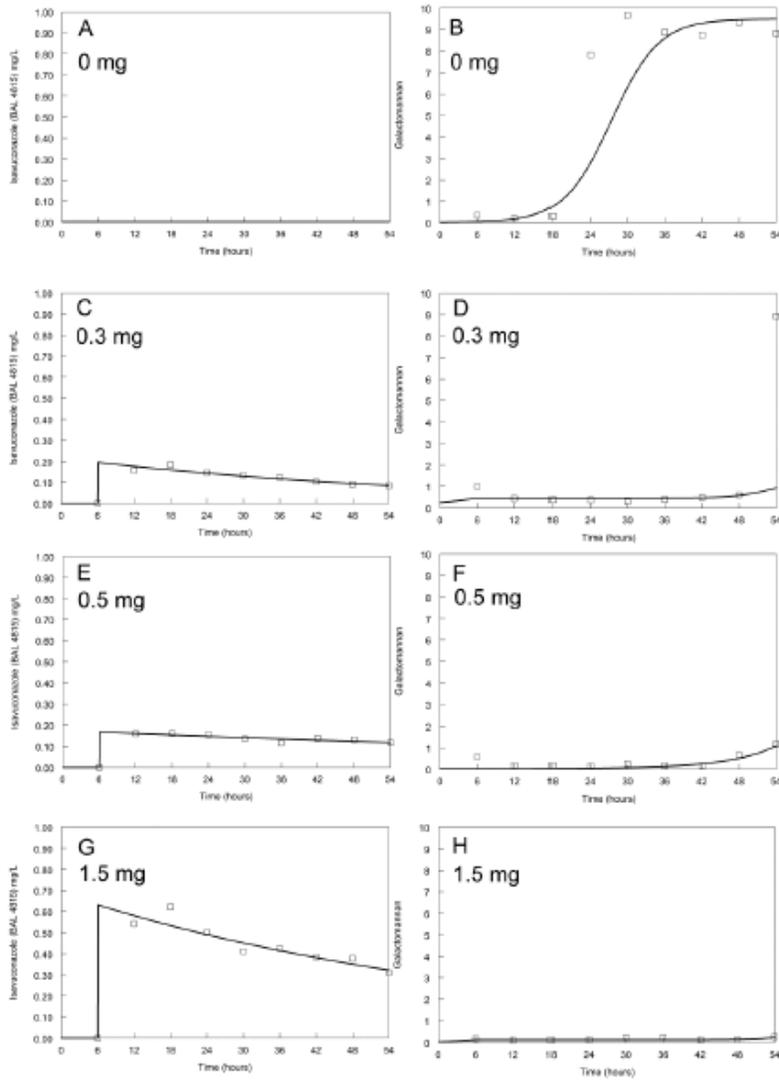


Figure 5: Pharmacokinetics and pharmacodynamics of isavuconazole against *A. fumigatus* NIH 4215



Modal MIC = 1 mg/L

Modal MIC = 1 µg/mL

The solid continuous line represents the fit of the Bayesian posterior estimates for each circuit.

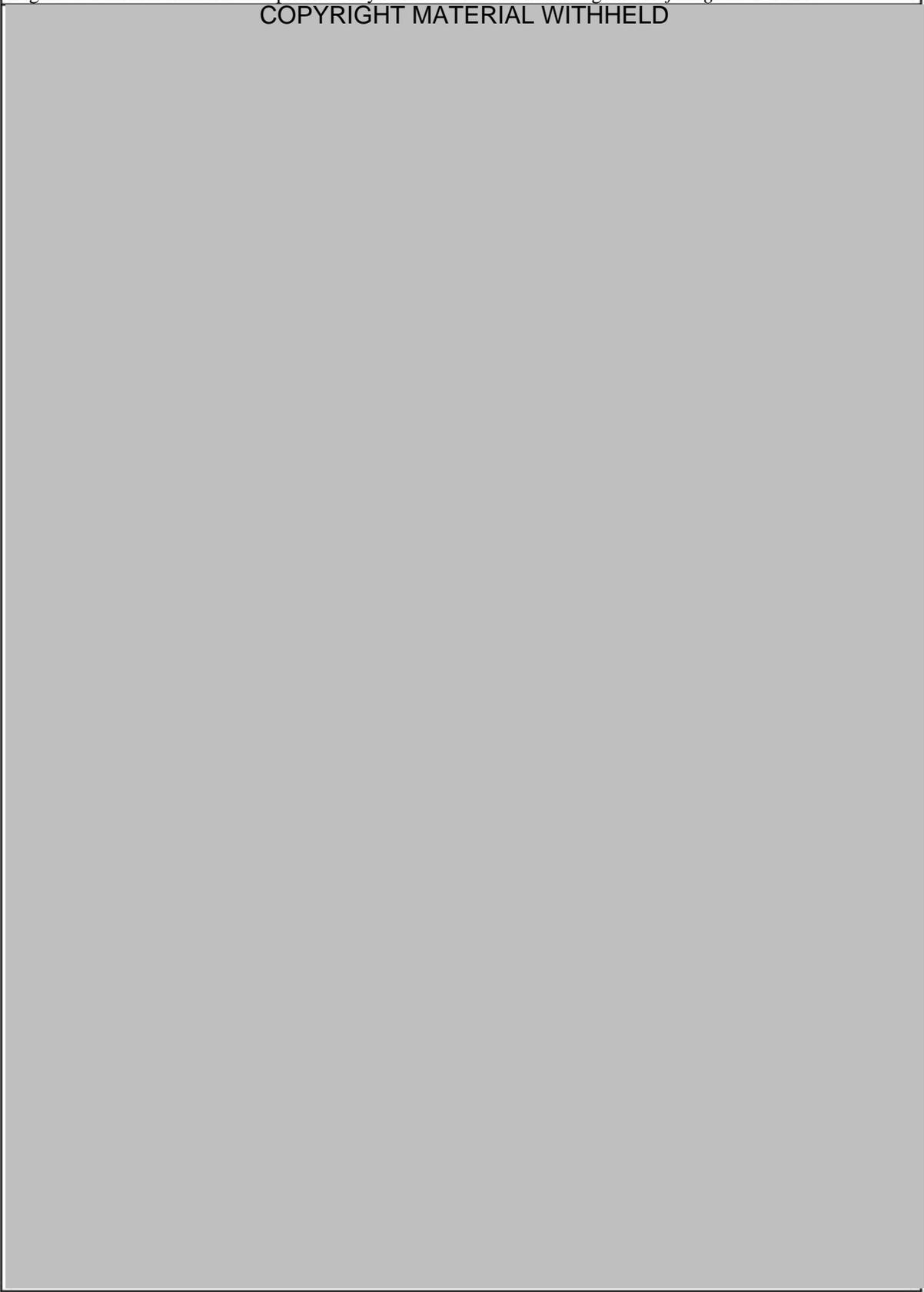
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Figure 6: Pharmacokinetics and pharmacodynamics of isavuconazole against *A. fumigatus* F/16216

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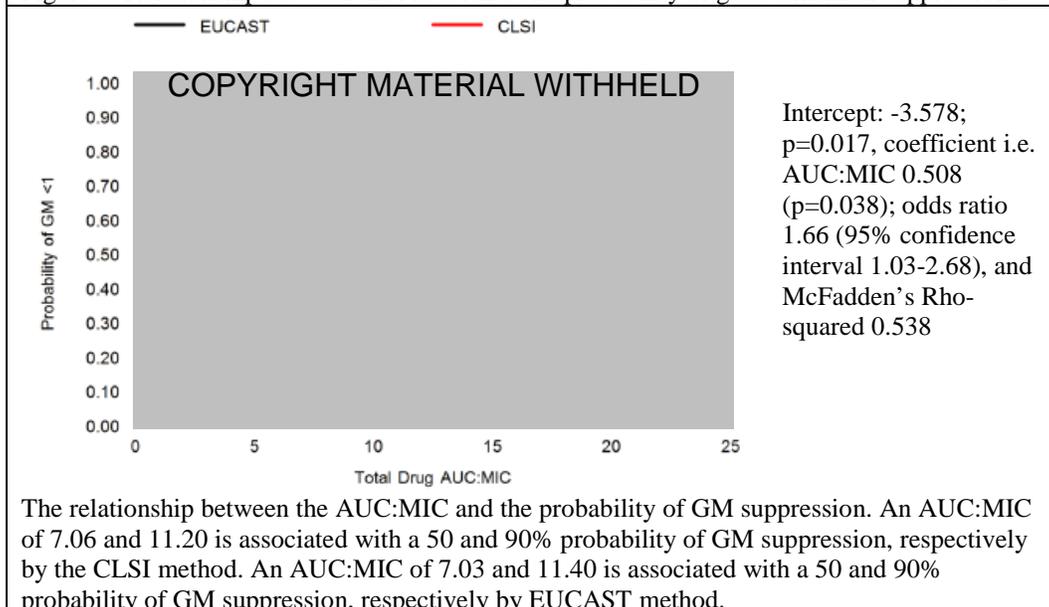
Figure 7: Pharmacokinetics and pharmacodynamics of isavuconazole against *A. fumigatus* F/11628

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Pharmacodynamic target associated with maximal antifungal activity: The logistic regression model showed the regression was statistically significant (Figure 8); AUC:MIC of 11.20 and 11.40 (by CLSI and EUCAST methods, respectively) resulted in a 90% probability of GM suppression <1. The results were identical if a GM endpoint of <0.5 was used to define a successful outcome.

Figure 8: Relationship between the AUC/MIC and probability of galactomannan suppression



Comments:

*The study suggests isavuconazole causes a concentration-dependent reduction in GM concentrations that are released from *A. fumigatus*. The MIC of the strain appears to be related to pharmacodynamic differences. An AUC:MIC target of 11.20 and 11.40 using CLSI and EUCAST methodology, respectively, was associated with near maximal antifungal activity in this *in vitro* model. The pharmacodynamic target associated with therapeutic success is likely to be lower for this early stage of disease compared with the later phases of infection.*

The applicant stated that this experimental model has several aspects that may be applicable to clinical contexts:

- *Protein has been added to the cell culture medium, which enables the binding of drug. This is likely to be important for isavuconazole because it is extensively bound in human plasma.*
- *There are no immunological effectors in this model. Consequently, the model simulated a profoundly neutropenic patient.*
- *A human-like concentration time profile of isavuconazole was used. This minimized the chance that errors will be made when the results are bridged to humans.*

*The dynamic *in vitro* model used in this report is representative of early invasive disease and mimics pathophysiological events before there is significant tissue necrosis or infarction.*

3.3.2. Mucormycosis

No studies were performed to measure activity of isavuconazole in a dynamic *in vitro* model against any of the Mucorales species.

3.4. Activity of isavuconazole in combination with other antifungals *in vitro*

3.4.1. *Aspergillus* species

The *in vitro* activity of isavuconazole, in combination with amphotericin B or micafungin, was measured against 3 clinical isolates of *Aspergillus* species [*A. fumigatus* (isolate number 4215²⁷), *A. terreus* (isolate number 95644²⁷), and *A. flavus* (isolate number 10B²⁷)] obtained from the (b) (4) by the CLSI-M38-A method (Katrakou *et al.*, 2013). Briefly, inocula were prepared in RPMI 1640 medium to obtain an initial inoculum; range of inoculum concentration was approximately 0.4 to 5 x 10⁴ CFU/mL. The three antifungal agents (isavuconazole with amphotericin B or micafungin) were tested by a two-dimensional (eight-by-twelve) checkerboard microdilution method and cultures incubated at 37°C for 48 hours. Each isolate was tested three times on different days. The choice of the appropriate range of drug concentrations was based on the MICs findings on the individual drug and isolate. The combined effects of antifungal agents were quantified by using two methods: a spectrophotometric method in which the optical density (OD) at 550 nm of each well was measured; and the metabolic reduction assay XTT (2,3-bis[2-methoxy-4-nitro-5-sulphophenyl]2H-tetrazolim-5-carboxanilide).

Interactions between antifungal agents were analyzed using the median fractional inhibitory concentration (FIC) index and the Bliss independence model. The **FIC index** was based on the following equation:

$$\Sigma FIC = FICA + FICB = C_A^{comb} / MIC_A^{alone} + C_B^{comb} / MIC_B^{alone}$$

where MIC_A^{alone} and MIC_B^{alone} represent the MICs of drugs A and B when acting alone and C_A^{comb} and C_B^{comb} are the concentrations of drugs A and B in combination, respectively, corresponding to a MIC (isoeffective combinations).

A median FIC index value of less than 1, in all 3 replicates, indicated significant synergy, and in all other cases indifference or additive effect was concluded.

In the **Bliss independence model**, the expected theoretical percentage of growth (E_{ind}) (compared to an antifungal-agent free control) describing the effect of the combination of two antifungal agents was calculated with the following equation:

$$E_{ind} = E_A \times E_B$$

where E_A and E_B are the experimental percentages of growth when each antifungal agent acts alone. For each combination of x µg/mL of antifungal agent A with y µg/mL of antifungal agent B in each of the independent replicate experiments, the experimental observed percentage of growth, E_{obs} , was subtracted from E_{ind} .

When the ΔE ($\Delta E = E_{ind} - E_{obs}$) was positive and its 95% confidence interval (CI) did not include 0, significant synergy was claimed for the specific combination of x µg/mL of antifungal agent A with y µg/mL of antifungal agent B. When the ΔE was negative without its CI overlapping 0, statistically significant antagonism was claimed. In any other case, indifference was concluded. Results presented as isobolograms showed the three dimensional relationship across the spectrum of interactions between all isavuconazole concentrations combined with amphotericin B or micafungin concentrations.

²⁷ The isolate numbers are from the registry of the (b) (4)

²⁸ Katrakou A, Meletiadis J, Petraitis V, Moradi PW, Strauss GE, Kovanda LL, Petraitiene R, and Walsh TJ. (2013) *In vitro* combination therapy of isavuconazole against medically important moulds. Internal report no. 9766-PH-0114.

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The isavuconazole MICs were 0.5 µg/mL against *A. terreus*, 1 µg/mL against *A. fumigatus* and 4 µg/mL against *A. flavus*. The amphotericin B MICs were 0.5 µg/mL against *A. fumigatus* and *A. flavus* and 1 µg/mL against *A. terreus*. Micafungin was inactive against all *Aspergillus* isolates (MICs: >32 µg/mL).

The activity of isavuconazole in combination with amphotericin B was additive or indifferent (median FIC index value was 1.18 against *A. terreus* and 1.5 against *A. fumigatus* and *A. flavus* (Table 29). By the Bliss model, the combination of isavuconazole and amphotericin B showed antagonistic interactions against *A. fumigatus* and *A. flavus* (Table 29). However, drug interaction against *A. terreus* was concentration-dependent (Figure 9); at high concentrations of amphotericin B (1–8 µg/mL) and isavuconazole (1–16 µg/mL), the interaction was antagonistic, while at low concentrations of amphotericin B (0.125–0.5 µg/mL) and isavuconazole (0.25–2 µg/mL), the interaction was synergistic (Figure 9).

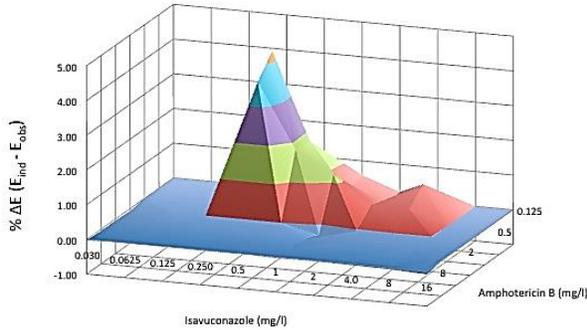
For isavuconazole and micafungin, the median FIC indices were 0.59 against *A. flavus*, 0.625 against *A. terreus*, and 0.75 against *A. fumigatus*, suggesting a synergistic effect (Table 29). Bliss analysis showed a synergistic interaction against *A. fumigatus* (Table 29 and Figure 9), *A. flavus* (Table 29 and Figure 9) and *A. terreus* (Table 29 and Figure 9). No antagonism was reported for isavuconazole in combination with micafungin against any of the *Aspergillus* species tested.

Table 29: *In vitro* interactions of isavuconazole with amphotericin B deoxycholate (AMB) or micafungin (MCA) against clinical isolates of *Aspergillus* species (A) FIC index (B) Bliss model

A: FIC index			
Strain	AMB + ISA Median FIC index (range)		MCA + ISA Median FIC index (range)
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B: Bliss model:			
Isolate	Isavuconazole and amphotericin B deoxycholate		
	Type of interaction	Mean %ΔE value (range)	Mean %SE (range)
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Isolate	Isavuconazole and micafungin		
	Type of interaction	Mean %ΔE value (range)	Mean %SE (range)
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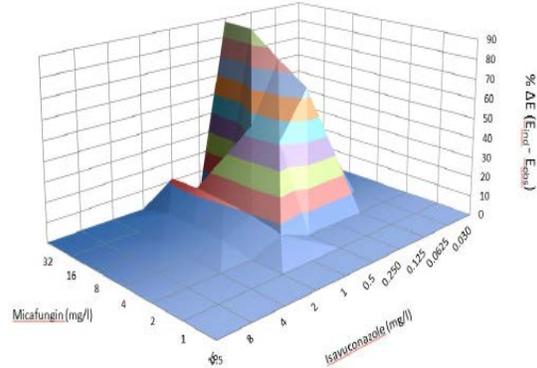
Figure 9: Interaction surface plots obtained from analysis with the Bliss independence model of isavuconazole and amphotericin B deoxycholate or micafungin interactions against *A. terreus* (strain 95644), *A. fumigatus* (strain 4215), or *A. flavus* (strain 10B).

Isavuconazole and amphotericin B ~ *A. terreus*



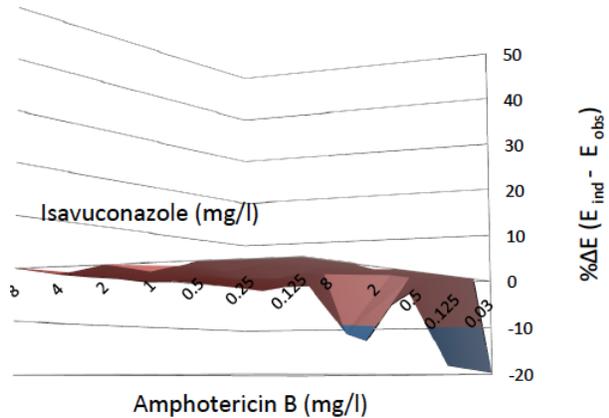
The combination of isavuconazole and amphotericin B deoxycholate resulted in drug-concentration-dependent interactions (the mean $\% \Delta E \pm SEM$ for the antagonistic interactions was $-0.04\% \pm -0.04\%$ and for the synergistic interactions $1.35\% \pm 1.55\%$).

Isavuconazole and micafungin ~ *A. terreus*



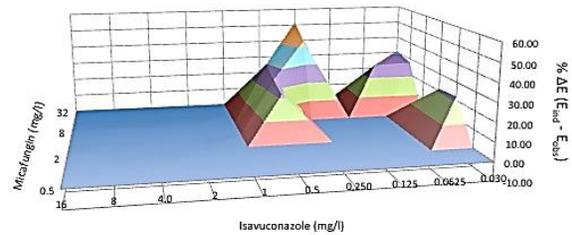
The combination of isavuconazole and micafungin resulted in synergistic interaction. The mean $\% \Delta E \pm SEM$ was $33.73\% \pm 0.06\%$.

Isavuconazole and amphotericin B ~ *A. fumigatus*



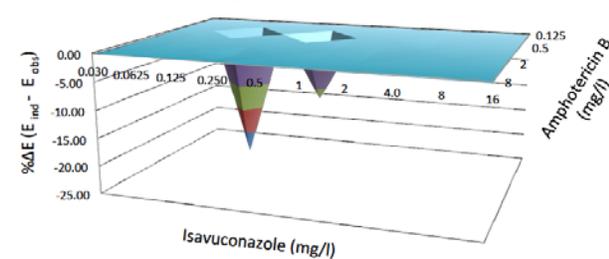
Antagonistic interactions – the mean $\% \Delta E \pm SEM$ was $-4.64\% \pm -0.47\%$.

Isavuconazole and micafungin ~ *A. fumigatus*



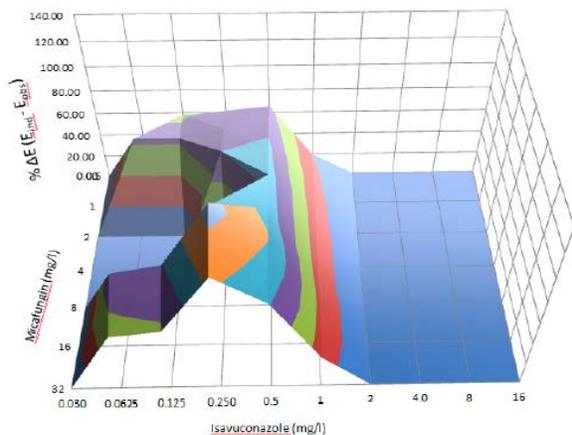
The combination of isavuconazole and micafungin resulted in synergistic interaction. The mean $\% \Delta E \pm SEM$ was $29.98\% \pm 6.16\%$.

Isavuconazole and amphotericin B ~ *A. flavus*



Antagonistic interactions were noted for *A. flavus*. The mean $\% \Delta E \pm SEM$ was $-1.4\% \pm -0.26\%$.

Isavuconazole and micafungin ~ *A. flavus*



The combination of isavuconazole and micafungin resulted in synergistic interaction. The mean $\% \Delta E \pm SEM$ was $65.59\% \pm 10.48\%$.

The zero plane ($\Delta E=0$) represents indifferent interactions whereas volumes above ($\Delta E>0$) zero plane synergistic interactions and below ($\Delta E<0$) zero plane represent synergistic and antagonistic interactions, respectively.

Comments:

A combination of isavuconazole and micafungin showed no antagonism against the strains of *A. fumigatus*, *A. flavus*, and *A. terreus* tested; the activity of the combination of isavuconazole and amphotericin B could be antagonistic depending on *Aspergillus* species and/or the drug concentrations. However, the clinical relevance of such findings is not known.

3.4.2. Mucorales species

Katragkou *et al.* (2013)²⁸ reported the *in vitro* activity of isavuconazole, in combination with amphotericin B or micafungin, against *R. oryzae* (isolate number 34²⁷), *Cunninghamella bertholletiae* (isolate number 182²⁷), *L. corymbifera* (isolate number 187²⁷), *M. circinelloides* (isolate number 234²⁷) and *R. microsporus* (isolate number 230²⁷) by the checkerboard assay. Experimental design was same as summarized above in section 3.4.1 for *Aspergillus* species. The MICs measured by the CLSI method are summarized in Table 30.

Organism	MIC (µg/mL)		
	Isavuconazole	Micafungin*	Amphotericin B
<i>C. bertholletiae</i>	16-32	>32	1
<i>R. oryzae</i>	1	>32	0.25
<i>R. microsporus</i>	4	>32	0.5
<i>L. corymbifera</i> ,	1-2	>32	0.125
<i>M. circinelloides</i>	16	>32	0.63

*The results are based on MEC; micafungin was inactive against all isolates of different Mucorales species

Based on the FIC index, a combination of isavuconazole with amphotericin B was additive or indifferent (the median FIC index values, against all species ranged from 1 to 1.12) (Table 31). However, based on the Bliss model analysis, the combination of isavuconazole and amphotericin B showed antagonistic interactions against *R. microsporus* (Table 31); against *L. corymbifera*, *M. circinelloides* and *R. oryzae*, the drug combination interactions were indifferent. Against *C. bertholletiae*, the activity of isavuconazole in combination with amphotericin B appeared to be concentration-dependent (Table 31 and Figure 10). For example, incubation with isavuconazole (2–32 µg/mL) and amphotericin B (4–8 µg/mL) at high concentrations showed antagonistic interaction with ΔE value of -0.31% (Table 31); whereas, at low concentrations of amphotericin B (0.125–0.5 µg/mL) and high concentrations of isavuconazole (4–32 µg/mL), the interaction was synergistic with mean ΔE value of 9.15% (Table 31 and Figure 10).

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Table 31: *In vitro* interactions of isavuconazole amphotericin B deoxycholate (AMB) or micafungin (MCA) against different mould isolates (A) FIC index (B) Bliss model

A: FIC index			
Strain	AMB + ISA Median FIC index (range)	MCA + ISA Median FIC index (range)	
<i>Cunninghamella bertholletiae</i> (182)	1.007 (0.5 – 2.12)	0.59 (0.52 – 2)	
<i>Lichtheimia corymbifera</i> (187)	1.12 (0.56 – 2.5)	0.75 (0.5 – 2.5)	
<i>Mucor circinelloides</i> (234)	1 (0.5 – 1.24)	1.06 (1 – 1.5)	
<i>Rhizopus microsporus</i> (230)	1.03 (0.5 – 2.1)	1.06 (1 – 1.5)	
<i>Rhizopus oryzae</i> (98)	1.09 (0.56 – 2.25)	1.06 (1 – 1.5)	

B: Bliss model			
Isolate	Isavuconazole and amphotericin B deoxycholate		
	Type of interaction	Mean %ΔE value (range)	Mean %SE (range)
<i>Cunninghamella bertholletiae</i> (182)	Antagonistic*	-0.31 (-0.13 – -0.54)	-0.052 (-0.01 – -0.1)
	Synergistic*	9.15 (6.39 – 16.23)	1.62 (1.04 – 2.83)
<i>Lichtheimia corymbifera</i> (187)	Indifferent	-	-
<i>Mucor circinelloides</i> (234)	Indifferent	-	-
<i>Rhizopus microsporus</i> (230)	Antagonistic	-3.04 (-0.11 – -50.59)	-0.24 (-0.009 – -3.69)
<i>Rhizopus oryzae</i> (98)	Indifferent	-	-
	Isavuconazole and micafungin		
	COPYRIGHT MATERIAL WITHHELD		3.55 (0.72 – 5.29)
			-2.45 (-0.68 – -5.87)
			2.96 (1.28 – 6.69)
			-
			-
			-

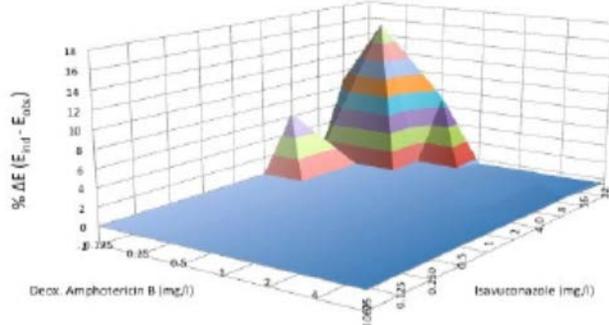
*depending on the range of concentrations tested

Based on FIC indices, a combination of isavuconazole and micafungin was synergistic against *C. bertholletiae* (FIC index 0.59) and *L. corymbifera* (FIC index 0.75); however, against, *M. circinelloides*, *R. oryzae* (FIC index), and *R. microsporus* the interaction was additive or indifferent (median FIC index 1.06 against all species) (Table 31).

By Bliss model analysis, the combination of isavuconazole and micafungin showed a synergistic interaction against *C. bertholletiae* and indifferent activity against *M. circinelloides*, *R. microspores*, and *R. oryzae* species. However, against *L. corymbifera* the interaction varied from antagonistic to synergistic depending on the concentration; micafungin (0.5–32 µg/mL) in combination with low concentrations isavuconazole (0.015–0.125 µg/mL) resulted in antagonistic interaction (mean ΔE value of -15.56%) whereas with high concentrations (0.25–2 µg/mL) of isavuconazole, the interaction (mean ΔE value of 26.32%) was synergistic (Table 31 and Figure 10).

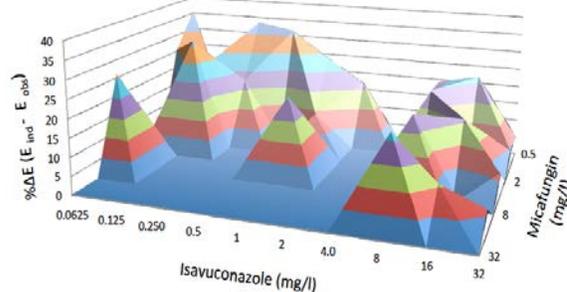
Figure 10: Interaction surface plots obtained from analysis with the Bliss independence model of isavuconazole and amphotericin B deoxycholate or micafungin interactions against (A) and (B) *C. bertholletiae* (182), (C) *R. microsporus* (230), (D) *L. corymbifera* (187)

A: Isavuconazole and amphotericin B ~ *C. bertholletiae*



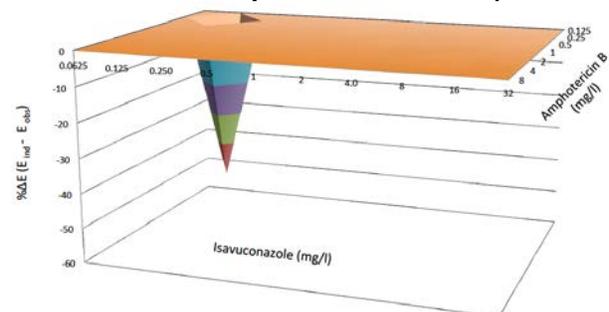
The combination of isavuconazole and amphotericin B deoxycholate resulted in drug-concentration-dependent interactions (-0.31% ± 0.052% for antagonistic interaction and 9.15% ± 1.62% for synergistic interaction).

B: Isavuconazole and micafungin ~ *C. bertholletiae*



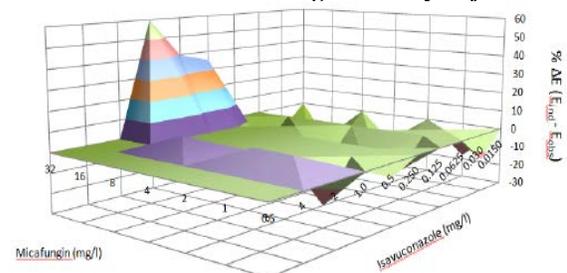
The combination of isavuconazole and micafungin was synergistic; the mean % ΔE ± SEM for the synergistic interactions was 19.3 % ± 3.5 %.

C: Isavuconazole and amphotericin B ~ *R. microsporus*



The combination of isavuconazole and amphotericin B deoxycholate resulted in antagonistic interaction (the mean %ΔE ± SEM -3% ± -0.2%,)

D: Isavuconazole and micafungin ~ *L. corymbifera*



The combination of isavuconazole and micafungin resulted in drug-concentration-dependent interactions. For *L. corymbifera* the values were -15.56% ± -2.45% for antagonistic interaction and 26.32% ± 7.43% for synergistic interaction.

The zero plane ($\Delta E=0$) represents indifferent interactions, whereas volumes above ($\Delta E>0$) and below ($\Delta E<0$) zero plane represent synergistic and antagonistic interactions, respectively

Comments:

- A combination of isavuconazole and micafungin showed indifferent or additive activity against the strains of *C. bertholletiae*, *R. oryzae*, *R. microsporus*, and *M. circinelloides* tested; however, against *L. corymbifera* strain the activity varied from synergy to antagonism depending on the drug concentrations.
- The activity of the combination of isavuconazole and amphotericin B could be antagonistic depending on *Mucorales* species and/or the drug concentrations.
- The clinical relevance of these findings is not known.

3.5. Activity in vivo

The activity of isavuconazole was measured in animal models of aspergillosis and mucormycosis.

3.5.1. Aspergillosis model

The activity of isavuconazole was measured in disseminated and pulmonary models of invasive aspergillosis.

3.5.1.1. Disseminated aspergillosis model

The activity of isavuconazole was measured in mice infected with *A. flavus*, *A. fumigatus*, or *A. terreus*.

3.5.1.1.1. *Aspergillus flavus*

Warn *et al.* (2006)²⁹ reported the activity of isavuconazole against *A. flavus* (strain AFL8; MIC 1 µg/mL by the CLSI⁵ method and MFC 2 µg/mL) in neutropenic CD-1 mice (4-5 week old). Neutropenia was induced by intravenous administration of cyclophosphamide (200 mg/kg) on Day -3; on Day 0, when profound neutropenia occurred (and persisted for additional 4 days), mice were infected intravenously with 2.4×10^4 conidia. Treatment with isavuconazium (pro-drug) or a comparator antifungal agent was initiated either 2 hours pre-infection, 4 hours post-infection, or 24 hours post-infection. A group of untreated infected mice were administered appropriate vehicle, and included as controls. The organ burdens were measured by both culture (cultured for 5 days - because of the possibility of airborne contamination, the detection limit was stated to be >30 CFU/organ) and quantitative real time PCR using fluorescence resonance energy transfer, oligonucleotide primers, and fluorogenic hybridization probes complementary to the *A. flavus* ITS1 region of the rDNA gene (Figure 11). The sensitivity of the quantitative PCR was ≤10 copies of the target DNA and was stated to be measurable in the range 10^1 – 10^6 template copies.

Figure 11: rDNA schematic showing the conserved 18S, 5.8S and 28S regions and less-conserved ITS1 and ITS2 regions of *A. flavus* (not to scale).

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The results were expressed as colony-forming units per gram (CFU/g) of tissue. The fungal burden in untreated mice was 1.3×10^4 CFU/g of tissue by culture and 3.4×10^4 genome equivalents by PCR (Table 32).

In one experiment, the effect of short term treatment (3 days; 3 mice/group) on kidney burden was determined. Mice were followed for 12 hours (isavuconazole or itraconazole treated mice) or 24 hours (voriconazole or caspofungin treated mice). Treatment with isavuconazole or other comparator drugs reduced fungal burden; activity decreased as the time of initiation of therapy was delayed from pre-exposure to 4 or 24 hours post-exposure (Table 32).

²⁹ Warn PA, Sharp A, Mosquera J, Spickermann J, Schmitt-Hoffmann A, Heep M, and Denning DW. (2006) Comparative *in vivo* activity of BAL4815, the active component of the prodrug BAL8557, in a neutropenic murine model of disseminated *Aspergillus flavus*. *J Antimicrob Chemother* **58** (6):1198-1207.

Table 32: Summary of *A. flavus* kidney tissue burden 3-4 days post infection by culture and quantitative PCR

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Pre=2 hours pre-infection; 4POST=4 hours post-infection; 24POST=24 hours post-infection.

*WSA=isavuconazole

The mice in PRE group were administered either the pro-drug shown as isavuconazole equivalent doses of 15, 6 or 3 mg/kg, orally, three times daily on days 1 and 2 followed by twice daily) OR caspofungin (1 mg/kg/day, intravenous), voriconazole (10 mg/kg/day, orally), itraconazole (10 mg/kg, orally, three times daily on days 1 and 2 followed by twice daily).

The mice in 4POST and 24POST groups were administered either the pro-drug as isavuconazole equivalent doses of 30, 15 or 6 mg/kg, orally, three times daily on days 1 and 2 followed by twice daily) OR caspofungin (1 mg/kg/day, intravenous), voriconazole (25 mg/kg/day, orally), itraconazole (25 mg/kg, orally), three times daily on days 1 and 2 followed by twice daily).

In another experiment, mice were treated either pre- or post-infection (4 hours or 24 hours) for 10 days and followed for survival up to Day 14 (n=6 per group); the fungal burden was measured in the kidneys, liver, lungs, brain, and spleen at Day 14 post-infection. A majority of infected untreated mice died by Day 8 post-challenge (Figure 12). In the pre-infection model, isavuconazole (6 mg/kg) and caspofungin were more effective in improving survival compared to itraconazole (Figure 12). Voriconazole was not very effective under the experimental conditions tested; the authors stated that this could be due to voriconazole PK as peak serum concentrations occurred before exposure to the challenge agent. In the 4-hour post-infection model, isavuconazole (≥ 15 mg/kg), voriconazole, or itraconazole were effective; however, in the 24 hour post-infection model, isavuconazole and caspofungin were more effective than voriconazole or itraconazole.

Organ burdens recovered from mice killed 14 days post-infection were stated to be relatively low with the kidneys being the most heavily infected.

Figure 12: Plots of survival against time in murine model of invasive aspergillosis

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Figure can be found at Warn et al, J Antimicrob Chemother 58 (6):1204

Fungal burden:

Pre-exposure*

Clearance of all organs was achieved in 2/5, 0/6 and 0/1 survivors at 15, 6 and 3 mg/kg/dose WSA (isavuconazium), and in 3/4 of the itraconazole and 2/6 of the caspofungin groups. Clearance rate in voriconazole treated mice was not specified.

In most mice, *A. flavus* was only recovered from the kidneys.

4 hour post-exposure*

Clearance of all organs was achieved in 3/4, 3/5 and 0/1 survivors at 30, 15 and 6 mg/kg/dose WSA (isavuconazium), and in 3/5 of the itraconazole, 2/5 of the voriconazole and 0/3 of the caspofungin groups.

No treatment was able to clear the burden completely from mice in any of the treatment groups.

24 hour post-exposure*

Clearance of all organs was achieved in 5/6, 3/6 and 3/4 survivors at 30, 15 and 6 mg/kg/dose WSA (isavuconazium), and in 3/5 of the itraconazole, 2/5 of the voriconazole and 6/6 of the caspofungin groups.

*Solvent-treated mice that survived to the end of the study had residual organ burdens.

PRE, survival of mice treated 2 h pre-infection; 4POST, survival of mice treated 4 hours post-infection; and 24POST, survival of mice treated 24 hours post-infection.

Solid black bar indicate duration of therapy. All isavuconazole concentrations refer to the active compound. CAS, caspofungin; ITC, itraconazole; VRC, voriconazole.

There was a good correlation between fungal burden measured by culture or PCR for control or any of the azole treated groups (Figure 13) but not for caspofungin treated mice. However, it is unclear if these results are in mice treated for 3 days or 10 days.

Figure 13: Scattergrams of the tissue burdens determined by conventional culture compared with tissue burdens determined by quantitative PCR (a) control mice or treated with either (b) WSA (isavuconazium), (c) voriconazole, (d) itraconazole, or (e) caspofungin. (f) Tissue burden and quantitative PCR results for all antifungals combined.

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Figure can be found at Warn et al, J Antimicrob Chemother 58 (6):1203

In a separate group of immunosuppressed infected and treated mice, blood was collected at different time intervals up to 8 to 9 hours post-dose (3 mice at each dose group) by cardiac puncture for PK measurements; plasma samples were stored at -20°C . Isavuconazole concentration was measured by LC-MS/MS method whereas voriconazole and itraconazole concentrations were measured by a bioassay using a San Antonio strain of *Candida kefyr*. Itraconazole levels (after 25 mg/kg/dose) peaked at $18.5\ \mu\text{g/mL}$, voriconazole levels (after 25 mg/kg/dose) at $16.4\ \mu\text{g/mL}$, and isavuconazole peak levels were in the range $0.67\text{--}3.72\ \mu\text{g/mL}$ for doses ranging from 3 to 30 mg/kg (Table 33) with undetectable or extremely low isavuconazole levels after 8 hours. Overall, there was good absorbance of all azole drugs, and rapid transformation of the pro-drug into the active compound isavuconazole. The exposure of mice treated with isavuconazium at the highest dose of 30 mg/kg active compound was substantially less than voriconazole and itraconazole.

Table 33: Drug exposure after treatment with WSA (isavuconazium), voriconazole (VRC) or itraconazole (ITC)
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Majithiya *et al.* (2008)³⁰ reported the activity of isavuconazole in CD-1 mice infected with *A. flavus* strain AFL8 (same strain as used for the study summarized above). The experimental design was similar to that summarized above except that treatment was initiated 4 hours post-infection; a range of one, two and three daily doses of oral isavuconazonium (as isavuconazole equivalent daily doses of 45, 60, 90, 120, or 150 mg/kg or daily doses of 10, 30, 75 or 225 mg/kg or twice daily doses of 5, 15, 37.5, or 112.5 mg/kg, or three times daily doses of 10, 25, or 75 mg/kg), amphotericin B (5 mg/kg/day intraperitoneal), caspofungin (1 mg/kg/day, intravenous), voriconazole (25 mg/kg/day intravenous; plus grapefruit juice), or vehicle (controls) were administered for two to four days. Isavuconazole was not effective in clearing the fungal burden in kidneys; all mice slowly died over time (Table 34). The duration of follow-up was not specified.

Table 34: MIC and MFC values, time-kill kinetics and *in vivo* clearance data of antifungal agents against nine isolates of *Aspergillus* spp.

Strain	Isavuconazole				Voriconazole				Amphotericin B			
	MIC mg/L	MFC mg/L	Time-kill	In vivo	MIC mg/L	MFC mg/L	Time-kill	In vivo	MIC mg/L	MFC mg/L	Time-kill	In vivo

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Comments:

- *The studies suggest that isavuconazole, like other azoles (itraconazole or voriconazole), was effective in improving survival and reducing fungal burden in neutropenic mice infected with A. flavus. The activity may decrease with a delay in time of initiation of treatment.*
- *High plasma concentration of isavuconazole were reported in neutropenic infected mice treated with ≥ 15 mg/kg/dose isavuconazole; The isavuconazole MIC for the A. flavus strain*

³⁰ Majithiya JB, Sharp A, Parmar A, Denning DW, and Warn PA. (2008) Correlation of *in vitro* MIC and MFC against isavuconazole, voriconazole and amphotericin B of *Aspergillus* with *in vivo* outcome in mice with disseminated aspergillosis. 18th European Congress of Clinical Microbiology and Infectious Diseases, Poster P-1915, Barcelona, Spain.

used in this study was 1 µg/mL; a ratio of 24 hour AUC/MIC in excess of 1 were obtained after treatment with ≥15 mg/kg/dose isavuconazole. The authors stated that although not yet clear, it is likely that trough levels maintained above the MIC will predict a good outcome.

- The half-life after oral administration in mice was about 3.8 hours, much less than in humans (135 hours).

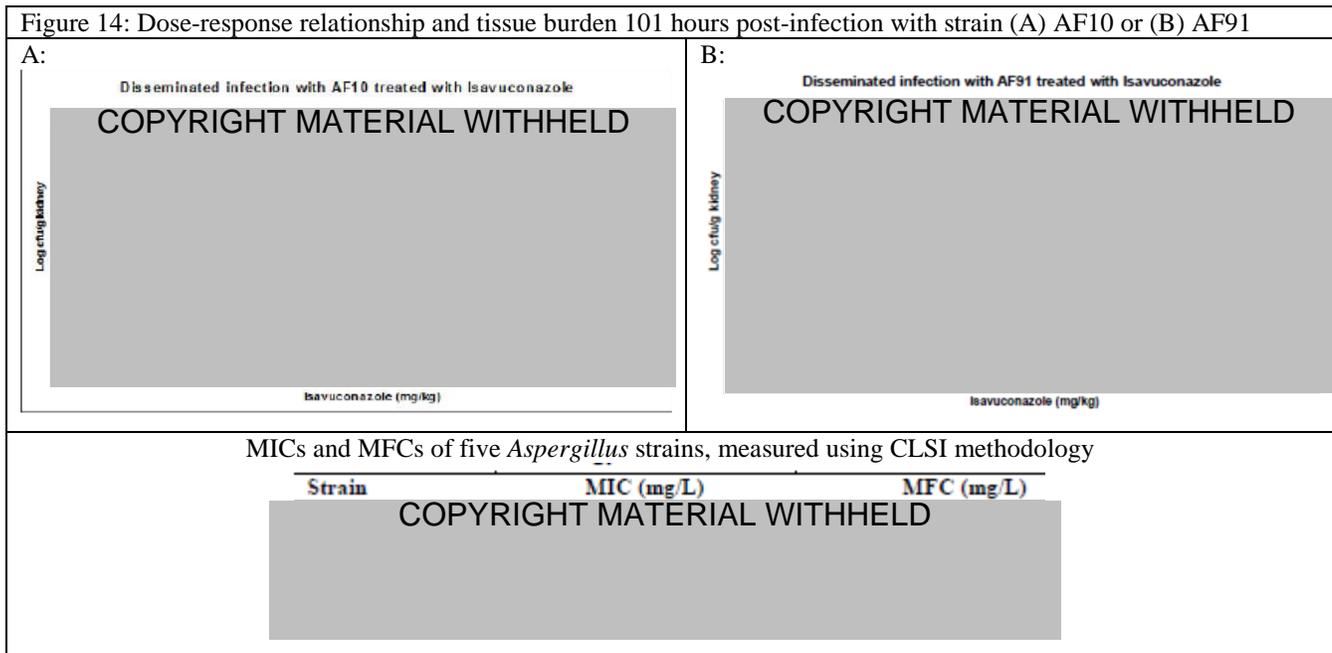
3.5.1.1.2. *Aspergillus fumigatus*

The activity of isavuconazole against *A. fumigatus* was measured in neutropenic and non-neutropenic (immunocompetent) mice.

• Neutropenic murine model

Warn *et al.* (2007)³¹ reported the activity of isavuconazole in neutropenic CD-1 mice infected intravenously with two strains (AF10 and AF 91) of *A. fumigatus*. The concentration of inoculum used for infection was not specified. Neutropenia was induced as summarized above for the *A. flavus* study (see section 3.5.1.1.1. above). The animals were monitored for 101 hours post-infection for the development of a heavy non-lethal fungal burden in the kidney. Groups of four mice were orally treated with isavuconazonium at isavuconazole equivalent doses ranging from 0.625-250 mg/kg/day at 5, 29, 53, 77, and 101 hours post-infection. It is unclear at what time the kidneys were collected for measuring fungal burden if the last dose was administered at 101 hours.

The results showed a reduction in fungal burden in the kidneys from mice treated with isavuconazole; the reduction in fungal burden was more in mice infected with the AF-10 strain (MIC 0.25 µg/mL) compared to the AF91 strain with MIC of 4 µg/mL (Figure 14).



³¹ Warn PA, Parmar A, Sharp A, and Denning DW. (2007) Dose response in neutropenic mice with disseminated infection of multiple strains of *Aspergillus fumigatus* with widely varying MIC and MFC values treated with isavuconazole. 17th European Congress of Clinical Microbiology and Infectious Diseases, Poster P-1951, Munich, Germany.

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Majithiya *et al.* (2008)³⁰ reported the *in vivo* activity of isavuconazole in neutropenic CD-1 mice infected with seven different strains (A1163, AF4, AF1108, AF82, AF210, AF293 and AF19) of *A. fumigatus* with different MIC and MFC values determined in accordance with the CLSI method M38A. Experimental design was similar to that summarized above. The results showed clearance of fungal burden in isavuconazole treated mice infected with strains AF4, AF210, and A1163 with rapid kill but not strain AF293 with slow time-kill; the results of mice infected with the other three strains (AF1108, AF19, and AF293) were not shown (Table 34). Overall, the activity of isavuconazole was similar to voriconazole and amphotericin B.

The applicant stated that mice infected with the A1163 strain and treated with isavuconazonium (as isavuconazole equivalent doses of 120 or 150 mg/kg/day) showed a >99% decrease in kidney fungal burden compared to control group of mice; lower doses of isavuconazonium (as isavuconazole equivalent ≤ 90 mg/kg/day) were ineffective. Mice infected with *A. fumigatus* strain AF4 and treated with isavuconazonium at isavuconazole equivalent doses of 225 mg/kg per day, 112.5 mg/kg twice daily, or 75 mg/kg three times daily showed >95% reductions in renal fungal burden. Mice infected with strain AF4 and treated with isavuconazonium at total isavuconazole equivalent daily doses of 30 or 75 mg/kg generally had lower fungal burden in kidneys. The reduction in fungal burden was enhanced by dividing the dose and administering it twice or three times daily. However, the data were not shown.

Warn *et al.* (2006)³² reported the activity, PK, dose response, and effect of dose fractionation of isavuconazole in neutropenic mice infected with the CAE10 strain of *A. fumigatus* (MIC 0.5 $\mu\text{g/mL}$ by the EUCAST method). Experimental design was similar to that summarized above except that the PK of isavuconazole was determined after administration of multiple (13) doses (once daily, subcutaneously, and orally) of isavuconazonium. To measure the dose-response relationship of isavuconazole, groups of four to six mice were treated with oral isavuconazonium at isavuconazole equivalent doses of 0.625-250 mg/kg per day oral once daily at 5, 29, 53 and 77 hours post-infection. The maximum effect (E_{max}) value from the generated dose response curve was then used to determine the concentrations of isavuconazole used in the dose fractionation study. Doses were chosen to give 20%, 40%, 60% and 80% of the E_{max} value, which equated to 25, 80, 180 or 250 mg/kg per day of oral isavuconazonium as isavuconazole equivalent doses, respectively. These doses were administered once every 2 days, once daily, or divided into two or four daily doses over 77 hours. Fungal kidney burden was measured in all mice at 101 hours post-infection. The isavuconazole concentrations in the lungs were approximately two-fold higher than in plasma, and were three to five-fold higher in the kidneys compared with plasma. The half-life of isavuconazole (1.1–2.3 hours) was similar in the plasma, lungs, and kidneys. At >80 mg/kg per day, multiple daily doses were more effective at lowering kidney burden, compared with the same doses administered once daily or once every two days (Table 35).

³² Warn PA, Sharp A, and Denning D. (2006). Dose response curves and dose fractionation studies with BAL8557 in a murine model of disseminated aspergillosis. 46th Interscience Conference on Antimicrobial Agents and Chemotherapy, Poster A-1120, San Francisco, CA, USA.

Table 35: The response of fungal burden in kidneys to dose fractionation of isavuconazonium (as isavuconazole equivalent doses) following oral administration (20%-80% of Emax)

Daily dose of isavuconazonium (mg/kg) ^a	Tissue burden (CFU/g kidney tissue)			
	Once every two days	Once daily	Twice daily	Four times daily
180	nt	1116	396	252
150	624	780	180	165
80	876	1380	1080	1020
25	3306	975	1764	2520

^a as isavuconazole equivalent dose; CFU = colony-forming units; nt = not tested

Overall, isavuconazole showed tissue penetration, with similar half lives in blood and other tissues.

The applicant stated that increasing efficacy was noted over a wide therapeutic range (up to 250 mg/kg/day). The dose fractionation data suggested that T>MIC, rather than AUC, was the PD parameter that best described the *in vivo* antifungal activity of isavuconazole. However, the degree of certainty of this finding is in question due to the extremely short half-life in the mouse as well as the small differences in the burdens following dose fractionation. The data were not available for an independent review.

- **Non-neutropenic murine model**

Seyedmousavi (2014)³³ reported the activity, dose-response, and exposure-response relationship of isavuconazole in immunocompetent CD-1 mice infected with either a WT isolate (AZN 8196) or one of the three azole-resistant isolates harboring substitutions in the *cyp51A*-gene: G54W (V 59-73), M220I (V28-37) and TR₃₄/L98H (V 52-35). Strain identification and the *cyp51A*-gene substitutions were confirmed by sequence-based analysis (Table 36). All isolates grew well after 48 hours of incubation at 35 to 37°C. The *in vitro* activity of isavuconazole was measured by the CLSI method.³⁴ Isavuconazole MICs were similar between WT strain (AZN8196, isavuconazole MIC, 0.25 µg/mL) and strain V59-73 (isavuconazole MIC, 0.25 µg/mL) with G54W mutation at the *cyp51A* target and lower than for strains V28-73 (isavuconazole MIC, 2 µg/mL) and V 52-35 (isavuconazole MIC, 8 µg/mL) with other mutations (M220I and TR34/L98H, respectively) in the *cyp51A* target gene.

The 90% lethal dose (LD₉₀) was determined for each isolate, separately and varied 2- to 5-fold with the strain (Table 37). Mice were infected intravenously with a conidial suspension corresponding to the LD₅₀ for each isolate and treatment initiated 24 hours post-infection. Isavuconazonium was administered orally, once daily with doses of 0.25, 1, 4, 16, 64, 128 and 256 mg/kg pro-drug isavuconazonium sulfate for 14 days; these dosages represented isavuconazole equivalent doses of 0.12, 0.48, 1.92, 7.68, 30.7, 61.4, and 122.9 mg/kg; control mice were administered saline.

³³ Seyedmousavi S. (2014) Pharmacodynamics of isavuconazole in an *Aspergillus fumigatus* mouse infection model. Internal report (Astellas report no. 9766-PH-0204).

³⁴ Represent MICs determined by the CLSI microdilution methodology.

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Table 36: *In vitro* antifungal susceptibilities of *A.fumigatus* isolates used in the study as well as disease classification, history of previous azole exposure, underlying azole resistance mechanisms.

ID number	<i>Aspergillus</i> disease*	Prior azole exposure	Cyp51A substitution	MIC or MEC (mg/L)**					
				AMB	ITC	VRC	POS	ISA	AFG

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The MIC (minimum inhibitory concentration) was defined as the lowest concentration that completely inhibited growth in comparison to the drug-free well (control) as assessed by visual inspection.
The MEC (minimum effective concentration) was defined as the lowest concentration in which abnormal, short, and branched hyphal clusters were observed in contrast to the long, unbranched hyphal elements that were seen in the growth control well

<i>A. fumigatus</i> strain	LD ₉₀ (conidia)	ED ₅₀ (mg/kg)**	ED ₁₀₀ (mg/kg)**
Wild-type (AZN8196)	2.4 x 10 ⁷	24.15	64
G54W	1 x 10 ⁷	28.93	128
M220I	5 x 10 ⁷	109	256
TR34/L98H	2.5 x 10 ⁷	483.8	Not achieved
TR46/Y121F/T289*	3.5 x 10 ⁷	Not done	Not done

* TR46/Y121F/T289strain was not included for further testing
**Isavuconazium sulfate ED₅₀ and ED₁₀₀: 50% and 100% effective dose based on survival

Pharmacokinetics: The PK profile in infected mice (infected with the WT strain (AZN8196) was determined on the second day of treatment corresponding to the third day of infection. BAL fluid samples were collected on the same day for determining concentrations of isavuconazole. Isavuconazole concentration in epithelial lining fluid (ELF) was then determined by the following equation:

$$\text{ISA concentration}_{\text{ELF}} = \text{ISA concentration}_{\text{BAL}} \times \frac{\text{Urea}_{\text{plasma}}}{\text{Urea}_{\text{BAL}}}$$

The dose normalized isavuconazole AUC₀₋₂₄ ratios in plasma ranged from 0.54 to 0.84 (Table 38A and Figure 15A). The isavuconazole concentrations in ELF were lower than plasma concentrations (Table 38B and Figure 15B); a significant correlation between mean isavuconazole concentrations in plasma and ELF was reported by linear regression analysis (Figure 15C). The authors stated that variable penetration ratio might be due to variation in measurements in different compartments as well as the difference in lysis of the cells available in interstitial spaces over the course of infection that limits passage through alveolar epithelial cells in various levels.

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Table 38: Pharmacokinetic parameters of isavuconazole following oral administration of various dosages (administered as pro-drug isavuconazonium sulfate) and penetration of isavuconazole in ELF versus plasma in infected mice

A: Pharmacokinetic parameters of isavuconazole

Dose group mg/kg ^a	C _{max} mg/liter	C _{last} mg/liter	Half-life (h)	AUCINF _{_pred} h.mg/liter	AUCINF _{_D_pred} (h.mg)/(liter.kg)	CL _{50_F} liter/(h.kg)	V _{z_F} liter/kg
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Accepted manuscript posted online 9 March 2015, doi: 10.1128/AAC.04907-14

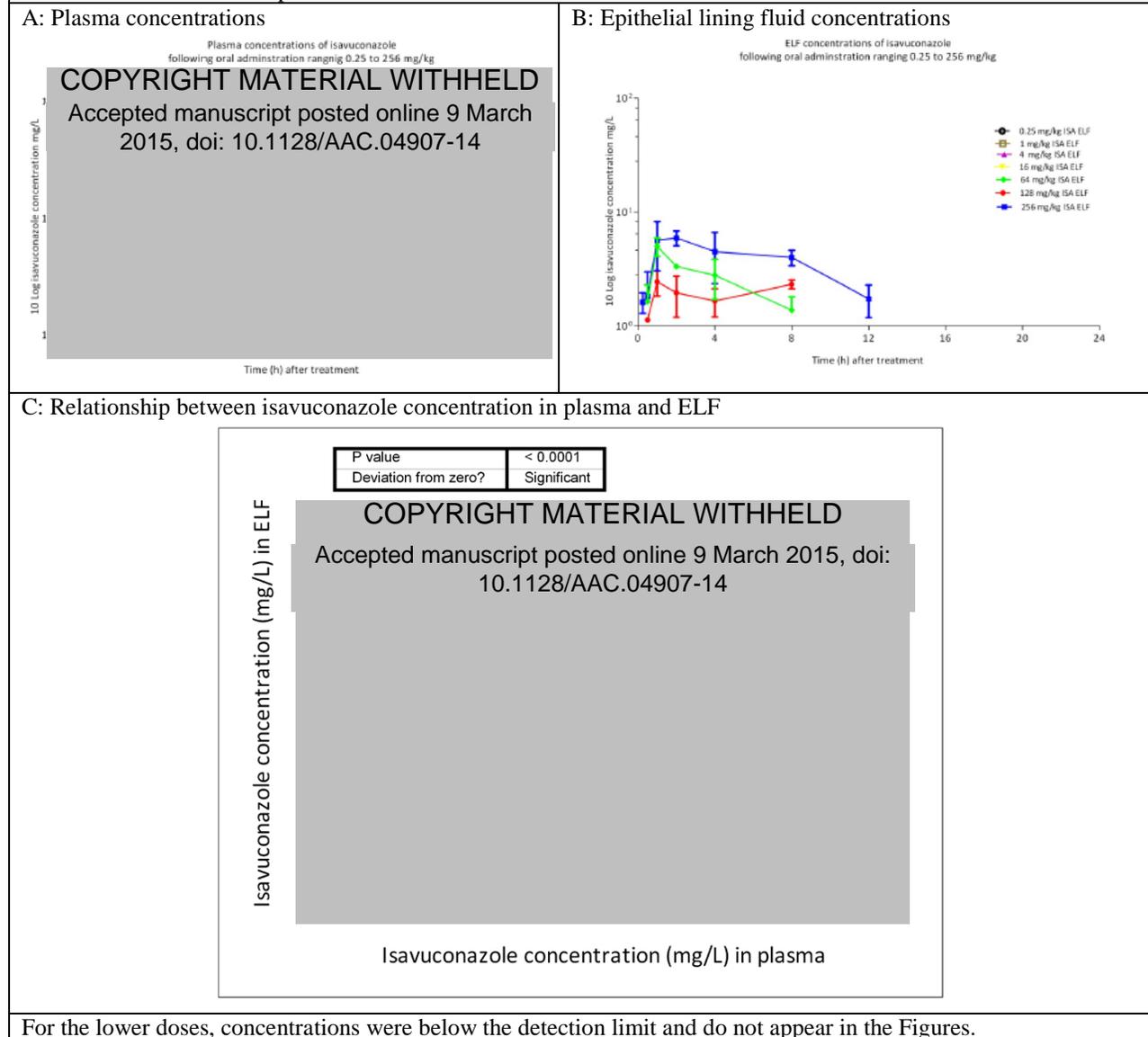
B: Penetration of isavuconazole in ELF versus plasma exposures

Dose group mg/kg	AUCINF _{_pred} h.mg/liter		AUCINF _{_pred} h ELF plasma Ratio (%)
	Plasma	ELF	

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Accepted manuscript posted online 9 March 2015, doi: 10.1128/AAC.04907-14

Figure 15: Concentrations of isavuconazole following oral administration of the pro-drug isavuconazonium sulfate to immunocompetent infected mice. (A) Plasma, (B) ELF, and (C) Relationship between isavuconazole concentration in plasma and ELF



In vivo activity: Survival and reduction in fungal burden were the primary endpoints in groups of 11 and 3 mice, respectively. Fungal burden in kidneys was determined by quantitative PCR at 72 hours post-infection. The reduction in kidney fungal burden was correlated with the survival of the remaining 11 mice from each corresponding group at Day 15 post-infection.

All untreated mice died within 8 days of infection. Isavuconazole was effective in improving survival and reducing fungal burden (Figures 16, 17, and 18). However, the activity varied with the MIC of the strain; the maximum effect (100% survival) was reached at a dose of 64 mg/kg for the WT strain (AZN 8196), 128 mg/kg for the G54W and 256 mg/kg for M220I mutants. A maximal response was not achieved with the TR₃₄/L98H strain at the highest dose of pro-drug isavuconazonium sulfate (256 mg/kg- 22.3% survival; isavuconazole equivalent dose of 122.9 mg/kg) tested.

Figure 16: Effect of treatment on survival of mice infected with four different strains of *A. fumigatus*

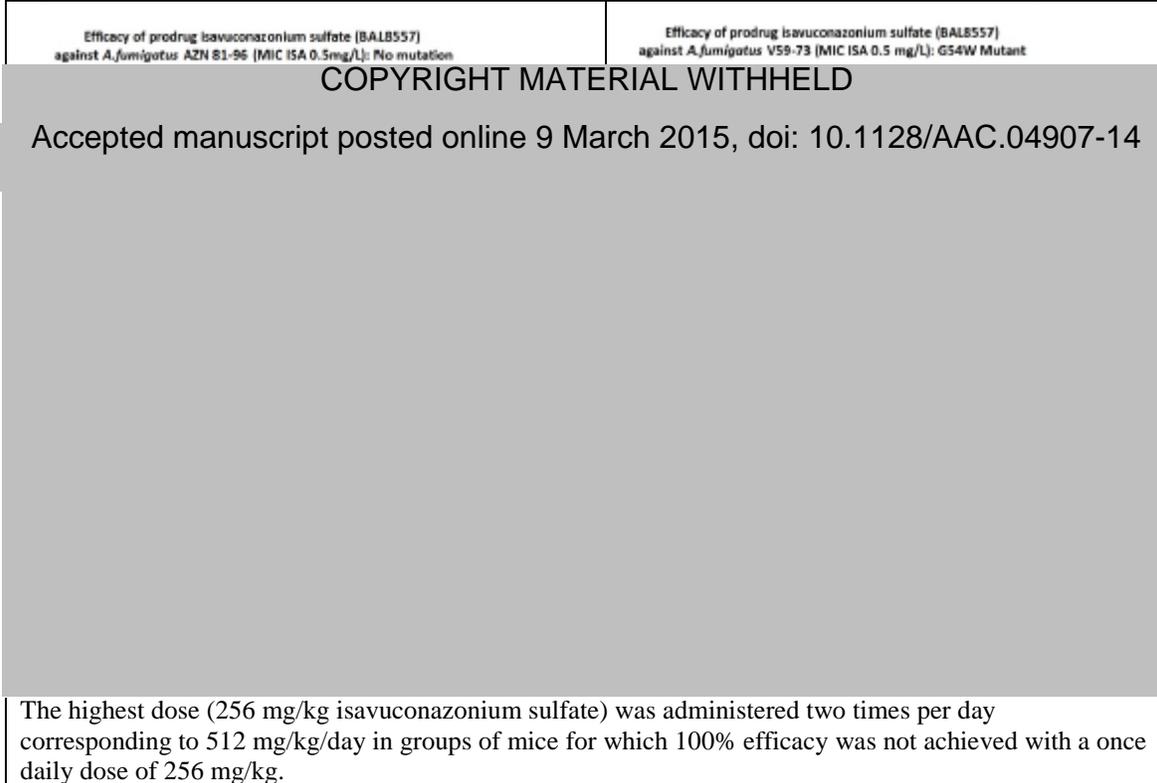
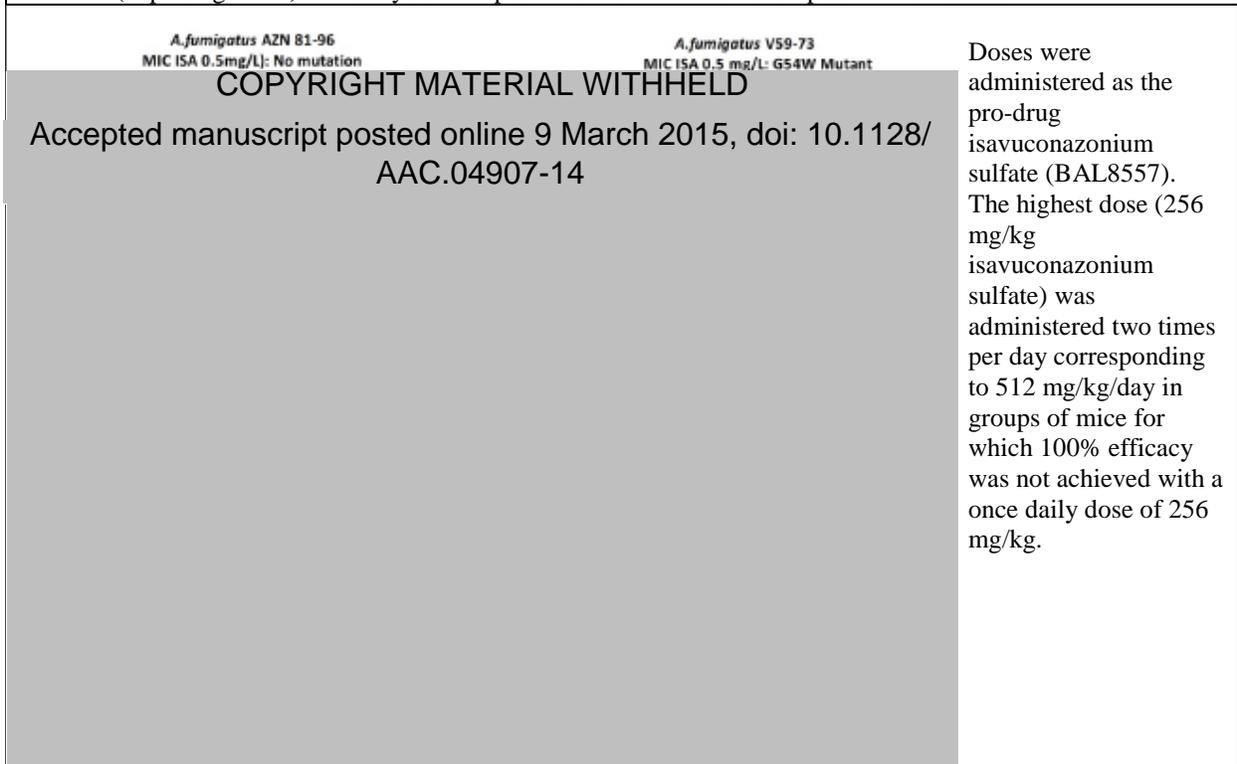
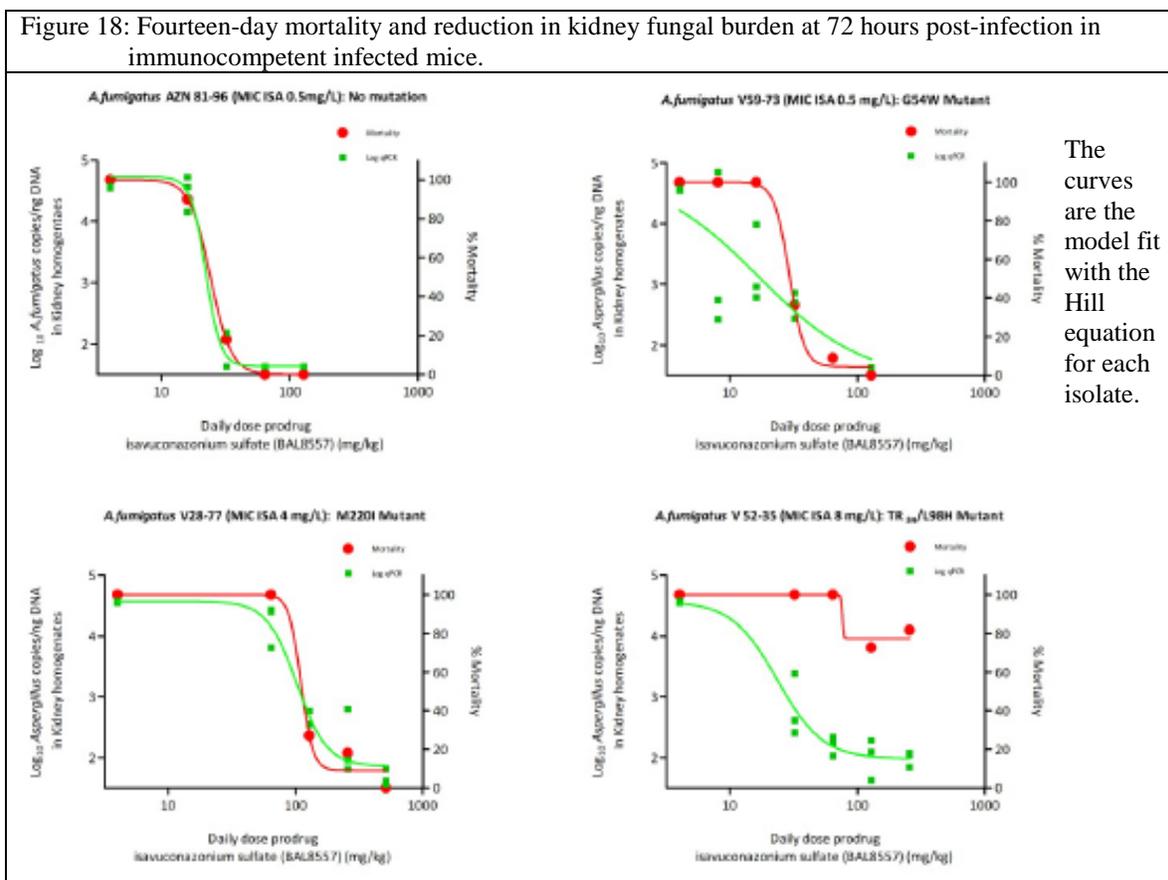


Figure 17: Activity of isavuconazole against four *A. fumigatus* isolates measuring *A. fumigatus* DNA load (copies/ng DNA) in kidneys at 72h post infection in immunocompetent infected mice.



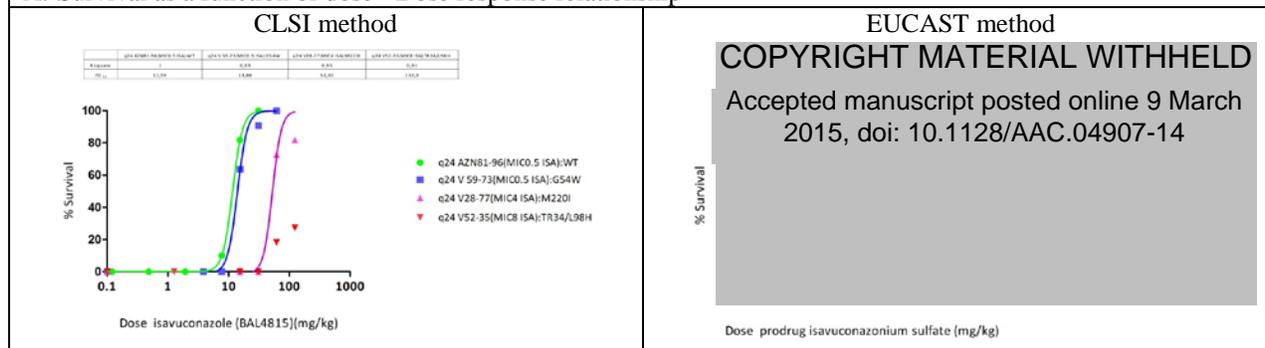


Dose-response analysis: Isavuconazole treatment improved survival of the mice in a dose-dependent manner (Figure 19). In addition to the isavuconazole dose, survival appears to depend on the azole-resistance mechanisms; the dose-response curve for mice infected with an isolate with higher isavuconazole MICs ($\geq 2 \mu\text{g/mL}$) was shifted to the right compared to those infected with the lower isavuconazole MIC ($0.25 \mu\text{g/mL}$), suggesting that higher doses of isavuconazole were required to achieve similar activity against the mutant strains. The ED_{50} and ED_{100} values were higher in mice infected with mutant strains compared to the WT strain (Table 37). Although the loss of activity was completely or partly compensated for by increasing the isavuconazole dose for treatment of the resistant strains, for mice infected with the $\text{TR}_{34}/\text{L98H}$ isolate, isavuconazole was not effective at the highest dose tested (Figures 18 and 19).

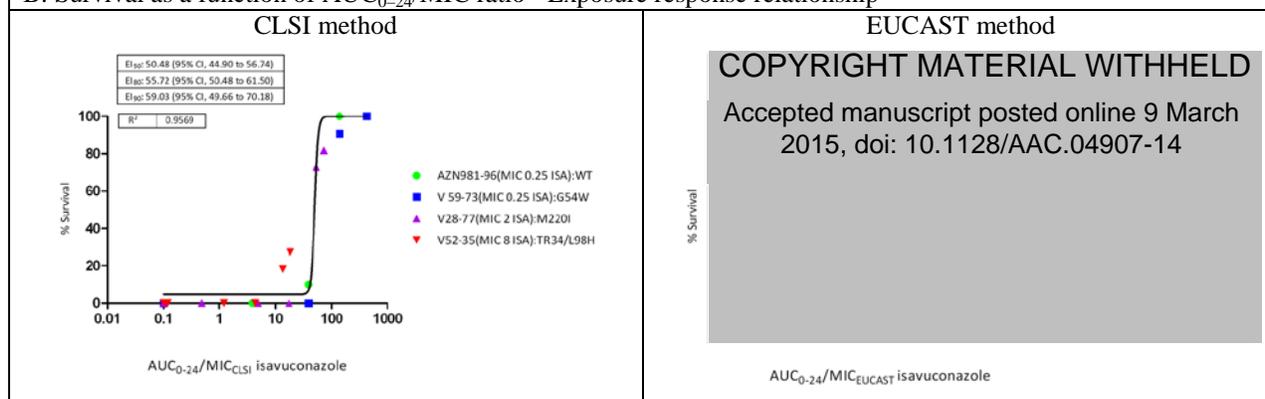
The Hill-type model with a variable slope fitted the relationship between the dose and 14-day survival well, with R^2 values of 1 for WT, 0.99 for G54W, 0.95 for M220I and 0.91 for $\text{TR}_{34}/\text{L98H}$ strains. The Hill equation with a variable slope fit the data well with a significant relationship between $\text{AUC}_{0-24}/\text{MIC}$ ratio and 14-days survival ($R^2 = 0.96$) ($P < 0.05$). For 50% survival, the effective $\text{AUC}_{0-24}/\text{MIC}$ ratio for isavuconazole total drug was 24.73 (95% confidence interval, 22.50 to 27.18) (Figure 19). The applicant stated that there was no difference between survival curves of groups treated with various dosing intervals (data not shown) suggesting that AUC/MIC was the driver of efficacy.

Figure 19: Fourteen-day survival of mice as a function of (A) pro-drug isavuconazonium sulfate dose, and (B) AUC_{0-24}/MIC ratio against four *A. fumigatus* isolates (MIC according to CLSI⁵ or EUCAST⁶ methodology).

A: Survival as a function of dose - Dose response relationship



B: Survival as a function of AUC_{0-24}/MIC ratio - Exposure response relationship



Exposure-response analysis: The AUC for each dose (Table 38A) was used to calculate the AUC_{0-24}/MIC ratio for each isolate. The exposure-response relationship curve was sigmoidal shape (Figure 19B). Increased isavuconazole exposure was required to obtain maximum efficacy in mice infected with the M220I (MIC 2 $\mu\text{g/mL}$) and TR₃₄/L98H mutants (MIC 8 $\mu\text{g/mL}$) compared to those infected with the WT and G54W mutant (MIC 0.25 $\mu\text{g/mL}$).

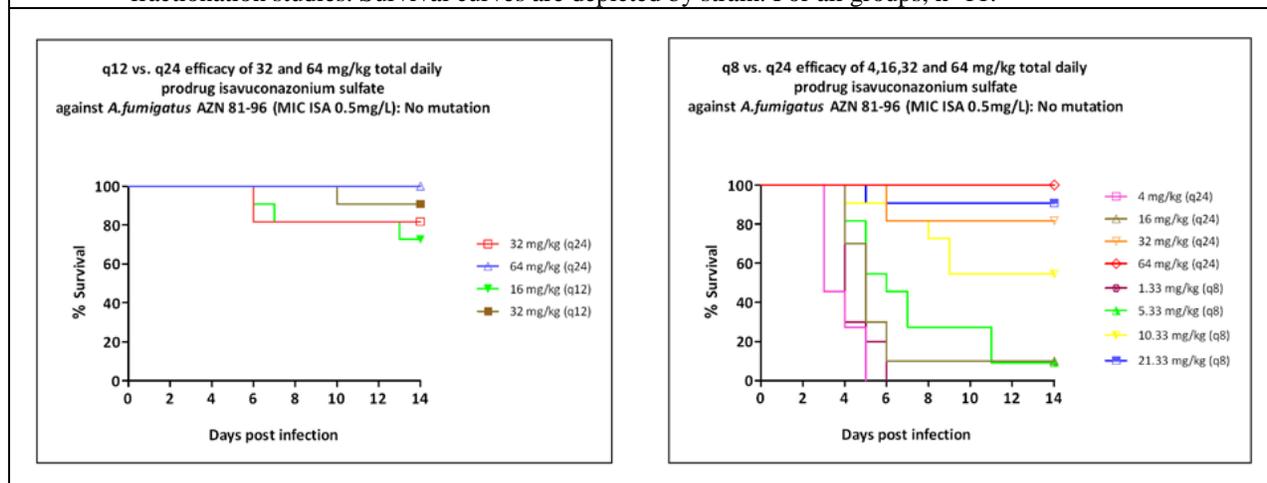
The Hill equation with a variable slope fitted the relationship between the 24-hour AUC/MIC ratio and 14-day survival well ($R^2=0.96$), as statistically significant PD indices for isavuconazole single-agent regimens ($P < 0.05$). The 50% effective PD index (total AUC_{0-24}/MIC) for isavuconazole was 24.73 (95% confidence interval, 22.50 to 27.18) and was considered the PD index most closely predictive of efficacy.

The peak level C_{max}/MIC was also determined (data not shown). The authors stated that the relationship between the *in vivo* efficacy and other PD indices, such as the cumulative percentage of a 24-hour period that the drug concentration exceeded the MIC under steady state PK conditions was determined. AUC_{0-24}/MIC appeared to be the most important pharmacodynamic index correlating with efficacy.

Dose fractionation studies: Dose fractionation studies were performed to determine the PD index predictive of therapeutic efficacy. For this, mice were infected with the WT *A. fumigatus* strain AZN 81-86 and treatment initiated 24 hours post-infection. Total daily dosing was every 8 (q8)

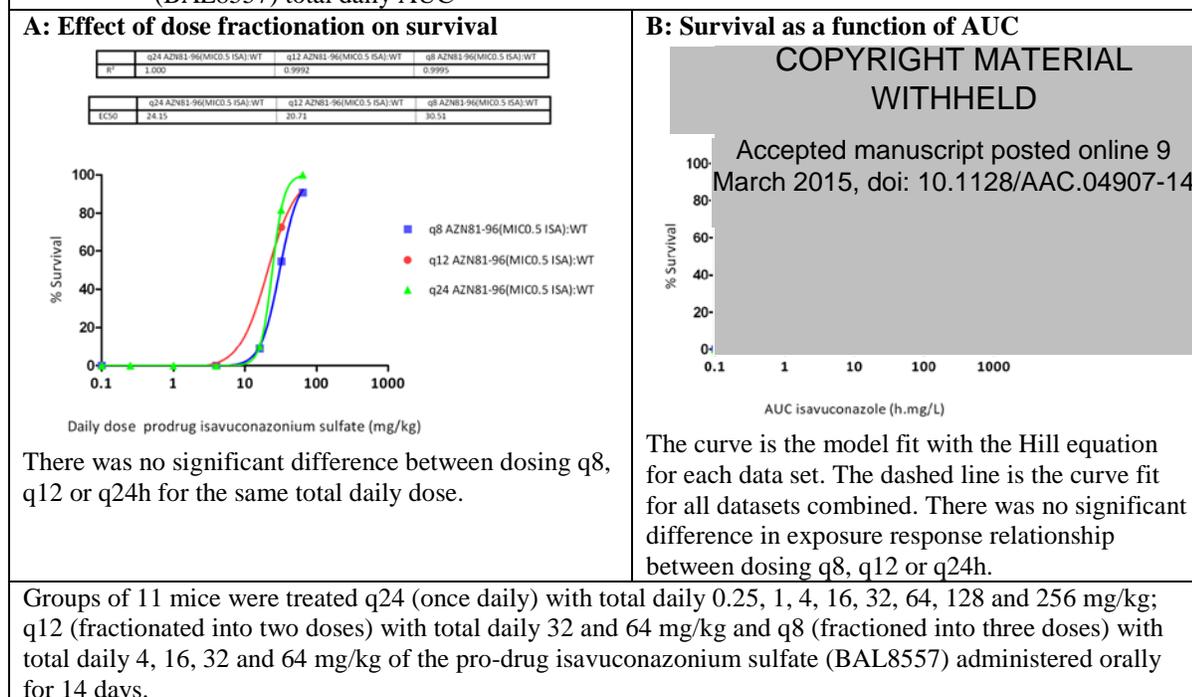
or 12 (q12) hours for 14 days. There was no difference in survival of mice treated at various dosing intervals (Figure 20).

Figure 20: Comparison of survival curves against wild-type *A. fumigatus* isolates for the q8, 12 and 24 dose-fractionation studies. Survival curves are depicted by strain. For all groups, n=11.



The three dose–response curves, as well as the exposure response curves were not significantly different suggesting the AUC/MIC as the driver of efficacy (Figure 21).

Figure 21: Dose fractionation study in mice infected with the wild-type *A. fumigatus* strain (MIC, 0.25 mg/L ISA) (A) Impact of dose fractionation on the *in vivo* activity of pro-drug isavuconazonium sulfate (BAL8557), and (B) Percent of survival as a function of the pro-drug isavuconazonium sulfate (BAL8557) total daily AUC



Comments:

The study suggests that MIC of the isolate had an impact on isavuconazole activity and AUC/MIC appears to be the driver of activity in mice. The loss of activity against the mutant

strains may be compensated by increasing the dose. The effect of host factors was not evaluated in this study.

3.5.1.1.3. *Aspergillus terreus*

Majithiya *et al.* (2008)³⁰ reported the *in vivo* activity of isavuconazole in neutropenic CD-1 mice infected with *A. terreus* strain AT49. The experimental design was similar to that summarized above (for details see section 3.5.1.1.1.) except that treatment with isavuconazole or other antifungals was initiated 4 hours post-infection. Isavuconazole was not effective in clearing the fungal burden in kidneys; all mice slowly died over time (Table 34).

3.5.1.2. Pulmonary aspergillosis model

The activity of isavuconazole was measured against *A. fumigatus* in animal models of pulmonary aspergillosis.

3.5.1.2.1. *Aspergillus fumigatus* – immunocompromised animals

The activity of isavuconazole was measured against the WT and/or mutant strains of *A. fumigatus* in mice, guinea pigs, and rabbits.

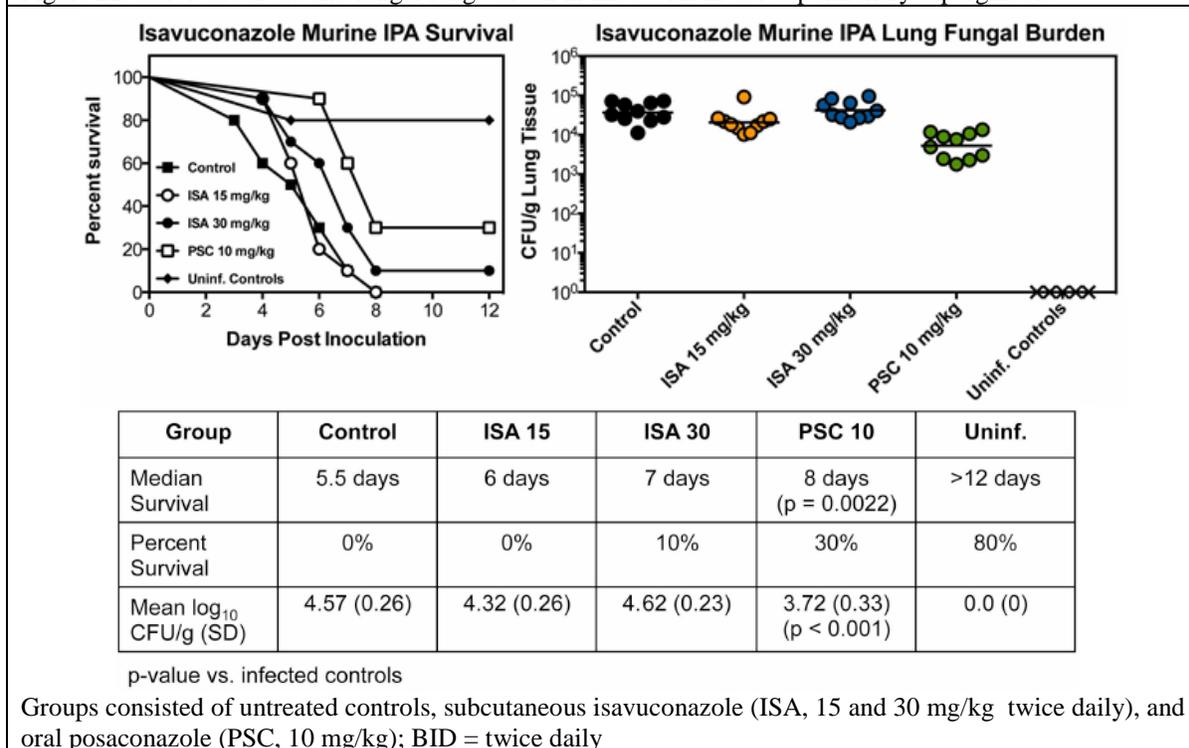
- **Mice**

Wiederhold (2012)³⁵ reported the activity of isavuconazium in immunocompromised ICR mice infected by aerosolization with the AF293 strain of *A. fumigatus*. Mice were immunosuppressed with cyclophosphamide and cortisone acetate prior to aerosolized inoculation; cyclophosphamide (250 mg/kg 2 days prior and 200 mg/kg 3 days following inoculation) and cortisone acetate (250 mg/kg, 2 days prior and 3 days following inoculation) were administered that maintained leukopenia up to 7 to 8 days post-infection and permitted the development of lethal infection. The authors stated that with this combination, uninfected mice had lost more than 20% of their initial body weight and were too ill to receive further immunosuppression. Anti-bacterial prophylaxis with ceftazidime was administered subcutaneously at 50 mg/kg beginning two days prior to inoculation and was continued daily until the end of the experiment.

Treatment with isavuconazonium (isavuconazole equivalent doses of 15 and 30 mg/kg, b.i.d.) was initiated 24 hours following inoculation (Day 1), subcutaneously, and continued through Day 7. Posaconazole was included as a positive control. Mice were followed until Day 12 for survival time, and pulmonary fungal burden (measured on Day 8). The results showed that treatment with isavuconazole twice-daily did not improve the median survival time or percent survival, compared with untreated controls (Figure 22). A reduction in lung fungal burden was not observed in the isavuconazole-treated mice. In contrast, treatment with posaconazole did improve median survival and decreased fungal lung burden, compared to control mice.

³⁵ Wiederhold NP. (2012) Evaluation of the investigational triazole isavuconazole in a guinea pig and murine models of invasive pulmonary aspergillosis. Internal report 9766-PH-0201.

Figure 22: Survival curves and fungal lung burden in mice with invasive pulmonary aspergillosis



Isavuconazole concentrations were measured by a bioassay in uninfected neutropenic mice administered single (15, 45, & 75 mg/kg) or multiple oral doses of isavuconazonium. The results showed low drug concentrations (<1 µg/mL) in serum at the lowest (15 mg/kg) dose tested while higher (45 and 75 mg/kg) doses resulted in higher isavuconazole serum levels. Serum isavuconazole levels were undetectable 12 hours after oral administration for all doses tested (Figure 23A).

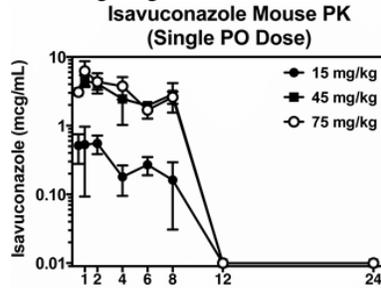
All of the mice administered aminobenzotriazole (ABT) alone or 45 mg/kg isavuconazole equivalent dose survived, but among 18 mice receiving 75 mg/kg drug, one death was recorded. There were 4/18 deaths among mice co-administered 45 mg/kg isavuconazole equivalent dose and ABT, and 7/18 deaths in mice administered 45 mg/kg drug and ABT (Figure 23).

Following administration of multiple doses of isavuconazonium, by oral gavage, serum drug concentrations did not increase; isavuconazole was undetectable by 8-12 hours after the last dose (Figure 23). C_{max} values observed following 15, 20 and 45 mg/kg subcutaneous administration of isavuconazole equivalent doses (2.76±0.7, 15.6±1.9 and 18.4±1.17 µg/mL, respectively) were higher than those achieved at the same dose levels by oral gavage (0.55±0.37, 0.67±0.1 and 1.79±0.71 µg/mL, respectively). Administration of ABT in combination with isavuconazonium resulted in higher drug serum concentrations and overall exposures as measured by AUC (Figure 23); these drug levels were also detectable for a longer period compared with animals that received isavuconazonium alone.

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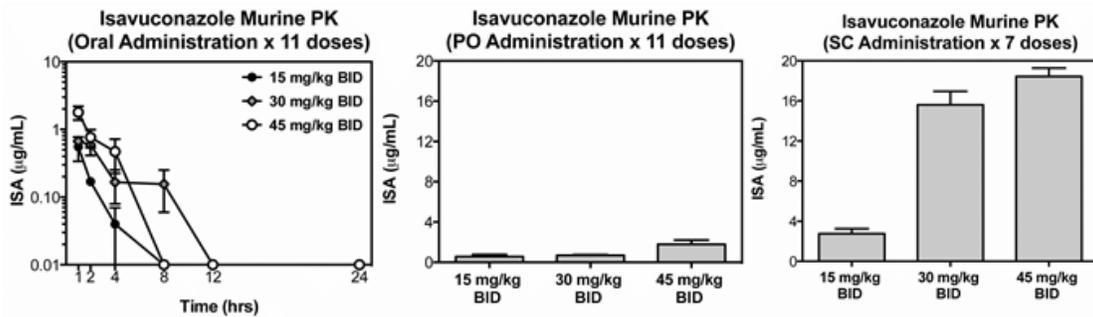
Figure 23: Isavuconazole serum concentrations following (A) single dose oral, and (B) twice daily (b.i.d.) dose administration by oral gavage (PO) and subcutaneous (SC) injection

A: Isavuconazole serum concentrations following single dose oral administration to uninfected mice

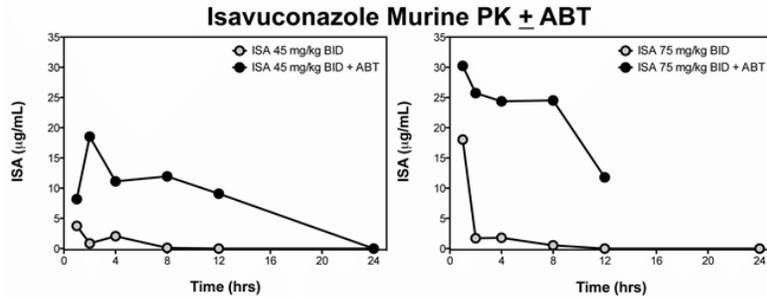


B: Twice daily (b.i.d.) dose administration by oral gavage (PO) and subcutaneous (SC) injection

- Without aminobenzotriazole – uninfected mice



- With aminobenzotriazole – infected mice



Group	ISA 45	ISA 45 + ABT	ISA 75	ISA 75 + ABT	ABT Controls
Percent Survival	100% (18/18)	77.8% (14/18)	94.4% (17/18)	61.1% (11/18)	100% (3/3)
Notes		Died b/w days 2 - 5	Died after last dose on day 7	Died b/w days 3 - 6 4 others moribund on day 7	No adverse effects or deaths observed

Comments:

Subcutaneous administration of isavuconazonium in mice was well tolerated and resulted in higher serum concentrations of isavuconazole compared with oral dosing. Co-administration of ABT, a cytochrome P-450 inhibitor, with isavuconazole led to higher serum concentrations of isavuconazole and longer periods of drug detection in mice, but was also associated with increased deaths at the higher doses.

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In infected mice, twice daily dosing of isavuconazole did not significantly improve survival time, the numbers of surviving animals, or pulmonary fungal burden, compared with untreated controls. The results of this study are inconsistent with the trends observed in other studies summarized above. It is unclear if such differences are due to the strain of A. fumigatus (known to exhibit slow-time kill), severe immunocompromised animals, or subcutaneous route of drug administration.

Lepak *et al.* (2013)³⁶ reported the activity of isavuconazole in ICR mice infected with 10 isolates of *A. fumigatus* (four WT isolates and six *cyp51* mutants; nine of the 10 isolates were stated to be clinical isolates and one a laboratory isolate with an *Fks1* mutation). *In vitro* susceptibility of all isolates was measured in accordance with CLSI method M38-A2.⁵ MICs varied from 0.25 to 8 µg/mL (Table 39). The organisms exhibited similar *in vivo* fitness.

Table 39: *In vitro* susceptibilities and *in vivo* fitness of *A. fumigatus* isolates

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^bEchinocandin MIC >16 mg/L

Mice were rendered neutropenic for four days by subcutaneous injection of 150 mg/kg cyclophosphamide on Days -4 and -1. Cortisone acetate (50 mg/kg) was also administered subcutaneously on Day -1, and an additional 150 mg/kg cyclophosphamide was injected on Day 3, to ensure neutropenia throughout the 7-day experiment. Ceftazidime (50 mg/kg) was administered subcutaneously on each day to prevent opportunistic bacterial infection. Mice were infected via pipetting of the inoculum into the anterior nares and subsequent aspiration into the lungs. Following infection, groups of mice were treated with 40-640 mg/kg oral isavuconazonium sulfate (corresponding isavuconazole dose = 19-307 mg/kg) twice daily for 7 days, starting on Day 0. Activity was determined by assessing fungal burden (expressed as C.E./mL of lung homogenate), by quantitative PCR, in the lungs of moribund animals or at the end of therapy on Day 7. At the start of therapy mice had 4.97 ± 0.33 log₁₀ conidia equivalent (C.E.)/mL of lung homogenate and the burden increased to 6.82 ± 0.51 log₁₀ C.E./mL of lung homogenate in untreated animals. Each isolate produced 100% mortality prior to study endpoint in untreated animals.

Single dose PK of isavuconazole was determined following oral administration of isavuconazonium at 10, 40, 160, and 640 mg/kg (isavuconazole equivalent doses of 4.8, 19, 77, and 307 mg/kg) by oral gavage. Isavuconazole concentrations were measured in plasma samples

³⁶ Lepak AJ, Marchillo K, Vanhecker J, and Andes DR. (2013). Isavuconazole (BAL4815) pharmacodynamic target determination in an *in vivo* murine model of invasive pulmonary aspergillosis against wild type and *Cyp51* mutant isolates of *Aspergillus fumigatus*. *Antimicrob Agents Chemother* **57**: 6284-6289.

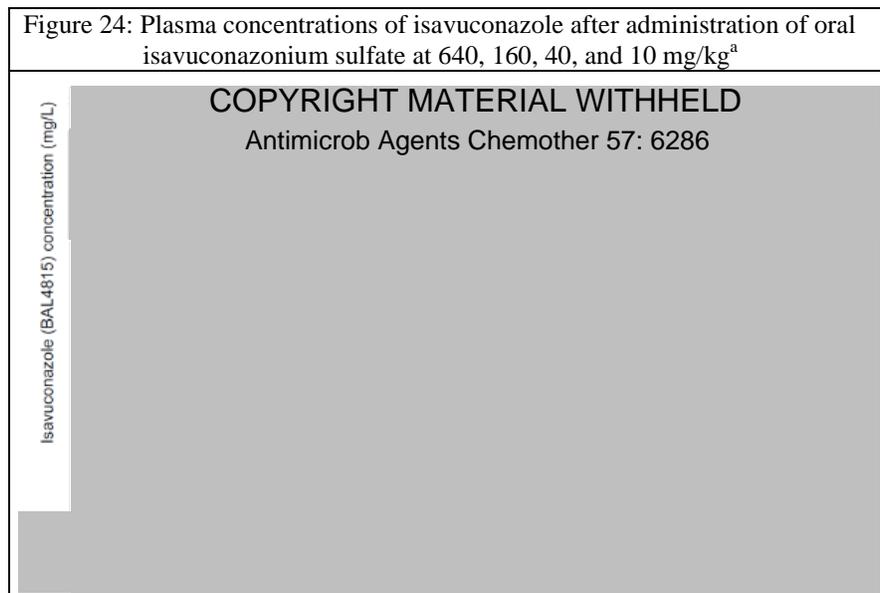
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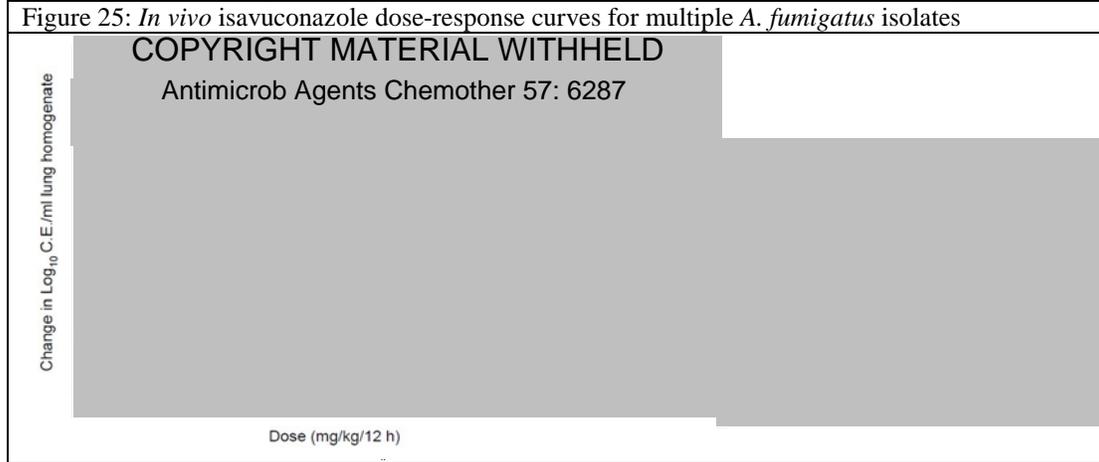
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collected from groups of three mice at 0.5, 1, 2, 4, 8, 12, and 24 hours. To assess PD, two-fold increases in oral isavuconazonium (40-640 mg/kg), were administered twice daily by oral gavage for 7 days. Doses were selected to range from no or little effect to maximal efficacy and included an exposure range expected in humans during clinical trials. Controls were utilized for each isolate and included a zero hour and an untreated group. Four mice were included in each treatment and control group. The PK-PD relationship of isavuconazole was examined and the optimal drug exposure for infection due to WT and *cyp51* mutant isolates defined.

Pharmacokinetics: Peak isavuconazole concentrations in plasma were achieved within two hours for each dosing regimen, and the C_{max} values ranged from 0.51-25.4 $\mu\text{g/mL}$ (Figure 24). The elimination half-life in serum increased in a dose-dependent fashion from 1 to 5 hours. The AUC ranged from 0.9 to 287 $\mu\text{g}\cdot\text{h/mL}$ and was relatively linear ($R^2 = 0.98$) over the dose ranges tested.



MIC ~ PK and dose-response: Higher doses were needed to achieve microbiologic effect against isolates with elevated isavuconazole MICs (Figure 25). Maximal effect against WT isolates was an approximate 2 \log_{10} reduction in lung fungal burden compared with the start of therapy, and an almost 4 \log_{10} decrease compared to untreated controls at the end of therapy. Overall, there was a dose-response relationship for each isolate, with higher drug doses achieving increased anti-fungal effect.



PD index and target magnitude: The AUC/MIC ratio was used as the PD index for exploring exposure-response relationships. The results showed isavuconazole suppressed net growth of isolates with an MIC of ≤ 1 $\mu\text{g/mL}$; whereas 1 \log_{10} kill was observed for strains with an MIC of ≤ 0.5 $\mu\text{g/mL}$ (Table 40). It was possible to estimate the static dose AUC/MIC target for all WT isolates, and the *cyp51* mutants exhibiting the low (≤ 1 $\mu\text{g/mL}$) MIC values (F14403 and F14532). The static dose for *cyp51* WT isolates ranged from 212-617 mg/kg twice daily; for the mutant isolates, F14403 and F14532, the dose was 65 and 515 mg/kg twice daily, respectively. The 1 \log_{10} kill PD target was achieved in 3 of 4 *cyp51* WT isolates at a dose that ranged from 302-455 mg/kg twice daily; for a mutant isolate F14403 the dose was 147 mg/kg twice daily.

Table 40: *In vivo* pharmacodynamic efficacy of isavuconazole in an immunocompromised murine pulmonary aspergillosis model

Organism	MIC (mg/L)	Static dose (mg/kg/12 h)	Static dose 24 h AUC ($\mu\text{g}\cdot\text{h/mL}$)	Static dose 24 h AUC/MIC	Static dose 24 h free drug AUC/MIC	1 log kill dose (mg/kg/12 h)	1 log kill 24 h AUC ($\mu\text{g}\cdot\text{h/mL}$)	1 log kill 24 h AUC/MIC	1 log kill 24 h free drug AUC/MIC
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The total drug AUC/MIC associated with net stasis for the *cyp51* WT group ranged from 415-1111; whereas for isolates F14403 and F14532 this endpoint was slightly lower at 361-367. The difference between the two groups was not statistically significant ($P = 0.18$).

For all isolates where net stasis was achieved the median static dose total drug AUC/MIC was 503. The 1 \log_{10} kill total drug AUC/MIC was roughly two-fold higher than the static dose PD target, with a median value of 1111. The AUC/MIC values and treatment outcome for all organisms was fit to the Hill sigmoid dose-response model (Figure 26). Overall, the results suggest AUC/MIC to be a predictor of observed outcome ($R^2 = 0.75$).

Figure 26: Relationship between PD index total-drug AUC/MIC and treatment efficacy for isavuconazole against 10 *A. fumigatus* isolates

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Comments:

The study showed that isavuconazole PD index AUC/MIC ratio correlated with treatment outcome in a murine model of invasive pulmonary aspergillosis (IPA). The MIC appeared to be a predictor of success or failure regardless of the presence or absence of a cyp51 mutation. Mutations that lead to elevated MICs of other triazoles did not universally correlate with elevated isavuconazole MICs. The median total- and free-drug 24-hour AUC/MIC ratio PD targets for net stasis were 503 and 5, respectively.

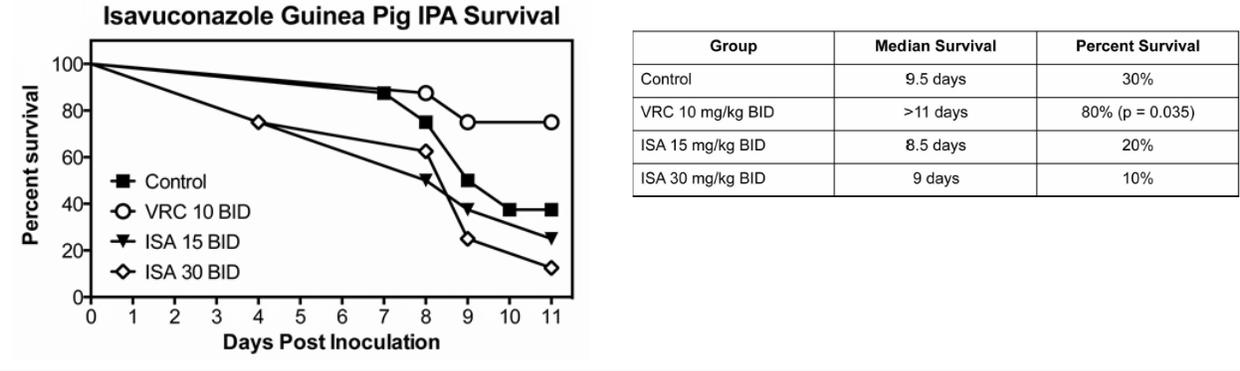
The static dose and 1-log₁₀ dose was the primary PD target endpoints used in this study. It is unclear which PD endpoint in the animal model correlates with the optimal treatment effect in patients.

• **Guinea pig**

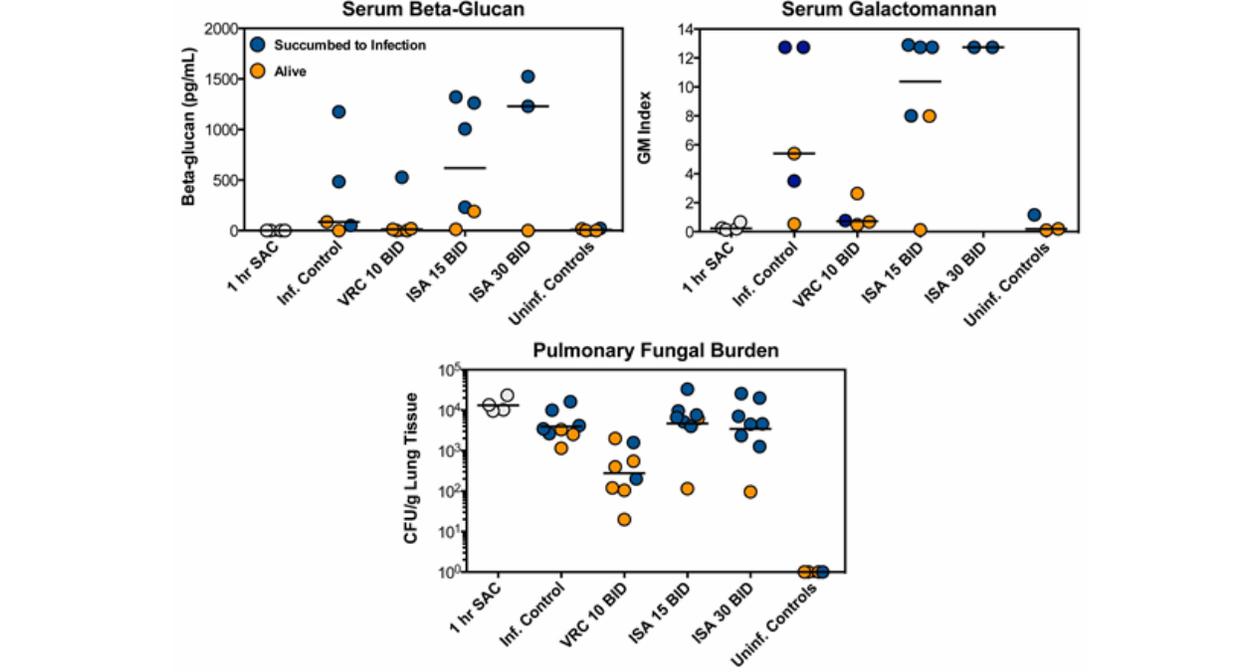
Wiederhold (2012)³⁵ reported the activity of isavuconazole in immunocompromised guinea pigs infected by aerosolization with the AF293 strain of *A. fumigatus*. Immunosuppressive regimen and experimental design was similar to the murine model summarized above (see section 3.5.1.2.1. above) except that animals were followed until Day 11 and voriconazole was included as a positive control. Isavuconazole was administered orally. Additionally, pulmonary fungal burden was measured on Day 12. Concentrations of (1-3)- β -D-glucan and GM in the serum were also measured as markers of fungal burden. The results showed no improvements in median survival time, the percentage survival, serum (1-3)- β -D-glucan and GM levels, as well as fungal lung burden in guinea pigs administered isavuconazole (15 or 30 mg/kg twice-daily), compared to untreated controls (Figure 27). Voriconazole significantly improved percent survival and reduced markers of infection compared with the control group ($P < 0.05$).

Figure 27: Effect of treatment on (A) survival and (B) serum (1-3)- β -D-glucan and galactomannan levels, and fungal lung burden in guinea pigs with IPA

A: Survival



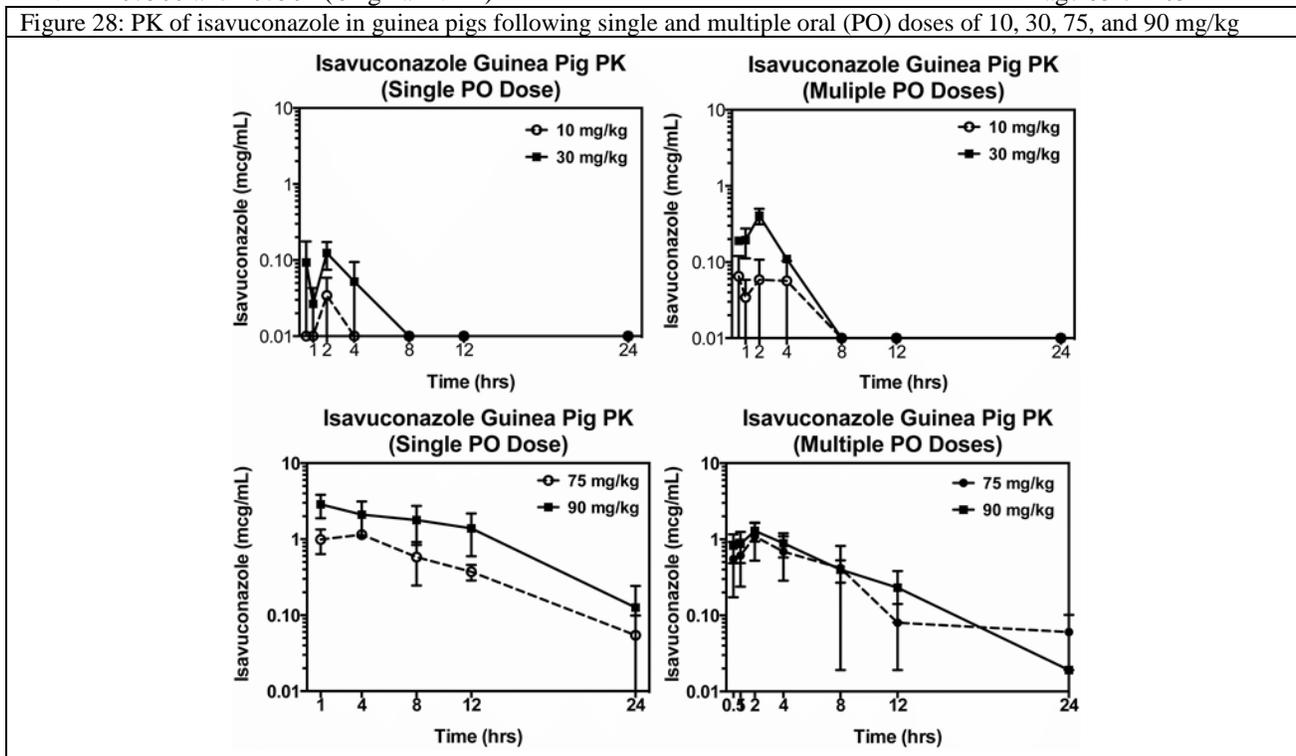
B: Serum (1-3)- β -D-glucan levels, galactomannan levels, and fungal burden in lung



Groups consisted of infected and untreated controls, oral isavuconazole (ISA, 15 and 30 mg/kg), oral voriconazole (VRC, 10 mg/kg), and uninfected controls; BID = twice daily

The PK of isavuconazole was measured in uninfected neutropenic guinea pigs. Following single or multiple dose oral administration of isavuconazonium (isavuconazole equivalent 10 or 30 mg/kg twice daily), the highest serum isavuconazole concentration measured was less than 1 μ g/mL and declined to undetectable levels 8 hours post-dose (Figure 28). Higher serum concentrations were reported following oral administration at 75 and 90 mg/kg but deaths were also observed (two and one, respectively).

Figure 28: PK of isavuconazole in guinea pigs following single and multiple oral (PO) doses of 10, 30, 75, and 90 mg/kg



All guinea pigs administered isavuconazonium subcutaneously died while placebo injected animal survived. Activity of subcutaneous administration of isavuconazole in infected animals was not measured.

Comments:

In infected guinea pigs, oral administration of isavuconazium did not significantly improve survival time, the number of surviving animals, or pulmonary fungal burden, compared with untreated controls. Voriconazole was effective in improving survival. Guinea pig does not appear to be a good model for measuring activity of isavuconazium.

• **Rabbits**

Petraitiene *et al.* (2013)³⁷ reported the activity of isavuconazole in persistently neutropenic female New Zealand White rabbits infected by endotracheal inoculation with the NIH 4215 (ATCC MYA-1163; isavuconazole MIC 1 µg/mL and voriconazole MIC 0.5 µg/mL by the CLSI method³⁴) strain of *A. fumigatus*. Briefly, immunosuppression and profound persistent neutropenia (a neutrophil cell count of <100 neutrophils/µL) was established and maintained using cytarabine and methylprednisolone; antibiotics (ceftazidime, gentamicin, and vancomycin) were used for prevention of opportunistic bacterial infections during neutropenia. On Day 2, rabbits were infected via endotracheal inoculation under general anesthesia. Oral treatment was initiated one day post-infection; a loading dose of oral isavuconazonium sulfate (isavuconazole

³⁷ Petraitiene R, Petraitis V, Moradi PW, Strauss GE, Huertas BT, Katragkou A, Petraityte E, Kovanda LL, Smart J, Hope WW, and Walsh TJ. (2013) Pharmacokinetics and efficacy of isavuconazole in comparison to voriconazole for treatment of experimental invasive pulmonary aspergillosis. Internal report no. 9766-PH-0207.

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equivalent doses of 90 mg/kg) followed by daily isavuconazole equivalent dosing of 20 (ISA20), 40 (ISA40), or 60 (ISA60) mg/kg per day for up to 12 days. Voriconazole (15 mg/kg) was included as a comparator and administered orally twice daily. Control animals received no treatment following infection. Rabbits were followed for survival, residual fungal burden, GM index in both serum and BAL fluid, and plasma (1-3)- β -D-glucan concentrations. Additionally, lung weights and numbers of pulmonary infarcts were monitored as indicators of organism-mediated pulmonary injury. Serum creatinine, urea nitrogen, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and potassium were also measured.

The single dose PK of oral isavuconazium (isavuconazole equivalent doses of 20, 40, and 60 and 90 mg/kg per day) was measured in non-infected rabbits (n=4 per group). Blood samples were collected immediately prior to dosing, and at 1, 2, 4, 8, 12, 18, and 24 hours post dose; time points for sampling were determined by inspection of full plasma concentration profiles obtained in normal rabbits following administration of similar dosages. The optimal sampling PK of isavuconazole was also studied in five to eight infected animals per dosage group. Plasma samples were collected on Day 6 of antifungal therapy immediately prior to dosing and again at 1, 4, 8, and 24 hours post dose.

The results showed improved survival and reductions in lung weight, infarct scores, fungal burden in lungs, GM concentration in serum and BAL fluid, as well as plasma (1-3)- β -D-glucan levels of rabbits treated with isavuconazole especially, isavuconazole 40 and isavuconazole 60 mg/kg, compared to voriconazole-treated animals (Figures 29, 30, and 31).

Figure 29: Mean (\pm standard error of the means (SEMs) pulmonary tissue residual fungal burden, lung weight, pulmonary infarct score and survival rates of treated and untreated neutropenic rabbits with primary pulmonary aspergillosis

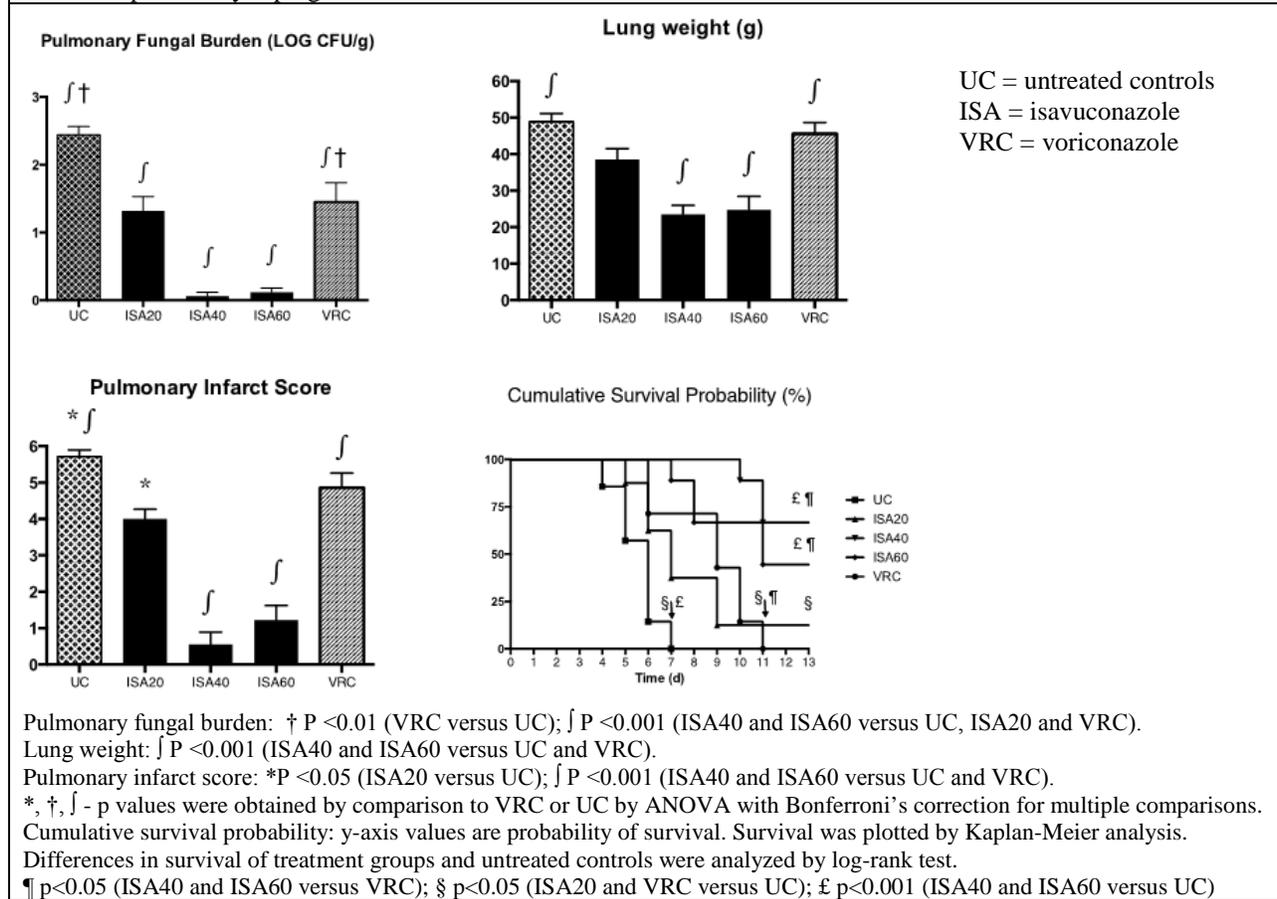


Figure 30: Concentrations of galactomannan in serum (A) and BAL fluid (B) in treated and untreated neutropenic rabbits with primary pulmonary aspergillosis

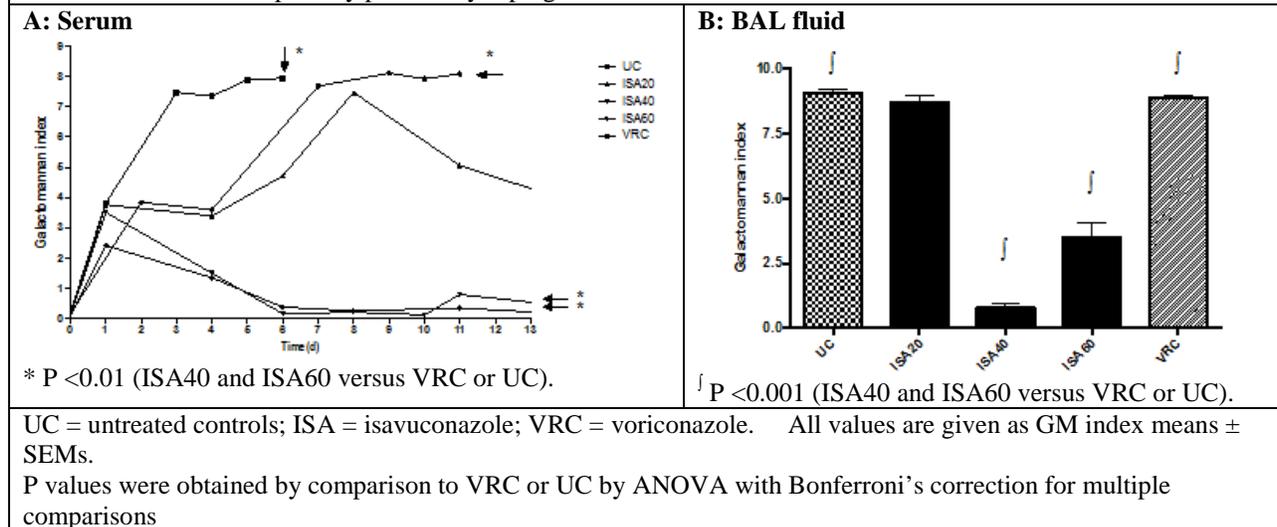
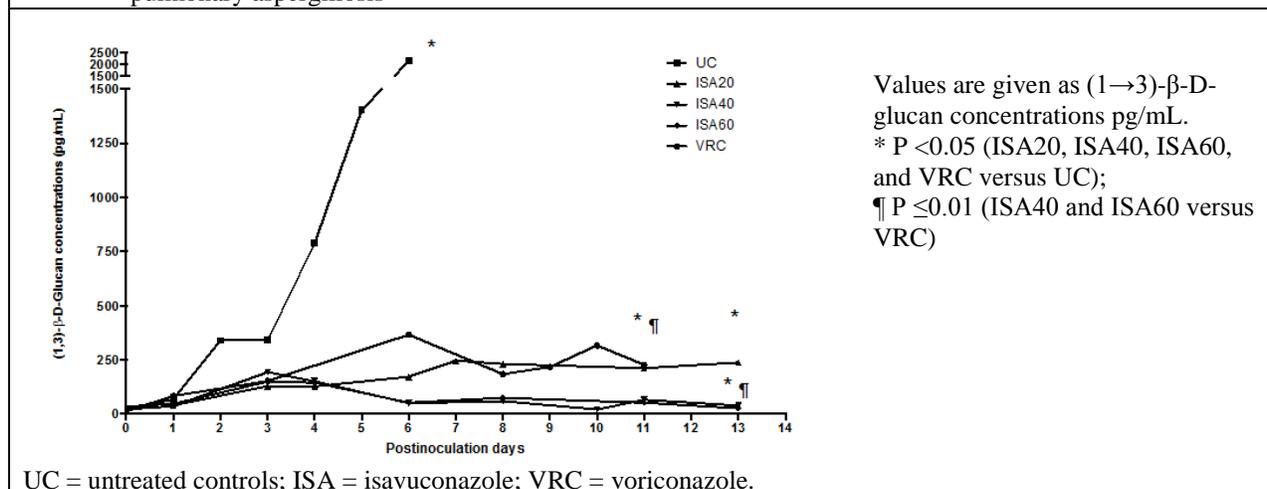


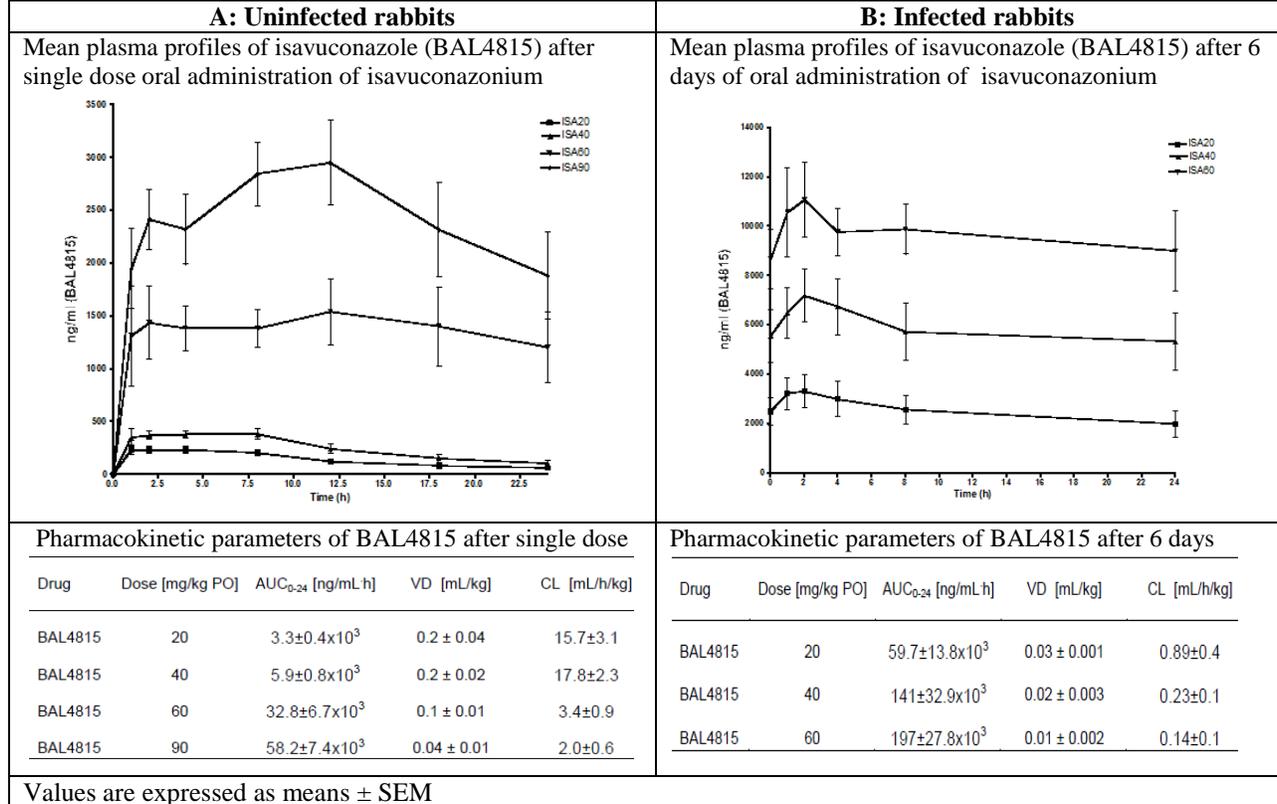
Figure 31: Concentrations of plasma (1-3)- β -D-glucan in treated and untreated neutropenic rabbits with primary pulmonary aspergillosis



After single oral doses, AUC_{0-24} values in uninfected animals from the isavuconazole 20, 40, 60, or 90 mg/kg treated groups were $3.3 \pm 0.4 \times 10^3$, $5.9 \pm 0.8 \times 10^3$, $32.8 \pm 6.7 \times 10^3$ and $58.2 \pm 7.4 \times 10^3$ ng.h/mL, respectively, and for clearance were 15.7 ± 3.1 , 17.8 ± 2.3 , 3.4 ± 0.9 and $2.0 \pm 0.6 \times 10^3$ mL/h/kg, respectively, consistent with non-linear saturation kinetics. The volume of distribution was relatively constant at 0.1-0.2 L across dosage groups (Figure 32A).

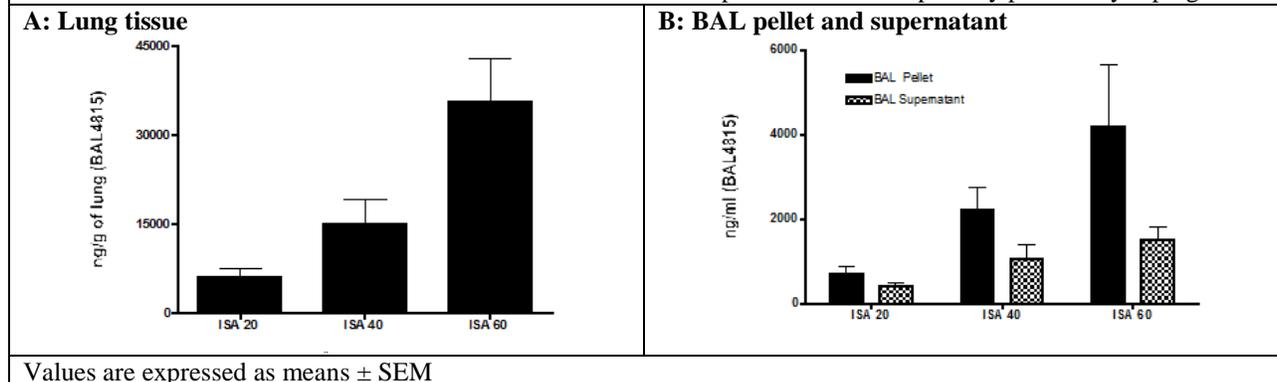
Following six days of oral administration in infected animals, AUC_{0-24} values were $59.7 \pm 13.8 \times 10^3$, $141.4 \pm 32.9 \times 10^3$ and $197.4 \pm 27.8 \times 10^3$ ng.h/mL for isavuconazole 20, 40, and 60 mg/kg treatment groups, respectively. Clearance values were 0.89 ± 0.4 , 0.23 ± 0.1 and 0.14 ± 0.1 mL/h/kg, for isavuconazole 20, 40, and 60 mg/kg treatment groups, respectively (Figure 32B).

Figure 32: Mean plasma profiles of isavuconazole (BAL4815) in (A) uninfected rabbits after single dose, and (B) infected rabbits after 6 days of treatment



There was a non-linear relationship between isavuconazole concentrations in the lung tissue and dose, with concentrations being disproportionately higher in the isavuconazole 60 mg/kg versus the 40 mg/kg treatment group (Figure 33A). To test the hypothesis that isavuconazole may accumulate in pulmonary alveolar macrophages (PAMs), the BAL fluid was centrifuged and isavuconazole concentrations measured in the pellet containing PAMs and supernatant. For each group of isavuconazole-treated animals, the pellet contained higher isavuconazole concentrations than the supernatant (Figure 33B) suggesting that drug may accumulate in alveolar macrophages.

Figure 33: Concentrations of isavuconazole (BAL4815) in lung tissue (A) and BAL fluid pellet and supernatant (B) after oral administration of isavuconazonium in neutropenic rabbits with primary pulmonary aspergillosis



Comments:

The study showed that rabbits treated with doses equivalent to 40 or 60 mg/kg/day isavuconazole which corresponds to exposure levels of 141.4×10^3 and 197.4×10^3 ng.h/mL, respectively, had prolonged survival, lower pulmonary fungal burdens and reduced lung injury compared with untreated controls.

3.5.2. Mucormycosis model

The activity of isavuconazium sulfate was measured in different murine models of mucormycosis (pulmonary or hematogenously disseminated infection models in neutropenic, diabetic ketoacidotic mice) infected with *R. oryzae*.

3.5.2.1. Pulmonary infection in neutropenic and diabetic ketoacidotic mice

Ibrahim (2013)³⁸ reported the *in vivo* activity of isavuconazole in an experimental pulmonary infection neutropenic and diabetic ketoacidotic (DKA) mouse (ICR mice) models of mucormycosis. Briefly, for the neutropenic model, neutropenia was induced by administering cyclophosphamide (200 mg/kg, intraperitoneal) and cortisone acetate (500 mg/kg, subcutaneous) on Days -2 and +3 relative to infection to facilitate the establishment of the pulmonary fungal infection. For the DKA model, mice were made diabetic by streptozotocin injection (210 mg/kg, intraperitoneal). Cortisone acetate (250 mg/kg, subcutaneous on Days -2 and +3 relative to infection) was administered to facilitate the establishment of the pulmonary fungal infection.

Eight isolates of *Rhizopus oryzae* were tested to determine *in vitro* activity of isavuconazole. Of the eight *R. oryzae* isolates tested, four were Type I lactic acid producers (99-892, and NRRL 13142, 13440 and 21251), and the other four were Type II fumaric acid producers (99-880, and NRRL 21447, 21446 and 21477). MICs and MFCs were measured after 24 or 48 hours of incubation by the CLSI M38-A2 method.⁵ The MICs and MFCs ranged from 0.125–1 µg/mL after 48 hours (Table 41); isolate 99-880 (actual inhaled inoculum was 4.1×10^3 spores) was used for infection of neutropenic and DKA mice by the intra-tracheal route.

³⁸ Ibrahim AS (2013) Isavuconazole therapy for treatment of murine mucormycosis. Internal report (9766-PH-0206).

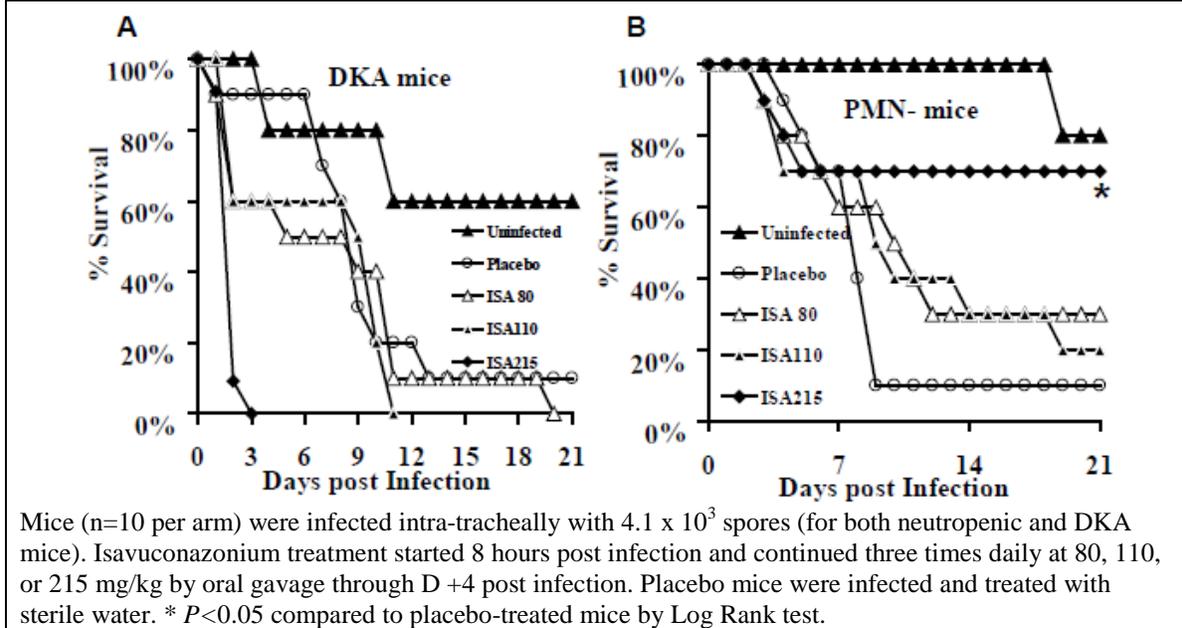
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Table 41: *In vitro* susceptibility of *R. oryzae* clinical isolate to isavuconazole (BAL4815)

	MICs mg/L		MFC mg/L	
	24 h	48 h	24 h	48 h
<i>R. oryzae</i> Type I lactic acid producers				
<i>R. oryzae</i> 99-892 (lung isolate from UTHSCSA)	0.125	0.125	0.125	0.125
<i>R. oryzae</i> NRRL 13142 (sphenoid isolate from LA County Hospital)	0.125	0.250	0.125	0.250
<i>R. oryzae</i> NRRL 13440 (tracheal isolate from Duke University)	0.125	0.250	0.125	0.250
<i>R. oryzae</i> NRRL 21251 (maxillary sinus isolate from Dept of Health NY)	0.125	0.250	0.125	0.250
<i>R. oryzae</i> Type II fumaric acid producers				
<i>R. oryzae</i> 99-880 (brain isolate from UTHSCSA)	0.250	0.50	0.250	0.50
<i>R. oryzae</i> NRRL 21447 (brain and ear isolate from McGill University)	0.250	1.0	0.250	1.0
<i>R. oryzae</i> NRRL 21446 (facial isolate NIH)	0.125	0.25	0.125	0.25
<i>R. oryzae</i> NRRL 21477 (facial isolate from NIH)	0.125	0.250	0.125	0.25

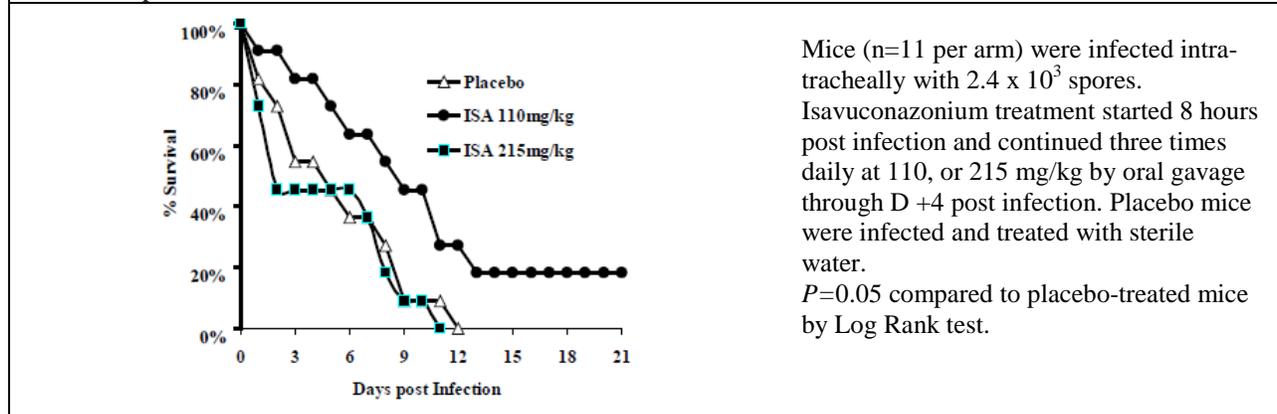
Mice were treated with isavuconazonium (80, 110, and 215 mg/kg, orally, three times daily corresponding to isavuconazole equivalent doses of 43, 59, and 116 mg/kg) starting eight hours post-infection for a total of five days. Sterile water was included as a vehicle control. There were 10 mice in each treatment group. Isavuconazole at the highest dose (215 mg/kg) tested was effective in improving survival of neutropenic infected mice (Figure 34B) but not of DKA infected mice (Figure 34A). No comparator was included for testing in this experiment.

Figure 34: Survival rates of uninfected, placebo-treated and isavuconazonium treated in murine models of mucormycosis pneumonia (A) DKA mice and (B) neutropenic mice



In another experiment, DKA mice (n=11 mice per group) were infected with lower inoculum concentration (actual inhaled inoculum was 2.4×10^3 spores). The results showed a trend towards increased survival after treatment with isavuconazonium at a dose of 110 mg/kg three times daily compared with placebo-group of mice (Figure 35). No comparator was included for testing in this experiment.

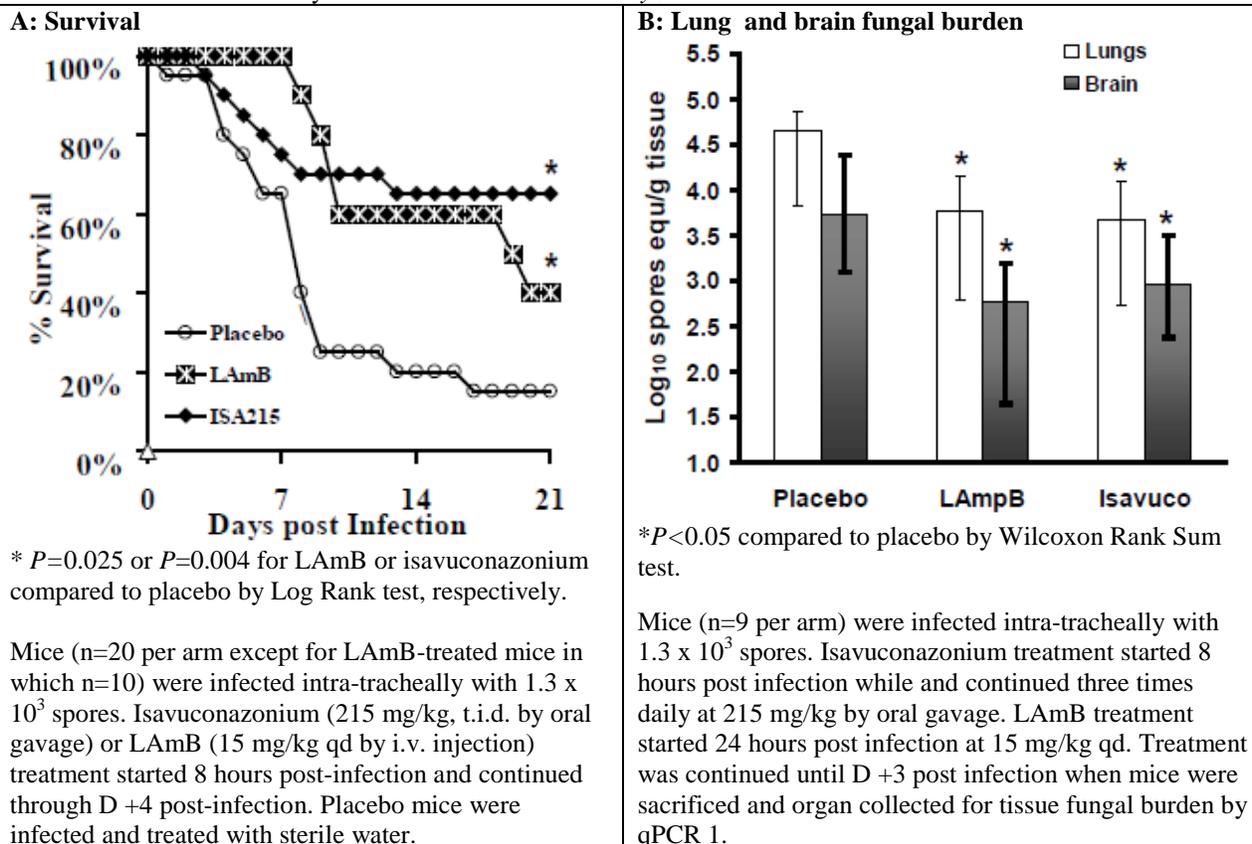
Figure 35: Survival rates of placebo- versus isavuconazonium-treated DKA mice in a model of mucormycosis pneumonia



In another experiment in neutropenic mice, the activity of isavuconazonium (215 mg/kg, orally, three times daily) was compared with high dose liposomal amphotericin B (15 mg/kg, once daily by the intravenous route; considered to be the standard therapy in this model). Experimental design was same as summarized above except that the inhaled inoculum concentration was 1.3×10^4 . It appears that for the fungal burden study, liposomal amphotericin B treatment was initiated 24 hours post-infection. The results showed isavuconazonium and liposomal amphotericin B were effective in improving survival (Figure 36A) and reducing fungal burden

by about one log in the lungs and brain (Figure 36B) of infected mice; reduction in fungal burden appears to be more in brain than lungs under the experimental conditions tested.

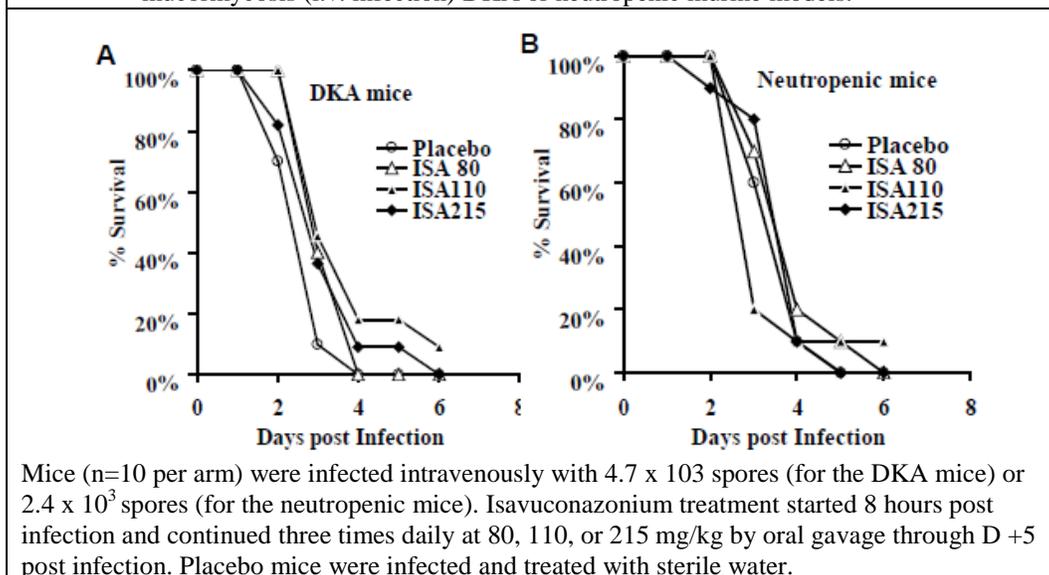
Figure 36: Effect of treatment with placebo, isavuconazonium (ISA) or LAmB on the protection of neutropenic mice from mucormycosis after intra-tracheal *R. oryzae* infection



3.5.2.2. Hematogenously disseminated infection in neutropenic and diabetic ketoacidotic mice

Ibrahim (2013)³⁸ reported the activity of isavuconazonium in a hematogenously disseminated mucormycosis model in neutropenic and DKA mice, representing a more severe or advanced disease state. Such an advanced disease state is due to the direct inoculation of *R. oryzae* into the bloodstream and is considered more difficult to treat due to the iron rich environment in the blood that supports *Mucorales* growth. Experimental design was same as summarized above except that mice were infected with *R. oryzae* (strain 99-880 same as above) spores intravenously through the tail vein instead by the intra-tracheal route. Isavuconazonium (80, 110, or 215 mg/kg, p.o. t.i.d.) was not effective in improving survival compared to placebo treated controls (Figure 37).

Figure 37: Activity of isavuconazonium measured by survival in hematogenously disseminated mucormycosis (i.v. infection) DKA or neutropenic murine models.



Comments:

*Overall, the study suggests that isavuconazole was effective in improving survival and reducing fungal burden in lungs and brain in DKA and neutropenic mice infected intra-tracheally. Isavuconazole was as effective as high dose liposomal amphotericin B in protecting neutropenic mice from *R. oryzae* infection.*

*Isavuconazonium treatment was not effective in the hematogenously disseminated model. The effectiveness may vary with the severity of infection and immune status of the host. The applicant stated that since patients are usually infected with *Mucorales* by inhalation, the clinical relevance of the hematogenously disseminated model is in question.*

3.6. Drug Resistance and Cross Resistance

3.6.1. Drug Resistance

3.6.1.1. Potential for development of drug resistance

Jiménez-Ortigosa and Perlin, 2014³⁹ reported a potential for development of resistance *in vitro* by incubating conidia (10^3 /mL) of *A. fumigatus* strains (ATCC 13073 and a clinical isolate R21; isavuconazole MIC was 0.25 µg/mL for both strains by the CLSI M38-A2 method⁵) on potato dextrose agar (PDA) plates containing 0.12 to 0.5 µg/mL of isavuconazole at 37°C for 5 days. Mutants (n=135; 81 for ATCC13073 and 54 for R21 strains) were screened for growth on PDA plates containing increasing concentrations of isavuconazole (0.5 - 16 µg/ml). Isavuconazole MIC values were 16-64 times higher than the negative controls for 13 mutants of the 2 WT strains (ATCC13073 and R21) after 48 hours of growth at 37°C (Table 42). All the 13

³⁹Jiménez-Ortigosa C, and Perlin DS. (2014) Assess the spectrum and frequency of drug resistance in *Aspergillus fumigatus* associated target (Cyp51A) following exposure to isavuconazole. Internal interim (9766-PH-0123).

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isavuconazole-resistant mutants were plated onto PDA plates containing 32 µg/mL of isavuconazole. Three (A3, R3* and R8*) of the 13 mutants grew on these plates; the isavuconazole MICs for these three isavuconazole-resistant mutants was ≥16 µg/mL.

Table 42: MIC distributions of isavuconazole, itraconazole, voriconazole and amphotericin B for the *A. fumigatus* mutants

Strain	Background	IR	MIC (mg/L)			
			ISA	ITR	VRC	AMB
ATCC13073	WT	no	0.25	0.06	0.125	1
A1	ATCC13073	yes	16	>16	8	1
A2	ATCC13073	yes	4	2	2	1
A3	ATCC13073	yes	16	>16	8	2
A4	ATCC13073	yes	4	2	2	1
A15	ATCC13073	yes	4	2	8	1
A37	ATCC13073	yes	8	2	4	1
A3 (32)	ATCC13073	yes	>16	>16	>16	2
R21	WT	no	0.25	0.06	0.125	1
RC2	R21	yes	4	4	4	1
RC4	R21	yes	4	2	4	1
R1	R21	yes	4	2	4	1
R1*	R21	yes	4	>16	4	1
R3*	R21	yes	4	>16	8	1
R8*	R21	yes	16	>16	16	1
R24*	R21	yes	8	2	8	1
R3* (32)	R22	yes	16	>16	8	2
R8* (32)	R23	yes	>16	>16	16	2

In another experiment, 10^5 conidia of both WT strains (ATCC13073 and R21) were inoculated onto PDA plates containing 5X MIC of isavuconazole (1.25 µg/mL), itraconazole (0.3125 µg/mL), or voriconazole (0.625 µg/mL) and incubated at 37°C. After 5 days of incubation, there were no or very few colonies of *A. fumigatus* on the isavuconazole plates compared to those of voriconazole or itraconazole (Figure 38A). Overall, the study suggested a low potential for development of resistance to isavuconazole compared to other azoles.

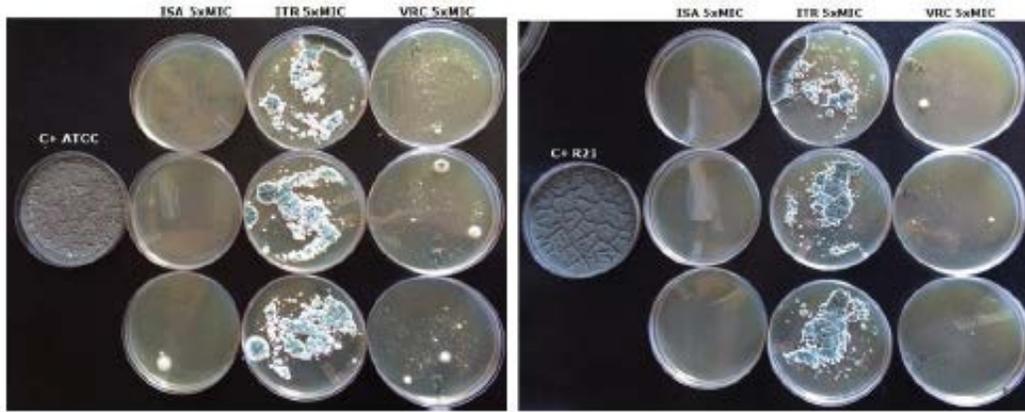
In another experiment, 10^8 conidia of both WT strains were inoculated onto PDA plates containing lower concentration of (0.5 µg/mL) of isavuconazole and incubated at 37°C. After 5 days of incubation, spores were harvested and then 10^5 conidia were inoculated onto PDA plates containing 5 or 10 times the MIC for isavuconazole, itraconazole, or voriconazole. After 5 days of growth at 37°C, colonies of *A. fumigatus* were detected on the plates with 10 times the MIC for the three azoles (Figure 38B). The results suggest that following a pre-exposure to low concentration of isavuconazole, the relative frequency of resistant isolates increased compared to 5X MIC of isavuconazole. The results also suggest cross-resistance between isavuconazole and other azoles.

In another experiment, spores of the WT strains were exposed to 0.12 µg/ml of itraconazole or 0.25 µg/ml of voriconazole and then inoculated onto PDA plates containing 2.5 µg/ml of isavuconazole (10 times the MIC for isavuconazole); colonies of *A. fumigatus* were detected on the plates (Figure 38C) thereby suggesting cross-resistance among azoles.

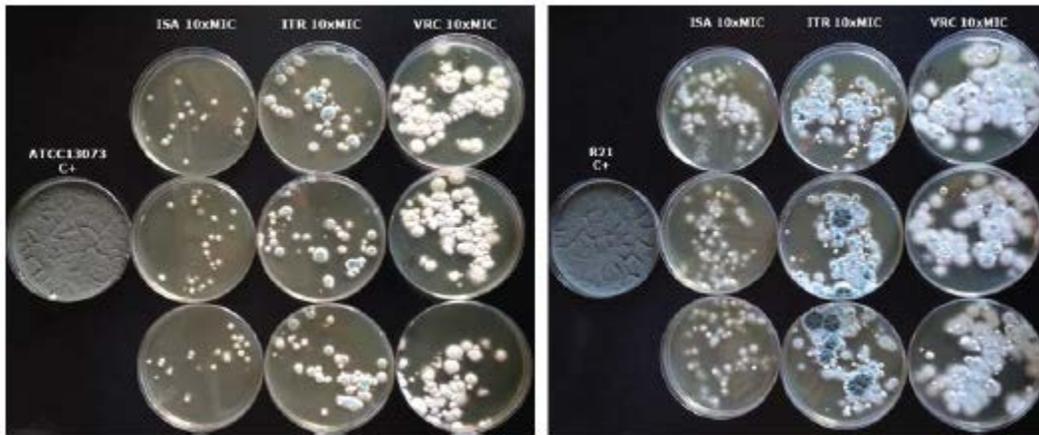
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Figure 38: Growth of ATCC13073 and R21 wild-type strains and mutants on PDA plates containing 5 or 10 times the MIC for isavuconazole, itraconazole, or voriconazole.

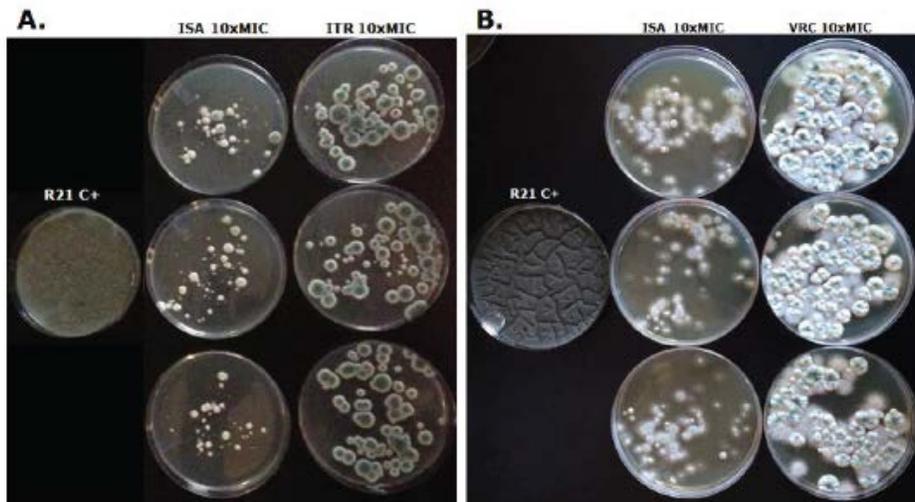
A: Growth of ATCC13073 and R21 wild-type strains on PDA plates containing 5 times the MIC for ISA (1.25 µg/ml), ITR (0.3125 µg/ml) and VRC (0.625 µg/ml).



B: Growth of isavuconazole resistant ATCC13073 and R21 spores on PDA plates containing 10 times the MIC for ISA (2.5 µg/ml), ITR (0.625 µg/ml) and VRC (1.25 µg/ml).



C: Growth of itraconazole resistant spores (A) or voriconazole resistant spores (B) of R21 onto PDA plates containing 10 times the MIC for ISA (2.5 µg/ml), ITR (0.625 µg/ml) and VRC (1.25 µg/ml).



Isavuconazole (ISA), itraconazole (ITR), or voriconazole (VRC)

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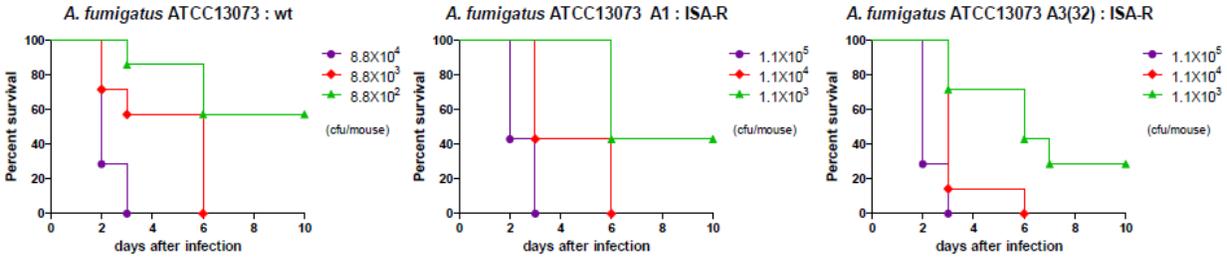
Matsumoto, 2014⁴⁰ evaluated the virulence i.e., fitness cost *in vivo*, of the two WT and some of the isavuconazole induced mutants in immunosuppressed mice as acquired resistance is often associated with a reduction of virulence. Briefly, mice were immunosuppressed by hydrocortisone treatment and intravenously infected with conidia of the WT *A. fumigatus* ATCC13703 or R21 as parent strains and two selected strains of isavuconazole-resistant mutants induced from each parent strain [isavuconazole-resistant A1 and A3(32) and R3*(32) and R8*(32) strains isolated from WT *A. fumigatus* ATCC13703 and R21, respectively]. Mice, infected with different inoculum concentrations ($8.8 \times 10^3 \sim 5.1 \times 10^4$ cfu/mouse) of WT or isavuconazole-resistant [A1, A3(32)] strains of *A. fumigatus* ATCC13073, died within 10 days of infection; the mortality rate was 90-100% (Figure 39A). Mice infected with $6.7 \times 10^3 \sim 3.2 \times 10^4$ cfu/mouse WT or isavuconazole-resistant [R3*(32), R8*(32)] strains of *A. fumigatus* R21, died with 86-100% mortality within 10 days post-infection (Figure 39B). The results suggest the susceptibility to infection was similar between the WT and isavuconazole induced mutants for the ATCC13703 and the R2 strains of *A. fumigatus*. The selection of clonal strain with phenotypic resistance, but with yet unknown mechanism, may not increase fitness cost *in vivo*.

⁴⁰ Matsumoto, S. (2014) Virulence study of isavuconazole-resistant *Aspergillus fumigatus* in mouse disseminated aspergillosis. Internal report (9766-PH-0123-A).

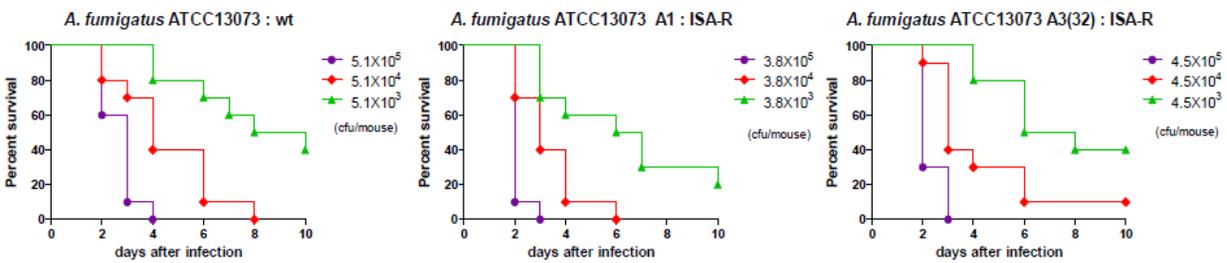
Figure 39: Survival curves after intravenous infection of immunosuppressed mice with *A. fumigatus* wild-type and resistant mutants of ATCC13073 and R2 strains

A: ATCC13073 WT and isavuconazole-resistant strains

Study 1:



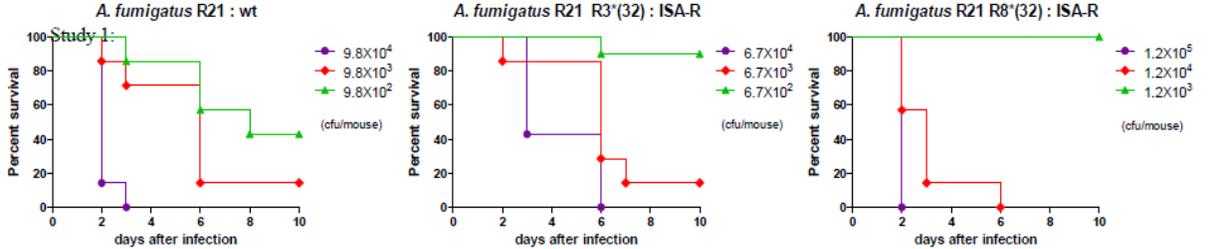
Study 2:



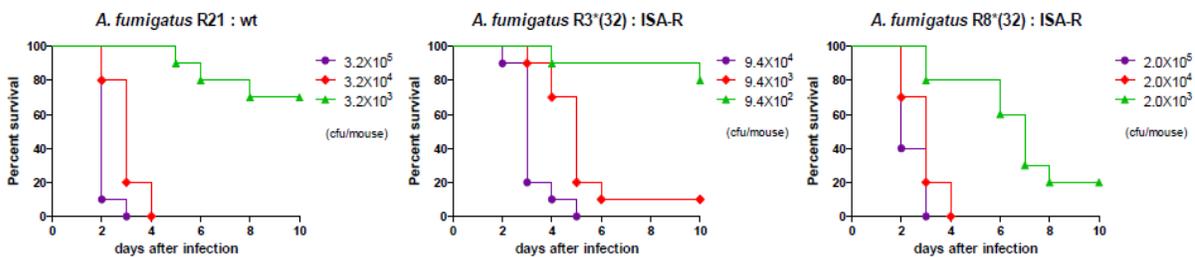
Mice were infected with $8.8 \times 10^3 \sim 5.1 \times 10^4$ cfu/mouse of WT or isavuconazole-resistant [A1, A3(32)] strains of *A. fumigatus* ATCC13073

B: R2 WT and isavuconazole resistant strains

Study 1:



Study 2:



Mice were infected with $6.7 \times 10^3 \sim 3.2 \times 10^4$ cfu/mouse WT or isavuconazole-resistant (R3*(32), R8*(32)) strains of *A. fumigatus* R21, N=7 in Study 1 and N=10 in Study 2

3.6.1.2. Mechanisms of drug resistance

- **Mutants induced *in vitro***

Jiménez-Ortigosa and Perlin (2014)³⁹ compared the sequence of the 2 WT strains and 16 mutants (induced *in vitro*; for details see section 3.6.1.1 above) for the *cyp51A* or *cyp51B* genes encoding the 14-alpha sterol demethylase enzyme and the multi-drug resistant 1-4 (*MDR 1-4*) genes (Table 43); these genes are known to belong to two major families of transporters ABC and MFS superfamilies and reflect an expression of efflux pumps. The results showed no point mutations in any of the 16 mutants after sequencing for the *cyp51A* or *cyp51B* genes. The authors stated that all isavuconazole resistant mutants showed none or a slight variation in the expression profile of the genes analyzed (*MDR1-4*). Moreover, if there were any variations, the genes were down-regulated instead of up-regulated.

Table 43: Oligonucleotides used for real time qPCR.

GENE		5'-3' Seq	DESCRIPTION
<i>A/MDR1</i>	F primer	(b) (4)	Multidrug transporter of ATP-binding cassette (ABC) superfamily
	R primer		
<i>A/MDR2</i>	F primer		Multidrug transporter of ATP-binding cassette (ABC) superfamily
	R primer		
<i>A/MDR3</i>	F primer		Plasma membrane multidrug efflux pump; MDR major facilitator transporter superfamily
	R primer		
<i>A/MDR4</i>	F primer		Multidrug transporter of ATP-binding cassette (ABC) superfamily
	R primer		

- **Isolates from Phase 3 clinical trial 9766-CL-0104 and 9766-CL-0103**

Ghannoum (2014)⁴¹ reported the alteration in sterol composition and efflux pump [(MDR1, MDR2, MDR3, and C-14 alpha sterol demethylase (*cyp51*)] in 18 clinical isolates collected from patients enrolled in clinical trials (Studies 9766-CL-0104 and 9766-CL-0103). Of the 18 isolates, 15 isolates [*A. fumigatus* (n=2) *A. niger* (n=3), *R. oryzae* (n=5), *R. pusillus* (n=1), *Mucor circinelloides* (n=1), *Fusarium subglutinans* (n=1), *Fusarium solani* (n=1), and *Fusarium oxysporum* (n=1)] had isavuconazole MICs ≥ 8 $\mu\text{g/mL}$ by the CLSI method and 3 isolates (1 isolate each of *A. fumigatus*, *A. niger*, and *R. oryzae*) had isavuconazole MICs between 0.25 and 1 $\mu\text{g/mL}$ (Table 44). In general, isolates with elevated MIC values to isavuconazole also had elevated MIC values to other azoles (Table 44). All isolates, except one, were collected at baseline (defined as up to Day 7 post-treatment); one isolate (#3018266-775-366-1) of *A. fumigatus* from isavuconazole treated patient (004320444) was obtained on Day 20.

⁴¹ Ghannoum MA (2014) Determine the mechanism of resistance of fungal isolates obtained from Phase 3 clinical trials of isavuconazole (003 and 004). Internal interim (9766-PH-0127).

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Table 44: List of Selected Fungal Isolates (MIC ≥ 8mg/L) Tested for Resistant Mechanisms from Studies 9766-CL-0104 and 9766-CL-0103, Patient Identification, MIC Values and Major Results

Organism†	Laboratory Accession Number	Isolate ID	Patient ID	Treatment Group	ISA MIC(mg/L) (100% inhibition)	VOR MIC(mg/L) (100% inhibition)	POS MIC(mg/L) (100% inhibition)	Role of Sterol Composition	Efflux Pumps – Functional Analysis	Efflux Pumps – Transcriptional Analysis
<i>Rhizopus oryzae</i> ‡	27710	3126629-774-115-1	003015001	ISA	1	8	1	↑ergosterol		NC
<i>Rhizopus oryzae</i>	19447	2162363-503-115-4	003970401	ISA	>16	>16	>16	↑squalene; †calciferol	No difference	NC
<i>Rhizopus oryzae</i>	19448	2162323-503-115-1	003490401	ISA	16	8	>16	↑squalene; †zymosterol	No difference	NC
<i>Rhizopus oryzae</i>	17825	1759822-405-366-1	004550701	VOR	>16	>32	1	↑squalene; †calciferol	No difference	NC
<i>Rhizopus oryzae</i>	19446	2176381-503-101-1	003491001	ISA	>16	>16	>16	↑squalene; †obtusifolial	No difference	NC
<i>Rhizopus oryzae</i>	28399	3188972	003011803	ISA	>16	>16	2	↑squalene; †calciferol; †4, 14-dimethylzymosterol	No difference	NC
<i>Mucor circinelloides</i>	19445	2162560-503-115-1	003970301	ISA	>16	>8	>16	↑squalene; †calciferol; †obtusifolial	No difference	NC
<i>Rhizomucor pusillus</i>	28404	3184895	003070201	ISA	8	>32	1	†calciferol	No difference	NC
<i>Fusarium subglutinans</i> ‡	17828	1905626-405-366-1	004011804	ISA	>16	4	1	†obtusifolial; †calciferol		NC
<i>Fusarium solani</i>	18749	1686168-405-367-1	004970402	VOR	>16	4	16	↑squalene; †obtusifolial	↑efflux activity	NC
<i>Fusarium oxysporum</i>	27718	3070323-775-366-2	004970411	ISA	>16	4	8	↑squalene; †obtusifolial	↑efflux activity	NC
<i>Aspergillus fumigatus</i> ‡ *	27722	3018266-775-366-1	004320444	ISA	0.25	2	0.12	squalene; calciferol; zymosterol; ergosterol; obtusifolial are present		NA
<i>Aspergillus fumigatus</i>	20438	1697778-405-366-1	004970911	ISA	8	1	0.25	↑squalene; †ergosterol; †zymosterol; †obtusifolial; †lanosterol	↑efflux activity	↑expression of <i>MDR2</i> ; no differences in <i>MDR1</i> , <i>MDR3</i> or <i>CYP51</i>
<i>Aspergillus fumigatus</i>	28500	3018268	004320455	ISA	>16	>16	2	‡zymosterol; †lanosterol	↑efflux activity	
<i>Aspergillus niger</i> ‡	28402	3184806	003970304	ISA	0.25	≤ 0.03	≤ 0.03	squalene; calciferol; ergosterol are present		NC
<i>Aspergillus niger</i>	27709	3078778-774-366-2	003320402	ISA	8	8	>16	↑squalene; †calciferol; †ergosterol	↑efflux activity	NC
<i>Aspergillus niger</i>	27713	3084252-774-366-1	003550301	ISA	8	4	1	↑squalene; †calciferol	↑efflux activity	NC
<i>Aspergillus niger</i>	27715	3019361-774-328-1	003970406	ISA	8	4	1	↑squalene; †calciferol	↑efflux activity	NC

ISA: isavuconazole; MIC: minimum inhibitory concentration; NA: not available; NC: transcriptional analysis not conducted due to gene-specific probes unavailable; POS: posaconazole; VOR: voriconazole.

MIC values obtained using CLSI methodology.

†All isolates obtained at baseline (up to day 7) except isolate ID 3018266-775-366-1 (patient 004320444; obtained on day 20).

‡ Bolded isolates represent the 3 wild-type isolates with ISA MIC < 8 mg/L except for *Fusarium subglutinans*

Source: [Study Report 9766-PH-0127, 9766-CL -0103 and 9766-CL-0104]

*Post-treatment isolate collected on Day 20 of isavuconazole treatment

Sterols were extracted from cells and analyzed using gas liquid chromatography. The results showed higher ergosterol levels in strains with isavuconazole MICs < 1 µg/mL compared to the isolates with increased isavuconazole MIC (≥ 8 µg/mL); squalene, calciferol, and/or zymosterol intermediates in the synthesis of ergosterol, were increased in isolates with MICs ≥ 8 µg/mL compared to those with lower isavuconazole MICs of ≤ 1 µg/mL (Tables 44 and 45).

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Table 45: Sterol profile of *Rhizopus* isolates sensitive to (≤ 1 $\mu\text{g/mL}$) or with increased MIC (≥ 8 $\mu\text{g/mL}$) to isavuconazole

<i>Rhizopus</i> isolates								
Sterol	Sterol Level (%)							
	<i>Rhizopus</i>							<i>Mucor spp.</i>
	27710 (S)	19447 (R)	19448 (R)	17825 (R)	19446 (R)	28399 (R)	19445 (R)	28404 (R)
Squalene	9.95	44.42	17.06	65.27	49.22	22.86	39.12	14.49
Calciferol	10.67	55.58	11.94	31.84	6.41	53.74	26.68	76.50
Zymosterol	0.00	0.00	68.70	2.88	0.00	0.00	0.00	0.00
Ergosterol	76.93	0.00	2.30	0.00	0.00	0.00	2.60	0.00
4,14-dimethyl zymosterol	2.46	0.00	0.00	0.00	0.00	23.40	0.00	0.00
Obtusifolial	0.00	0.00	0.00	0.00	44.37	0.00	31.60	9.01
Lanosterol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

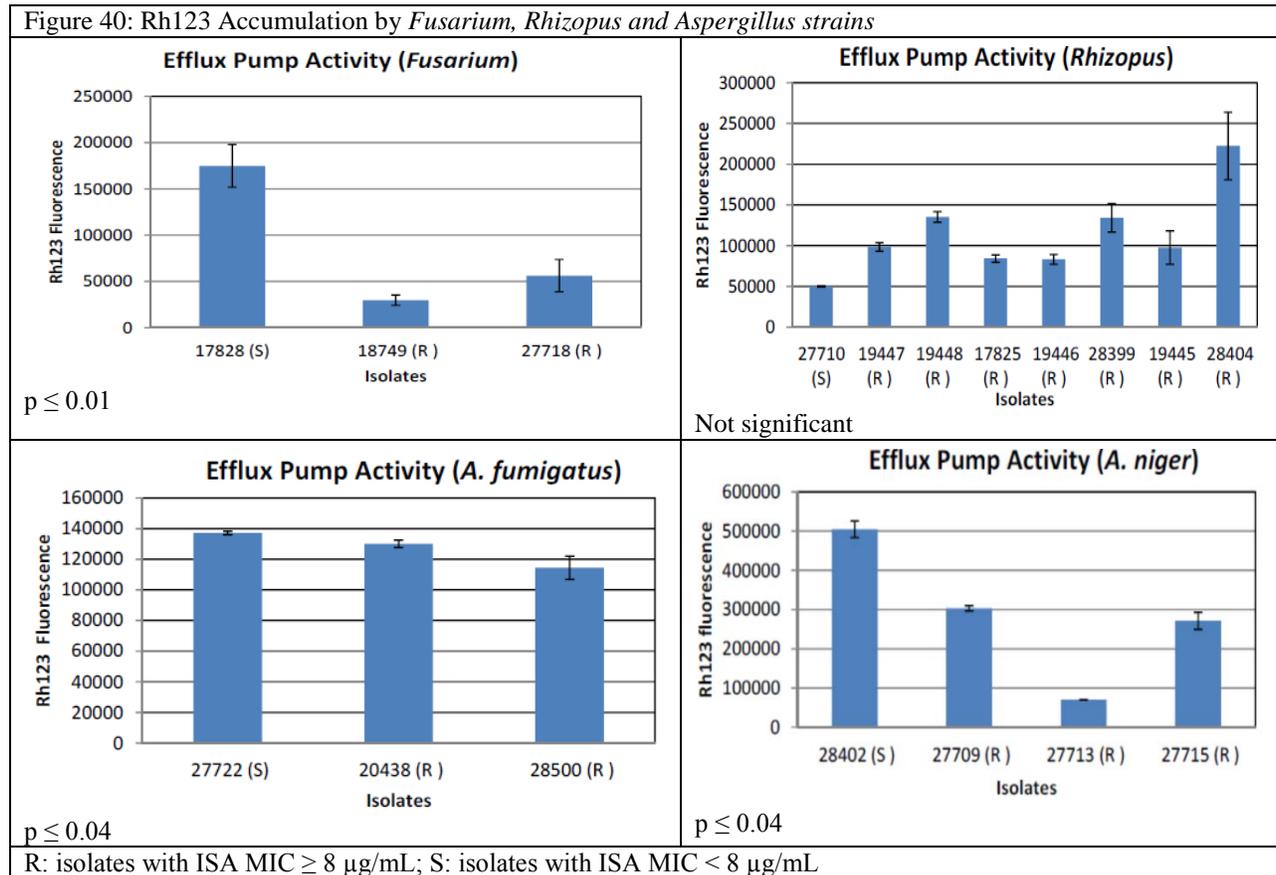
<i>Fusarium</i> isolates			
Sterol	Sterol Level (%)		
	<i>Fusarium</i>		
	17828 (S)	18749 (R)	27718 (R)
Squalene	28.62	58.04	55.92
Calciferol	22.77	26.22	20.71
Zymosterol	0.00	0	5.57
Ergosterol	0.71	0	17.80
4,14-dimethylzymosterol obtusifolial	0.00	0	0.00
Obtusifolial	47.90	15.74	0.00
Lanosterol	0	0	0.00

<i>A. fumigatus</i> isolates			
Sterol	Sterol Level (%)		
	<i>A. fumigatus</i>		
	27722 (S)	20438 (R)	28500 (R)
Squalene	21.99	81.31	27.84
Calciferol	28.73	10.18	37.33
Zymosterol	18.21	0.00	0.79
Ergosterol	12.64	4.66	15.69
4,14-dimethylzymosterol obtusifolial	0.15	0.00	0.52
Obtusifolial	10.80	3.85	13.15
Lanosterol	7.48	0.00	4.68

<i>A. niger</i> isolates				
Sterol	Sterol Level (%)			
	<i>A. niger</i>			
	28402 (S)	27709 (R)	27713 (R)	27715 (R)
Squalene	26.89	78.23	60.38	60.06
Calciferol	59.59	13.83	31	31.86
Zymosterol	0.00	0	0	0
Ergosterol	9.59	2.66	8.15	8.08
4,14-dimethylzymosterol obtusifolial	0.00	0	0	0
Obtusifolial	3.93	8.58	0	0
Lanosterol	0.00	1.18	0	0

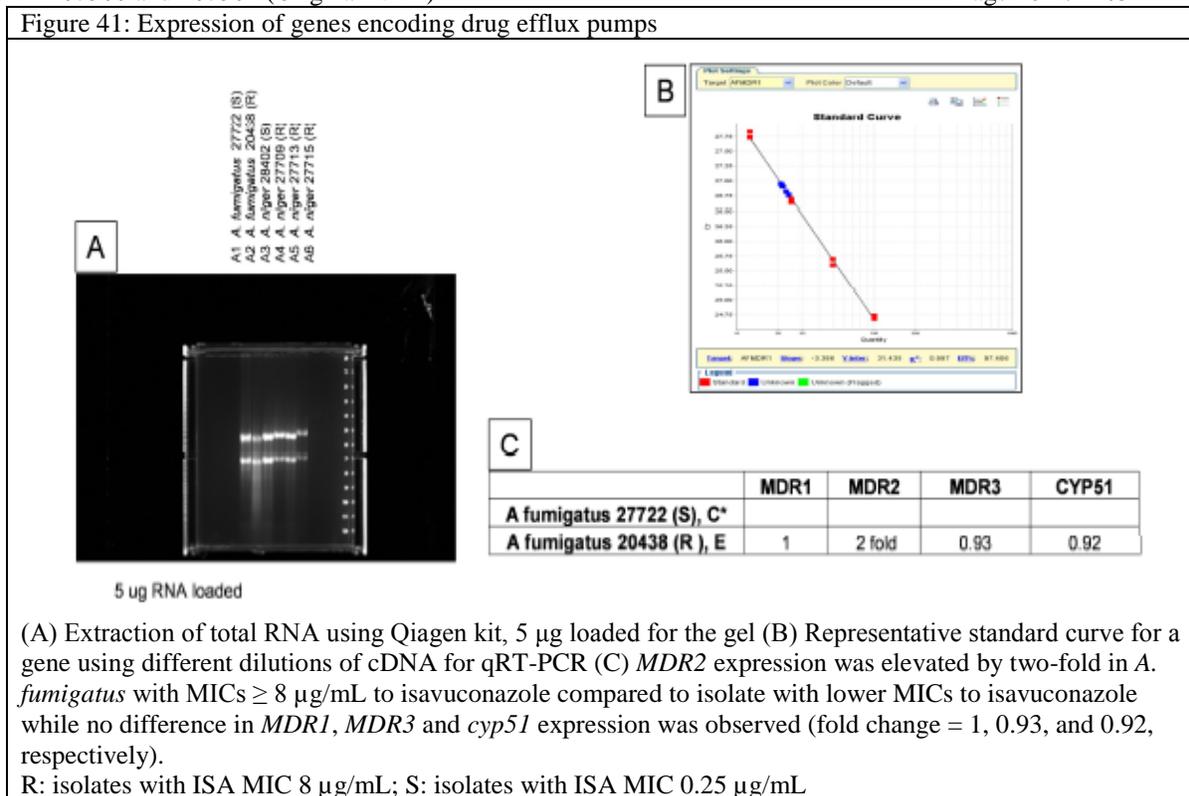
ISA: isavuconazole; MIC: minimum inhibitory concentration
R: isolates with ISA MIC ≥ 8 $\mu\text{g/mL}$; S: isolates with ISA MIC < 8 $\mu\text{g/mL}$

Functional activity of efflux pumps was assayed by uptake of a fluorescent substrate, Rhodamine 123, (Rh123) using a spectrofluorimeter; drug-resistant microbial cells express elevated pump activity, leading to lower retention of the antifungal and Rh123 whereas, susceptible strains do not have robust efflux pump activity leading to accumulation of elevated levels of Rh123 and anti-fungal drug. The results showed the retention of Rh123 was significantly higher in *Fusarium* isolates and *Aspergillus* isolates (*A. fumigatus* and *A. niger*) with lower ($\leq 1 \mu\text{g/mL}$) isavuconazole MICs compared to the isolates with increased isavuconazole MICs ($\geq 8 \mu\text{g/mL}$) (Figure 40). In contrast there was no significant difference in Rh123 levels for isolates of *Rhizopus* with lower ($\leq 1 \mu\text{g/mL}$) or higher ($\geq 8 \mu\text{g/mL}$) isavuconazole MICs (Figure 40). Overall, the results suggest that efflux pumps contributed to the higher MICs against isavuconazole in *Fusarium* and *Aspergillus* isolates but not for the *Rhizopus* species tested.



For efflux pumps, total RNA was isolated from all isolates and reverse transcribed to obtain cDNA, and analyzed using gene-specific primer/probes for *A. fumigatus* efflux pumps (*MDR1*, *MDR2*, *MDR3*, and *cyp51*) by quantitative reverse transcription polymerase chain reaction (RT-PCR). The results showed no difference in the expression of *MDR1*, *MDR3* or *cyp51* for *A. fumigatus* strain with isavuconazole MIC $0.25 \mu\text{g/mL}$ (Table 44). The expression of *MDR2* was elevated two-fold in *A. fumigatus* isolate with increased isavuconazole MIC ($\geq 8 \mu\text{g/mL}$) compared to the isolate with isavuconazole MIC of $0.25 \mu\text{g/mL}$ (Figure 41). The authors stated that since gene-specific primers/probes were not available for *Fusarium*, *Rhizopus* and *A. niger*, transcriptional analysis was not conducted.

Figure 41: Expression of genes encoding drug efflux pumps



Comments:

Overall, the study suggests a potential for development of resistance to isavuconazole exists. Increased MICs to isavuconazole and other azoles in *Rhizopus*, *Fusarium* and *Aspergillus* isolates are likely due to multiple mechanisms involving substitutions in the target gene *cyp51* and alterations in sterols and/or efflux pumps. Changes in sterol profile, and not efflux pump activity, were associated with increased MICs against *Rhizopus* isolates. However, for *Fusarium* and *Aspergillus* isolates, an increase in MIC was associated with elevated activity of efflux pumps. There does not appear to be any impact of the presence of resistance mechanisms on clinical response (for details see sections 5 and 6.1.4. below); however, these findings should be interpreted with caution due to a small number of isolates tested.

3.6.2. Cross Resistance

Jiménez-Ortigosa and Perlin, 2014³⁹ tested *in vitro* susceptibility of the two WT strains and 16 isavuconazole-resistant mutants (for details, see section 3.5.1.1 above) to itraconazole, voriconazole, and amphotericin B; the experimental design was same as summarized above. The results showed cross-resistance with other azoles (itraconazole and voriconazole); however, amphotericin B MICs were similar between the WT and mutant strains (Table 42).

The susceptibility to the three echinocandins (anidulafungin, caspofungin and micafungin) reported as minimum effective concentration (MEC) was determined for the isavuconazole resistant mutants obtained *in vitro*. The MEC values for any of the echinocandins (at 24 hour and 48 hour of growth) were similar against the isavuconazole resistant mutants and the WT strains (Table 46).

Table 46: MEC distributions for the echinocandins (ANF, CSF and MCF) for the isavuconazole resistant mutants isolated in the study.

Strain	Background	IR	MEC (mg/L)					
			ANF		CSF		MCF	
			24h	48h	24h	48h	24h	48h
ATCC13073	WT	no	0.06	0.06	0.06	0.06	0.03	0.03
A1	ATCC13073	yes	0.03	0.03	0.03	0.03	0.03	0.03
A2	ATCC13073	yes	0.12	0.12	0.12	0.06	0.03	0.03
A3	ATCC13073	yes	0.03	0.03	0.03	0.06	0.03	0.03
A4	ATCC13073	yes	0.12	0.12	0.06	0.06	0.03	0.03
A15	ATCC13073	yes	0.06	0.06	0.06	0.12	0.03	0.03
A37	ATCC13073	yes	0.03	0.03	0.12	0.06	0.03	0.03
A3 (32)	ATCC13073	yes	0.03	0.03	0.06	0.06	0.03	0.03
R21	WT	no	0.06	0.06	0.06	0.06	0.03	0.03
RC2	R21	yes	0.06	0.06	0.12	0.12	0.03	0.03
RC4	R21	yes	0.06	0.06	0.12	0.12	0.03	0.03
R1	R21	yes	0.06	0.06	0.12	0.12	0.03	0.03
R1*	R21	yes	0.06	0.06	0.06	0.06	0.03	0.03
R3*	R21	yes	0.12	0.12	0.12	0.12	0.03	0.03
R8*	R21	yes	0.03	0.03	0.03	0.06	0.03	0.03
R24*	R21	yes	0.12	0.06	0.12	0.12	0.03	0.03
R3* (32)	R22	yes	0.12	0.12	0.06	0.12	0.03	0.03
R8* (32)	R23	yes	0.06	0.06	0.06	0.06	0.03	0.03

IR=isavuconazole resistant; ANF=Anidulafungin; CSF=caspofungin; MCF=micafungin

Gregson *et al.* (2013)⁴² reported the activity of isavuconazole against 40 clinical isolates of *A. fumigatus* obtained from the (b) (4) a majority of the isolates were obtained from cases following azole exposure (patients had received 1 to 30 months of azole therapy) and 33 of the 40 isolates were known to have reduced *in vitro* susceptibility to either itraconazole, voriconazole, or posaconazole and a putative molecular mechanism of resistance defined (*cyp51A* mutations). All isolates were tested for *in vitro* antifungal susceptibility according to the CLSI M38-A2 methodology;⁵ however, the quality control strains *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019 were used for testing. The results showed isavuconazole MICs were higher against strains with reduced susceptibility to other triazoles; isavuconazole MICs differed depending on the *cyp51A* substitution (Table 47). Overall, isavuconazole MICs mirrored voriconazole MICs; isavuconazole and voriconazole MICs were lower against isolates with substitutions at position G54, whereas isolates with M220 alterations had more variable isavuconazole and voriconazole MICs.

There was no significant difference between the activity of amphotericin B against the *cyp51A*-mutated and WT strains.

⁴² Gregson L, Goodwin J, Johnson A, McEntree L, Moore CB, Richardson M, Hope WW, and Howard SJ. (2013) *In vitro* susceptibility of *Aspergillus fumigatus* to isavuconazole: correlation with itraconazole, voriconazole and posaconazole. *Antimicrob Agents Chemother* **57** (11):5778-5780.

Table 47: Minimum inhibitory concentrations performed by CLSI method

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Espinel-Ingroff *et al.* (2013)²⁵ reported cross-resistance between isavuconazole and other azoles against *Aspergillus* species *in vitro* (Table 48).

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Howard *et al.*, (2013)⁴³ reported the activity of isavuconazole against 30 *A. fumigatus* strains with *cyp51A* alterations at codons G54, TR34/L98H and M220 as well as 10 WT isolates; testing was done in 4 centers by the EUCAST method. There was variability in the MICs based on testing in four laboratories. The results showed that isavuconazole MICs against strains with mutations in the TR34/L98H codons were higher than the WT strains; isavuconazole MICs against strains with mutations in the G54 and M220 codons were lower than those with mutations in the TR34/L98H codons (Table 49). Other anti-fungal drugs were not included for testing.

⁴³ Howard SJ, Lass-Flörl C, Cuenca-Estrella M, Gomez-Lopez A, and Arendrup MC. (2013) Determination of isavuconazole susceptibility of *Aspergillus* and *Candida* species by the EUCAST method. *Antimicrob Agents Chemother.* **57** (11):5426-5431.

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Table 49: Isavuconazole MICs for *A. fumigatus* strains, with or without *cyp51A* gene alterations, tested in four laboratories

CYP51A alteration	Laboratory	No. of isolates	No. of isolates with indicated MIC (mg/L)							
			0.125	0.25	0.5	1	2	4	8	>8
None	1	10		4	4	2				
None	2	10		1	3	6				
None	3	10			5	5				
None	4	10			2	3	5			
None	Combined	40		5	14	16	5			
TR ₃₄ /L98H	1	10					2	6	2	
TR ₃₄ /L98H	2	10						8	2	
TR ₃₄ /L98H	3	10					2	5	3	
TR ₃₄ /L98H	4	10				4	3		3	
TR ₃₄ /L98H	Combined	40				4	7	11	16	2
G54E	1	5		4	1					
G54E	2	5		4	1					
G54E	3	5		1	1	3				
G54E	4	5			2	1	1	1		
G54E	Combined	20		9	5	4	1	1		
G54R	1	1		1						
G54R	2	1		1						
G54R	3	1			1					
G54R	4	1			1					
G54R	Combined	4		2	2					
G54V	1	2	1		1					
G54V	2	2		1		1				
G54V	3	2		1		1				
G54V	4	2				1			1	
G54V	Combined	8	1	2	1	3			1	
G54W	1	2		2						
G54W	2	2		2						
G54W	3	2		2						
G54W	4	2				1			1	
G54W	Combined	8		6		1			1	
M220I	1	2				1			1	
M220I	2	2					1			1
M220I	3	2				1	1			
M220I	4	2				2				
M220I	Combined	8				4	2		1	1
M220K	1	3		1	1	1				
M220K	2	3				2	1			
M220K	3	3			1	2				
M220K	4	3				3				
M220K	Combined	12		1	2	8	1			
M220T	1	2			1	1				
M220T	2	2				2				
M220T	3	2				2				
M220T	4	2							2	
M220T	Combined	8			1	5			2	
M220V	1	3				1	2			
M220V	2	3					1	2		
M220V	3	3				2	1			
M220V	4	3				1	1	1		
M220V	Combined	12				4	5	3		

The red line indicates the ECOFF proposed as described in [section 4.1.2.2](#)

Comments:

- Isavuconazole MICs were higher against strains of *A. fumigatus* with reduced susceptibility to other triazoles, and tended to mirror changes in voriconazole susceptibility; the increased MICs were against isolates with L98, TR₃₄/L98H, M220, or G138/Y431/G434/G448 mutations but not G54 mutation (affected by itraconazole and posaconazole). The clinical relevance of such findings is not known.

4. OVERVIEW OF CLINICAL PHARMACOLOGY

4.1. Studies in healthy subjects

Studies in healthy subjects showed the PK of oral CRESEMBA was dose proportional up to 600 mg per day (Table 50); maximum plasma concentration (C_{max}) was achieved in 2 to 3 hours after single and multiple dosing. No significant concentrations of the pro-drug or inactive cleavage product were observed in plasma after oral administration.

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Parameter Statistic	Isavuconazole 200 mg (n = 37)	Isavuconazole 600 mg (n = 32)
C_{max} (ng/mL)		
Mean	7499	20028
SD	1893.3	3584.3
CV %	25.2	17.9
t_{max} (h)		
Median	3.000	4.000
Range	2.0 – 4.0	2.0 – 4.0
AUC (h•ng/mL)		
Mean	121402	352805
SD	35768.8	72018.5
CV %	29.5	20.4

Following **IV** administration of CRESEMBA, maximal plasma concentrations of the pro-drug and inactive cleavage product were detectable during infusion and declined rapidly following the end of administration. The pro-drug was below the level of detection by 1.25 hours after the start of a 1 hour infusion. The total exposure of the pro-drug based on AUC was less than 1% that of isavuconazole. The inactive cleavage product was quantifiable in some subjects up to 8 hours after the start of infusion. The total exposure of inactive cleavage product based on AUC was approximately 1.3% that of isavuconazole.

4.2. Study ISN 9766-CL-0104 / Protocol WSA-CS-004

Median isavuconazole trough plasma concentrations on Day 7, Day 14 and at assumed steady-state were 3029.545 ng/mL, 2927.000 ng/mL and 3709.110 ng/mL, respectively, after CRESEMBA (200 mg) administration to patients in the PK analysis set (PKAS) with invasive fungal disease (Table 51).

Table 51: Study ISN 9766-CL-0104 - Clinical pharmacokinetic parameters

Statistic	ISA Trough Plasma Concentrations (ng/mL)		
	Day 7	Day 14	Assumed Steady-state†
n	68	66	65
Mean (SD)	3316.256 (1440.628)	3354.179 (1816.432)	3910.992 (1909.305)
%CV	43.4	54.2	48.8
Median	3029.545	2927.000	3709.110
Min - Max	1391.00 - 6953.71	813.13 - 9952.50	451.50 - 8645.91
Geometric Mean	3037.252	2954.431	3382.657

Concentrations below LLOQ (5 ng/mL) were set to zero. Geometric mean not calculated if one or more concentrations were < LLOQ. SD and %CV were not calculated if half or a majority of the concentrations at a given time point were < LLOQ.

LLOQ: lower limit of quantification; PKAS: pharmacokinetic analysis set.

† Trough concentrations from day 21 through 24 hours after the last dose of isavuconazole. Troughs were averaged for patients with more than one trough sample within this time frame.

Source: Clinical efficacy report

4.3. Study ISN 9766-CL-0103 / Protocol WSA-CS-003

The PKAS consisted of a subset of the ITT population administered the same dose as for Study ISN 9766-CL-0104, who had at least one isavuconazole plasma concentration. Isavuconazole trough concentrations on Days 7, 14, 28 (+/- 2 days) and at assumed steady state (> day 30) were >3000 ng/mL (Table 52).

Table 52: Study ISN 9766-CL-0103 - Isavuconazole trough plasma concentrations (PKAS)

Statistic	ISA Trough Plasma Concentrations (ng/mL)			
	Day 7	Day 14	Day 28	Assumed Steady-state†
n	55	40	69	78
Mean (SD)	3223.5 (1477.1)	3860.2 (2396.5)	3924.1 (1950.7)	3803.9 (1825.0)
%CV	45.8	62.1	49.7	48.0
Median	3166.9	3786.6	3914.6	3678.9
Min - Max	570.50–9676.2	0–14143.5	778.61–7795.9	325.42–8961.0
Geometric Mean	2898.1	NA	3333.7	3342.8

Concentrations < LLOQ (5 ng/mL) were set to zero. Geometric mean not calculated if one or more concentrations are < LLOQ. SD and %CV are not calculated if half or a majority of the concentrations at a given time point are < LLOQ.

ISA: Isavuconazole; LLOQ: lower limit of quantification; NA: not applicable; PKAS: pharmacokinetic analysis set.

† Trough concentrations from day 30 through 24 hours after the last dose of isavuconazole. Troughs were averaged for patients with more than 1 trough sample within this time frame.

Comments:

Based on PK data collected from phase 1 (189 phase 1 subjects provided 5828 concentrations) and phase 3 (232 phase 3 patients provided 535 concentrations) studies, a population PK model was developed. The half-life was 135 hours and AUC 2495 µg·hr/mL (Table 53).

Table 53: Population pharmacokinetic parameter estimates for isavuconazole based on the Phase 3 clinical trial data

PK Parameter	PK Parameter Values (mean ± SD)
$t_{1/2}$ (h)	135
AUC ₀₋₂₄ (µg·h/mL)	97.9 ± 57.16
Cl (mL/h)	2495 ± 1104.7
Steady state trough concentration (µg/mL) †	3.91 ± 1.909

Source: [9766-PK-0005; †9766-CL-0104]

5. CLINICAL MICROBIOLOGY

The applicant conducted two phase 3 clinical trials (ISN 9766-CL-0104 ~ Protocol WSA-CS-004 and ISN 9766-CL-0103 ~ Protocol WSA-CS-003) to support the efficacy and safety of isavuconazole for the treatment of invasive aspergillosis and invasive mucormycosis.

5.1. Study ISN 9766-CL-0104

This was a phase 3, randomized, double blind, active controlled, multi-center (approximately 150 centers) trial to evaluate the efficacy and safety of isavuconazole compared to voriconazole for the primary treatment of invasive fungal disease (IFD) caused by *Aspergillus* species or other filamentous fungi.

Primary objective

The primary objective of the study was to compare all-cause mortality through Day 42 following primary treatment with isavuconazole versus voriconazole in patients with IFD caused by *Aspergillus* species or other filamentous fungi.

Secondary objectives

The secondary objectives of the study were to characterize the safety and tolerability while assessing additional efficacy of treatment with isavuconazole versus voriconazole.

Exploratory objectives

- To summarize the concentration-time profiles of isavuconazole and metabolite(s) if warranted in patients from the PK sub study.
- To characterize PK trough values of isavuconazole and metabolite(s) if warranted.

Primary endpoint

All-cause mortality through Day 42.

Secondary endpoints

- Overall outcome at Day 42, end of treatment (EOT), and Day 84 (if different from EOT / if applicable).
- Response at Day 42, EOT, and Day 84 (if different from EOT / if applicable) in patients with mycologically confirmed pulmonary disease.
- Overall outcome in the subpopulations defined by the stratification variables.
- Mycological response at Day 42, EOT, and Day 84 (if different from EOT / if applicable).
- Survival rate at Day 42 and Day 84.
- Overall incidence of adverse events (AEs).
- Clinically significant changes in laboratory assessments (hematology, biochemistry and urinalysis) during the treatment period compared to baseline.
- Physical examination.
- Vital signs.
- 12-Lead electrocardiogram (ECG).

Study design

Inclusion criteria

- Either patients and/or legally authorized representative(s), if applicable, who had been fully informed and who gave voluntary written informed consent and Health Insurance Portability and Accountability Act (HIPAA); authorization for US centers or equivalent privacy language as per national regulations or patients unable to write and/or read but who fully understood the oral information given by the Investigator (or nominated representative) and who had given oral informed consent and HIPAA. Authorization for US centers or equivalent privacy language as per national regulations, witnessed in writing by an independent person.
- Ability and willingness to comply with the protocol.
- Male and female patients aged ≥ 18 years.
- Female patients must be non-lactating and at no risk for pregnancy for one of the following reasons:
 - Postmenopausal for at least 1 year.
 - Post-hysterectomy and/or post-bilateral ovariectomy.

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- If of childbearing potential, having a negative urine or serum human chorionic gonadotropin pregnancy test at the screening visit and be using a highly effective method of birth control throughout the course of the study. Reliable sexual abstinence throughout the course of the study was acceptable as a highly effective method of birth control for the purposes of this study.
- Patients with proven or probable IFD caused by *Aspergillus* species or other filamentous fungi were defined as summarized below [if, however, it was not possible to confirm IFD by culture, histology/ cytology or GM antigen within the 7 days after the first administration of study medication subjects were withdrawn from the study]:
 - *Proven invasive fungal disease:*

Patients with a positive diagnostic test obtained within the 7 days after the first administration of study medication:

 - Either histopathologic, cytopathologic, or wet mount examination of a needle aspiration or biopsy specimen showing hyphal forms with evidence of associated tissue damage (either microscopically or as an infiltrate or lesion by imaging).
 - OR
 - Recovery of a mould by culture from a sample obtained by a sterile procedure from a normally sterile and clinically or radiologically abnormal site consistent with an infectious disease process, excluding BAL and cranial sinus cavity.
 - *Probable invasive fungal disease:*

	At least one host factor [a] as below
PLUS	At least one clinical feature [b] as below
PLUS	At least one mycological criterion [c] as below
	NB not required for patients with neutropenia or allogeneic BMT and lower respiratory tract disease

a. HOST FACTORS:

- Either recently resolved (up to 2 weeks prior) or ongoing neutropenia [neutropenia defined as absolute neutrophil cell count $<0.5 \times 10^9/L$ ($<500/mm^3$) for ≥ 10 days, temporally related to the onset of fungal disease; or
- Receipt of an allogeneic hematopoietic stem cell transplant (BMT); or
- Prolonged use of corticosteroids [excluding patients with allergic bronchopulmonary aspergillosis (ABPA), autoimmune diseases] at an average minimum dose of 0.3 mg/kg/day prednisone equivalent for > 3 weeks; or
- Treatment with other recognized T-cell immune suppressants such as cyclosporine, tacrolimus, tumor necrosis factor- α blockers, specific monoclonal antibodies such as alemtuzumab or nucleoside analogues during the past 90 days.

b. CLINICAL FEATURES:

Lower respiratory tract disease:

The medical history was established to exclude different etiology and to distinguish between a primary and chronic pulmonary infection. Onset within 2 weeks prior to the first dose of study medication defines a primary pulmonary infection.

Either the presence of **at least one** of the following “**specific**” imaging signs on CT:

- Well defined nodule(s) with or without a halo sign.
- Wedge-shaped infiltrate.

- Air crescent sign.
 - Cavity.
- OR

The presence of a new “**non-specific**” **focal infiltrate PLUS** at least one of the following (not necessary if there is mycological evidence):

- Pleural rub.
- Pleural pain.
- Hemoptysis.

Note: Patients with neutropenia or allogeneic BMT (as defined above) who met criteria for clinical features of lower respiratory tract disease (as defined above), could be classified as Probable IFD even in the absence of mycological criteria.

Tracheobronchitis:

- Tracheobronchial ulceration; nodules; pseudomembrane; plaque or eschar seen on bronchoscopy.

Sino-nasal infection:

Imaging showing **sinusitis PLUS** at least one of the following:

- Acute localized pain (including pain radiating to eye).
- Nasal ulcer, black eschar.
- Extension from the paranasal sinus bony barriers, including into the orbit.

Central nervous system infection:

At least one of the following:

- Focal lesions on imaging.
- Meningeal enhancement on magnetic resonance imaging.

c. MYCOLOGICAL CRITERIA (cytology, direct microscopy, culture, antigen detection):

- Either sputum, BAL or bronchial brush samples demonstrating the presence of fungal elements either by recovery by culture of a mould (e.g., *Aspergillus* species) or detection by cytology or direct microscopy of hyphal forms; Or
- Recovery by culture of moulds or detection of hyphal forms by cytology or direct microscopy from a sinus aspirate.
- If invasive diagnostic procedures were not successful or not possible (e.g., problematic location of the infection or clinical conditions prohibit successful sampling), serum GM (a single value of ≥ 0.7 or two consecutive values of ≥ 0.5 - < 0.7 i.e., from two separate blood draws) was acceptable mycological evidence for enrollment as probable IFD except in patients receiving concomitant amoxicillin-clavulanate or piperacillin-tazobactam.

Note: GM in BAL, pleural fluid, or cerebrospinal fluid was not acceptable as mycological evidence for enrollment.⁴⁴

⁴⁴ In amendment 4 of the protocol, the applicant stated that BAL galactomannan was amended as allowable mycological criteria for enrollment of *Aspergillus* patients. Additional follow-up criteria for enrollment of these patients were also added.

- BAL GM (*Aspergillus* only): two values of > 1.0 (2 aliquots of the same sample) were allowed to enroll as possible IFD. Plasma-Lyte™ (Baxter) was not used as lavage fluid.
- Culture and histology/cytology was obtained from the same sample and serum GM was obtained per protocol requirements.

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- Possible invasive fungal disease:

	At least one host factor [a] - As above
PLUS	At least one clinical feature [b] – As above

Exclusion criteria

- Women who were pregnant or breastfeeding.
- Known history of allergy, hypersensitivity to or any serious reaction to the azole class of antifungals or to any component of the study medication.
- Patients for whom voriconazole is contra-indicated, including cardiovascular findings.
- Patients at high risk for QT/QTc prolongation such as a family history of long QT syndrome or other known pro-arrhythmic conditions.
- Evidence of moderate to severe hepatic or renal dysfunction with any of the following abnormal laboratory parameters at the screening visit:
 - Total bilirubin ≥ 3 times the upper limit of normal (ULN).
 - Alanine transaminase or aspartate transaminase ≥ 5 times ULN.
 - Patients with known cirrhosis or chronic hepatic failure.
- Concomitant use of sirolimus, efavirenz, ritonavir, astemizole, cisapride, rifampin/rifampicin, rifabutin, ergot alkaloids, long acting barbiturates, carbamazepine, pimozone, quinidine, neostigmine, terfenadine, ketoconazole, valproic acid or St. John's Wort in the 5 days prior to first administration of study medication.
- Patients with an invasive fungal infection other than *Aspergillus* species or other filamentous fungi and patients with zygomycosis/mucormycosis or *Scedosporium prolificans* infection not expected to respond to voriconazole treatment.
- Patients with either chronic aspergillosis, aspergilloma or allergic bronchopulmonary aspergillosis (ABPA).
- Microbiological (e.g., virological) findings or other potential conditions that were temporally related and suggested a different etiology of the clinical features in the absence of evidence of systemic aspergillosis infection.
- Patients who received more than 4 cumulative days of systemic antifungal therapy other than fluconazole or posaconazole within the 7 days prior to the first administration of study medication.
 - Patients with applicable host factors who developed new evidence of IFD while on prophylactic therapy, for at least 14 days, with either an amphotericin B product or an echinocandin, were eligible for enrollment.
 - Prior use of fluconazole of any duration and for any reason were eligible for enrollment.
- Advanced HIV infection with CD4 count < 200 or acquired immunodeficiency syndrome-defining condition.
- Any known or suspected condition of the patient that could jeopardize adherence to the protocol requirements or impeded the accurate measurement of efficacy, e.g., neutropenia not expected to resolve, patients with fungal endocarditis, fungal osteomyelitis, fungal meningitis, palliative therapy only for underlying condition.
- Patients with a concomitant medical condition that, in the opinion of the Investigator, was an unacceptable additional risk to the patient should he/she had participated in the study.
- Patients previously enrolled in a phase 3 study with isavuconazole.

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- Treatment with any investigational drug in any clinical trial within 30 days prior to the first administration of study medication except open label protocols.
- Patients who were unlikely to survive 30 days or patients on mechanical ventilation.
- Patients with a body weight (BW) \leq 40 kg.
- Patients with evidence of moderate to severe renal dysfunction with any of the following:
 - Calculated creatinine clearance $<$ 50 mL/minute at screening.
 - Currently on dialysis or likely to require dialysis during administration of study medication.

Drug administration and assessments

A loading dose (200 mg at 8 hour interval by infusion) of isavuconazole sulfate was administered followed by a dose of 200 mg once daily. Voriconazole loading dose (6 mg/kg b.i.d. by infusion and a placebo infusion to maintain the blind were administered for the first 24 hours followed by maintenance dose of 4 mg/kg dose, IV or 200 mg oral from Day 2 onwards. Switch to oral therapy was made as early as possible. Patients were treated until they reached a defined treatment endpoint.

Patients were followed for clinical and radiological outcome, PK measurements, and laboratory parameters that include microbiological measurements at different time intervals (Table 54). Survival status was recorded at EOT, Day 42, Day 84, and at the follow-up visits. Information on survival status on Days 42 and 84 was to be collected for all patients, irrespective of when treatment was discontinued.

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Table 54: Study ISN 9766-CL-0104 - Schedule of assessments.

Period	Screening		Study Treatment										Follow-up
	-96 Hours Prerandomization	D1 ¹	D2 ¹	D3 ¹	D7 ¹ (+1d)	D14 ¹ (±3d)	D28 ¹ (±7d)	D42 ² (±7d)	D63 ¹ (±7d)	D84 ^{2,6} (±7d)	EOT ^{2,3,6} (±3d)	Posttreatment FU ⁷ (4w after EOT [±7d])	
General													
Written informed consent	X												
Inclusion/Exclusion	X												
Demographics / Body Height	X												
Pregnancy test (if applicable) ⁴	X						X	X	X	X	X	X	
Underlying disease or condition	X												
Infectious disease history (4 wks prior)	X												
Medical history (3 mos prior)	X												
Prior and concomitant medication (4 wks prior)	X								X ongoing		→		
Nonmedication procedures (2 wks prior)	X								X ongoing		→		
Randomization	X ⁵												
Study drug								X daily ⁶			→		
Drug accountability								X ongoing			→		
Hospitalization status								X ongoing			→		
Efficacy													
Investigator's assessment of overall response								X		X	X		
Assessment of clinical symptoms and physical findings	X			X	X	X	X	X	X	X	X	X	
Radiological assessment ²²	X ¹⁶			X ¹⁶		X ¹⁹	X ¹⁹	X ¹⁷		X ¹⁷	X ¹⁷	X ¹⁵	
Bronchoscopic assessment ²⁰								X ongoing			→		
Serum GM antigen ¹⁸	X	X	X			X ¹	X ¹	X ¹		X ¹	X	X	
Mycological assessment (4 wks prior)	X	X ongoing		→				X		X	X		
Neutropenic status/ANC (4 wks prior)								X ongoing			→		
Survival status								X ⁷		X ⁷	X	X	
Safety													
Adverse Events								X ongoing			→		
Laboratory Tests:													
Hematology	X				X	X	X	X			X	X	
Chemistry	X			X	X	X	X	X			X	X	
Urinalysis/urine chemistry	X				X	X	X	X			X	X	
Body Weight	X							X		X	X		
Vital signs (SBP/DBP, PR, BT)	X	X ⁹	X ⁹	X ⁹	X ¹⁰			X ¹⁰		X ¹⁰	X ¹⁰	X ⁸	
12-lead ECG	X	X ¹¹				X ¹¹		X ¹¹		X ^{1,11}	X	X ⁸	
Physical Examination	X							X		X	X	X ⁸	
Eye Exam ²¹	X					X	X ¹	X ¹			X	X ⁸	
Whole Blood Sample for Optional Genotype Analysis ²³		X											
Pharmacokinetics													
Blood sampling for trough levels					X ¹²	X ¹²		X ¹			X ¹³	X	
Pharmacokinetic Substudy:													
Blood sampling					X ¹⁴	X ¹⁴							
24-hour Holter ECG	X ¹⁵				X ¹⁴	X ¹⁴						X ¹⁵	

ANC: absolute neutrophil count; BAL: bronchoalveolar lavage; BT: body temperature; CNS: central nervous system; CT: computed tomography; DBP: diastolic blood pressure; ECG: electrocardiogram; eCRF: electronic case report form; EOT: end of treatment; FU: follow-up; GM: galactomannan; HRCT: high resolution computed tomography; IFD: invasive fungal disease; LRTD: lower respiratory tract disease; MRI: magnetic resonance imaging; PR: pulse rate; SBP: systolic blood pressure.

1. Patients continuing on study drug
2. EOT, day 42, day 84 and follow-up assessments were required for all patients. Patients with a successful overall outcome were required to return for all three visits. Patients withdrawn from study drug or with an unsuccessful overall outcome were required to return for the EOT visit but were not asked to return for the day 42 and day 84 visits; however, survival status was obtained at these time points
3. If EOT occurred within 1 week of a scheduled visit, the visits and assessments could be combined; however, the schedule of assessments for EOT had to be applied
4. Women of childbearing potential
5. Randomization occurred up to and including day 1 prior to first study drug administration
6. Maximum duration of treatment was 84 days
7. For all patients irrespective of when study drug administration was discontinued
8. Only in patients with abnormalities observed at EOT
9. On day 1, day 2 and day 3, vital signs were measured within 1 hour before and 1 hour after the end of each study drug administration
10. For study visits from day 7 onwards, vital signs were measured once prior to study drug administration
11. 12-lead ECG was performed 15 minutes prior to the end of the first daily infusion or approximately 3 hours post oral dose
12. Trough level blood samples were taken immediately prior to the first daily dose, but no earlier than 1 hour prior
13. For EOT, trough level blood sample may have been taken up to 3 days before the last morning dose of study drug or 24 hours after the last morning dose of study drug.
14. Pharmacokinetic Substudy assessments were done either on day 7 or on day 14. Note that blood sampling and 24-hour Holter ECG was to be done on the same day.
15. Baseline 24-hour Holter ECG was done either at screening or at follow-up visit
16. If plain x-rays were used to diagnosis IFD during screening, these findings had to be confirmed by CT scan (or HRCT) or MRI within the 7 days after the first administration of study drug. If a CT scan (or HRCT) or MRI was performed at screening, an additional radiological examination between day 1 and day 7 was not required. (This served as the baseline radiological exam).
17. Radiological examination of the site of IFD utilized the same CT/HRCT/or MRI modality as the baseline radiologic examination
18. Serum GM antigen was drawn at screening and on days 1 and 2. A single value of ≥ 0.7 or two consecutive values each of $\geq 0.5 - < 0.7$ (i.e., from two separate blood draws) was considered a positive result except in patients receiving amoxicillin-clavulanate, piperacillin-tazobactam or Plasma-Lyte™ Baxter. Serum GM meeting the protocol defined requirements within 10 days prior to first dose of study drug were eligible for enrollment.
19. If clinically indicated in the treatment of the patient.
20. If bronchoscopic assessments were clinically indicated, samples were obtained for the following tests: culture and histology (or cytology). If BAL was sent for GM antigen testing, the results were recorded in the patient's eCRF. For BAL GM, two consecutive values of > 1.0 were considered a positive result, which in the absence of any other acceptable mycological evidence allowed the patient to be classified as Possible IFD. Plasma-Lyte™ Baxter was not used as lavage fluid.
21. Eye Exam consisted of visual acuity, confrontational visual field testing and color perception.
22. If clinically indicated (such as for patients with LRTD, CNS infection or sino-nasal IFD)
23. A separate patient consent was required

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• ***Mycological assessments***

Mycological assessment of invasive fungal infection included fungal culture, histology / cytology, and GM antigen detection by Bio-Rad Platelia™ *Aspergillus* EIA in serum as well as BAL fluid, performed at the local laboratories according to best local practice. Culture and histology/cytology were obtained from the same sample and serum GM must have been obtained per protocol requirements. GM in cerebrospinal fluid and other methods (e.g., PCR or β-D-glucan) were not acceptable as mycological evidence for enrollment.

All cultured pathogens associated with the IFD as well as serum specimens for GM testing were stored and shipped to a central laboratory (Table 55) for isolation of the fungal species and susceptibility testing. If the identities of the pathogens differed between the central and local laboratories, the central laboratory results took precedence over the local results for all study purposes. BAL fluid for GM specimens may have been processed locally, but an additional aliquot of BAL fluid was collected for shipment to the central laboratory as well.

Sponsor	Initially Basilea Pharmaceutica International, Ltd., followed by Astellas Pharma, Inc.
Coordinating Investigator	(b) (4)
CRO for Center Monitoring and Study Management	
Central Laboratory	
Central Radiology Review	
Central ECG Review	
Central Microbiological analysis	
GM analysis	
Measurement of isavuconazole concentrations	
Data Management	
Statistical Analysis	
Study Drug Product Supplies	

CRO: Clinical Research Organization; ECG: electrocardiogram; GM: galactomannan

The Investigator was asked to record any microbiological assessments confirming or excluding a different etiology for the clinical infection that were performed during the course of the study.

In vitro susceptibility testing:

Baseline and post-treatment isolates were tested for *in vitro* susceptibility testing against isavuconazole and other antifungal drugs by the CLSI-M38-A2⁵ and EUCAST⁶ methods.

• ***Definition of clinical, radiological, and mycological response***

Complete and partial overall responses were classified as a successful overall outcome (Table 56); an overall response of failure was classified as an unsuccessful overall outcome.

Following ‘Eradication’ at EOT and in the absence of signs of inflammation or infection, a subsequent positive culture for *Aspergillus* species or other filamentous fungi from a non-sterile site was described as ‘residual colonization’ and was not considered as part of the evaluation of mycological response.

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Table 56: Study ISN 9766-CL-0104 - Definition of clinical, radiological, and mycological response.

	Clinical Response	Mycological Response	Radiological Response
Success	<ul style="list-style-type: none"> • Resolution of all attributable clinical symptoms and physical findings • Partial resolution of attributable clinical symptoms and physical findings 	<ul style="list-style-type: none"> • Eradication • Presumed Eradication 	<ul style="list-style-type: none"> • $\geq 90\%$ Improvement from screening • $\geq 50\% - < 90\%$ Improvement from screening • $\geq 25\% - < 50\%$ Improvement from screening¹ • No signs on radiological images at screening (Proven IFD only)
<p>➤ Any one criterion from each response column is required to be considered to have an overall outcome of success. ¹ At Day 42 for patients with proven or probable invasive aspergillosis. At Day 84 however, this would be considered unsuccessful.</p>			

A data review committee (DRC), consisting of experts in infectious diseases, was established to adjudicate (independently from the Applicant and the Investigator) the categorization of each patient's IFD at enrollment (including data up to Day 7 as relevant) and to evaluate clinical, mycological, radiological and overall response at EOT, Day 42, and Day 84, as well as to assess attributable mortality.

The DRC assessments of IFD at baseline were summarized for the following categories:

- Presence of adequate host factor(s): (Yes/No/Not applicable)
- Presence of adequate radiologic/clinical feature(s): (Yes/No/Not applicable)

Mycological criteria at baseline that were assessed by DRC include the following categories:

- No mycological evidence available
- Serum GM (2 consecutive values of ≥ 0.5)*
- Serum GM (at least one value of ≥ 0.7 to < 1.0)
- Serum GM (at least one value of ≥ 1.0)*
- BAL GM (at least one value ≥ 1.0)**
- Histopathology
- Culture evidence of IFD
- Autopsy

* FDA proposed cut-offs in conjunction with clinical and host factors.

**Unclear if BAL GM index cut-off is based on testing of 2 aliquots or a single aliquot of the same specimen of BAL fluid.

DRC adjudicated each patient was adjudicated for the following IFD categories at baseline:

- Proven
- Probable
- Possible
- No IFD/No invasive mould infection

Number and percentage of patients for each category of pathogen causing IFD and location as assessed by DRC were summarized based on the following IFD categories:

- *Aspergillus* species only.
- Non-*Aspergillus* species only.
- Mould species, not otherwise specified (NOS)
- *Aspergillus* species plus other mould species
- No pathogen identified

Table 57 summarizes the definitions for clinical, mycological and radiological responses assessed by the DRC at day 42, day 84, and EOT.

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Table 57: Study ISN 9766-CL-0104 - Definitions for clinical, mycological and radiological responses based on DRC assessment	
Outcome	DRC-assessed Clinical Response
Success	<ul style="list-style-type: none"> - Resolution of all attributable clinical symptoms and physical findings - Partial resolution of attributable clinical symptoms and physical findings
Failure	<ul style="list-style-type: none"> - No resolution of any attributable clinical symptoms and physical findings and/or worsening
Not Applicable	<ul style="list-style-type: none"> - No attributable signs and symptoms present at baseline and no symptoms attributable to IFD developed post baseline
Outcome	DRC-assessed Mycological Response
Success	<ul style="list-style-type: none"> - Eradication - Presumed Eradication
Failure	<ul style="list-style-type: none"> - Persistence - Presumed Persistence
Not Applicable	<ul style="list-style-type: none"> - No Mycological Evidence Available at Baseline
Time point	DRC-assessed Radiological Response
Day 42	<ul style="list-style-type: none"> - Success (Improvement of at least 25% from baseline) - Failure - No postbaseline radiology available for patient with baseline evidence of radiologic disease - Radiology not applicable at baseline
Day 84	<ul style="list-style-type: none"> - Success (Improvement of at least 50% from baseline) - Failure - No postbaseline radiology available for patient with baseline evidence of radiologic disease - Radiology not applicable at baseline
EOT	<ul style="list-style-type: none"> - Success (Improvement of at least 25% from baseline, if EOT occurs prior to day 42; If EOT occurs after day 42, at least 50% improvement from baseline) - Failure - No postbaseline radiology available for patient with baseline evidence of radiologic disease - Radiology not applicable at baseline

Results

The intent-to-treat (ITT) population consisted of 516 randomized subjects who received at least one dose of isavuconazole (n=258) or voriconazole (n=258); the baseline characteristics of patients were balanced in the two treatment groups (Table 58). The total study drug administration duration was similar between the isavuconazole and voriconazole treatment groups; 400 patients (77.5%) switched from IV to oral dosing. The duration of study drug administration was 5 days for IV dosing and 56 days for oral dosing (60 days for isavuconazole treated subjects and 53 days for voriconazole treated subjects).

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Table 58: Study ISN 9766-CL-0104 – Patient disposition

Populations for Analysis	ISA	VRC	Total
Randomized	263 (100%)	264 (100%)	527 (100%)
ITT	258 (98.1%)	258 (97.7%)	516 (97.9%)
mITT	143 (54.4%)	129 (48.9%)	272 (51.6%)
mITT-FDA	147 (55.9%)	128 (48.5%)	275 (52.2%)
PPS-ITT	172 (65.4%)	175 (66.3%)	347 (65.8%)
PPS-mITT	108 (41.1%)	96 (36.4%)	204 (38.7%)
SAF	257 (97.7%)	259 (98.1%)	516 (97.9%)

Percentages were calculated based upon the Randomized population.

ISA: isavuconazole; VRC: voriconazole.

ITT = Intent-to-treat population consisted of patients who received at least one dose of trial medication.

mITT = Modified ITT population consisted of a subset of the ITT population of patients who had confirmation of definite or probable diagnosis (based on applicant’s criteria and FDA’s criteria of mycological findings serum or BAL GM findings as the sole microbiological criteria) of invasive aspergillosis.

MyITT = Mycological ITT population consisted of mITT patients with proven or probable invasive aspergillosis based on cytology, histology, culture or GMc set forth in the protocol and assessed by the DRC.

PPS = Per protocol set; population was a subset of ITT (or mITT) patients who did not deviate from the pre-specified classification criteria.

The baseline diagnosis of IFD was categorized by the Investigators as proven, probable, or possible. The DRC categorized the baseline IFD as proven, probable, possible or no IFD independent from the Investigator’s assessment, based on a blinded review of the eCRF data and the radiological assessments from Central Radiology. Of 516 ITT patients, the DRC categorized 272 (52.7%) patients as having proven or probable disease; based on Investigator assessments, 280 (54.3%) patients were categorized as having proven/probable baseline IFD (Table 59). Approximately, 40% of the subjects were characterized as probable and 13% as proven IFD. Overall, there was about 81% (420/516) concordance between DRC and Investigator assessments.

Table 59: Study ISN 9766-CL-0104 - concordance between Investigator and DRC categorization of IFD at baseline (ITT Population)

DRC Assessment	Investigator Assessment				Total
	Proven	Probable	Possible	Missing	
Proven	51 (9.9%)	9 (1.7%)	5 (1.0%)	0	65
Probable	4 (0.8%)	185 (35.9%)	17 (3.3%)	1 (0.2%)	207
Possible	0	12 (2.3%)	184 (35.7%)	0	196
No IFD/no invasive mould infection	10 (1.9%)	9 (1.7%)	29 (5.6%)	0	48
Total	65	215	235	1	516

IFD was categorized by the Investigator during screening (up to day 7), where a patient with more than one IFD category was identified with the highest IFD category by the following order: Proven followed by Probable followed by Possible. The IFD category was assigned as Missing if it was not provided by the Investigator.

DRC: Data Review Committee; IFD: invasive fungal disease; ITT: intent-to-treat.

Based on the DRC categorization of IFD, all-cause mortality rates were similar between the isavuconazole and voriconazole treated patients including mITT (based on applicant’s criteria⁴⁵) and mITT-FDA (based on Agency’s criteria⁴⁶) population at Day 42 and Day 84 (Table 60).

⁴⁵ Applicant’s criteria: Patients with appropriate host factor and clinical features were considered to have probable IFD based on the GM criteria (GMc) set forth in the protocol (i.e., 2 consecutive serum GM values ≥ 0.5 or at least one serum GM value ≥ 0.7).

⁴⁶ FDA criteria: The mITT-FDA population consisted of ITT patients who had proven or probable IFD. Patients with an appropriate host factor and clinical features were considered to have probable IFD based on the GM criteria (GMc) recommended by the FDA (i.e., 2 consecutive serum GM values ≥ 0.5 or at least 1 serum or BAL GM value ≥ 1.0); For more details see section 2.2 (Introduction and Background; Biology of invasive aspergillosis).

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Table 60: Study ISN 9766-CL-0104 – All-cause mortality through Day 42 and Day 84 for various populations

	ISA		VRC		Treatment Difference (%) 95% CI (%)†
	n	n (%)	n	n (%)	
Through Day 42					
mITT	143	28 (19.6)	129	30 (23.3)	-2.6 (-12.184, 6.916)
mITT-FDA‡	147	28 (19.0)	128	28 (21.9)	-2.1 (-11.422, 7.215)
PPS-ITT	172	26 (15.1)	175	31 (17.7)	-2.6 (-10.283, 5.079)
PPS-mITT	108	16 (14.8)	96	19 (19.8)	-5.1 (-15.166, 5.024)
ITT-excluding no IFD§	231	43 (18.6)	237	49 (20.7)	-1.3 (-8.424, 5.830)
myITT¶	123	23 (18.7)	108	24 (22.2)	-2.7 (-12.893, 7.542)
Through Day 84					
ITT	258	75 (29.1)	258	80 (31.0)	-1.4 (-9.150, 6.340)
mITT	143	43 (30.1)	129	48 (37.2)	-5.5 (-16.059, 5.148)
mITT-FDA‡	147	41 (27.9)	128	43 (33.6)	-4.7 (-15.099, 5.748)
PPS-ITT	172	43 (25.0)	175	48 (27.4)	-2.8 (-11.861, 6.234)
PPS-mITT	108	29 (26.9)	96	31 (32.3)	-5.7 (-17.735, 6.303)
ITT-excluding no IFD	231	67 (29.0)	237	75 (31.6)	-1.9 (-10.055, 6.216)
myITT¶	123	35 (28.5)	108	39 (36.1)	-5.7 (-17.062, 5.577)

A patient with unknown survival status was treated as a death.

BAL: bronchoalveolar lavage; BMT: bone marrow transplant; CMH: Cochran-Mantel-Haenszel;
GM: galactomannan; GMc: galactomannan criteria; IFD: invasive fungal disease; ISA: isavuconazole;
VRC: voriconazole.

† The adjusted treatment difference (ISA-VRC) was calculated by a stratified CMH method with Geographical Region, Allogeneic BMT Status and Uncontrolled Malignancy Status as the stratification factors. The 95% CI for the adjusted treatment difference was based on a normal approximation.

‡ The GMc used for the mITT population was 2 consecutive serum GM values ≥ 0.5 or at least one serum GM value ≥ 0.7 as defined in the protocol. The GMc used for the mITT-FDA population was 2 consecutive serum GM values ≥ 0.5 or at least 1 serum or BAL GM value ≥ 1.0 .

§ The ITT-excluding no IFD population is the ITT population excluding those who were assessed by the DRC as not having adequate evidence of proven, probable or possible IFD.

¶ The myITT population consisted of mITT patients with proven or probable invasive aspergillosis based on cytology, histology, culture or GMc.

ITT = Intent-to-treat population consisted of patients who received at least one dose of trial medication.

mITT = Modified ITT population consisted of a subset of the ITT population of patients who had confirmation of definite or probable diagnosis (based on applicant's criteria and FDA's criteria of mycological findings serum or BAL GM findings as the sole microbiological criteria) of invasive aspergillosis.

MyITT = Mycological ITT population consisted of mITT patients with proven or probable invasive aspergillosis based on cytology, histology, culture or GMc set forth in the protocol and assessed by the DRC.

PPS = Per protocol set; population was a subset of ITT (or mITT) patients who did not deviate from the pre-specified classification criteria.

Aspergillus species was identified in approximately 30% subjects and *A. fumigatus* was the most common pathogen identified. The number of patients with filamentous fungi other than *A. fumigatus* and *A. flavus* were small (<10). About 2% of the subjects had mixed infections with *Aspergillus* and other moulds and about 5% had other mould infections.

In patients with *A. fumigatus* as the baseline pathogen, the all-cause mortality, clinical and mycological responses were similar or better in isavuconazole and voriconazole treated subjects the mITT and PP populations (Tables 61, 62, and 63); the number of subjects with baseline pathogen other than *A. fumigatus* and *A. flavus* was small (Table 63). Overall, a higher percentage of failures were observed at Day 84 relative to Day 42.

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Table 61: Study ISN 9766-CL-0104 – All-cause mortality, clinical response and mycological response by baseline pathogen in mITT population												
Baseline Pathogen	Day 42						Day 84					
	Isavuconazole			Voriconazole			Isavuconazole			Voriconazole		
	All-cause mortality n/N (%)	Clinical Response n/N (%)	Mycological Response n/N (%)	All-cause mortality n/N (%)	Clinical Response n/N (%)	Mycological Response n/N (%)	All-cause mortality n/N (%)	Clinical Response n/N (%)	Mycological Response n/N (%)	All-cause mortality n/N (%)	Clinical Response n/N (%)	Mycological Response n/N (%)
Single infection												
Aspergillus species												
<i>A. flavus</i>	0/6	6/6	4/6	1/11 (9 1)	9/11 (81 8)	7/11 (63 6)	0/6	6/6	3/6	1/11 (9 1)	8/11 (72 7)	7/11 (63 6)
<i>A. fumigatus</i>	2/28 (7 1)	21/28 (75 0)	11/28 (39 3)	6/21 (28 6)	11/21 (52 4)	7/21 (33 3)	4/28 (14 3)	15/28 (53 6)	9/28 (32 1)	10/21	9/21 (42 9)	5/21 (23 8)
<i>A. niger</i>	2/6	4/6	4/6	-	-	-	3/6	2/6	2/6	-	-	-
<i>A. sydowi</i>	0/1	1/1	1/1	-	-	-	1/1	0/1	0/1	-	-	-
<i>A. terreus</i>	0/2	2/2	1/2	0/1	1/1	1/1	0/2	2/2	0/2	0/1	1/1	0/1
<i>A. ustus</i>	-	-	-	0/1	1/1	1/1	-	-	-	0/1	1/1	0/1
<i>A. westerdijkiae</i>	0/1	0/1	0/1	-	-	-	0/1	0/1	0/1	-	-	-
<i>Aspergillus</i> NOS	0/1	1/1	0/1	1/3	1/3	0/3	0/1	0/1	0/1	2/3	1/3	0/3
Aspergillus species (Total)	4/45 (8 9)	35/45 (77 8)	21/45 (46 7)	8/37 (21 6)	23/37 (62 2)	16/37 (43 2)	8/45 (17 8)	25/45 (55 6)	14/45 (31 1)	13/37 (35 1)	20/37 (54 1)	12/37 (32 4)
Pathogens other than Aspergillus species												
<i>Rhizopus</i> NOS	1/1	0/1	0/1	-	-	-	1/1	0/1	0/1	-	-	-
<i>Exserohilum rostratum</i>	-	-	-	0/1	0/1	0/1	-	-	-	0/1	0/1	0/1
<i>Fusarium</i> NOS	-	-	-	0/2	1/2	0/2	-	-	-	1/2	0/2	0/2
<i>Fusarium solani</i>	1/2	1/2	1/2	-	-	-	1/2	1/2	1/2	-	-	-
<i>Penicillium</i> NOS	-	-	-	0/1	1/1	0/1	-	-	-	0/1	0/1	0/1
<i>Penicillium marneffi</i>	-	-	-	0/1	0/1	0/1	-	-	-	0/1	0/1	0/1
<i>Trichosporon inkin</i>	0/1	1/1	1/1	-	-	-	0/1	0/1	0/1	-	-	-
Total	6/48 (12 5%)	37/48 (77 1)	23/48 (47 9)	8/42 (19 0)	25/40 (62 5)	16/42 (38 1)	10/48 (20 8)	26/48 (54 2)	15/48 (31 3)	14/42 (33 3)	20/41 (48 8)	12/42 (28 6)
Mixed infections												
<i>A. flavus</i> + <i>A. fumigatus</i>	1/3	2/3	2/3	-	-	-	2/3	1/3	1/3	-	-	-
<i>A. flavus</i> + <i>A. niger</i>	-	-	-	0/1	0/1	0/1	-	-	-	1/1	0/1	0/1
<i>A. flavus</i> + <i>A. terreus</i>	0/1	1/1	0/1	-	-	-	0/1	1/1	0/1	-	-	-
<i>A. fumigatus</i> + <i>A. terreus</i>	0/1	1/1	0/1	-	-	-	0/1	1/1	0/1	-	-	-
<i>A. flavus</i> + <i>Absidia</i> NOS	1/1	0/1	0/1	-	-	-	1/1	0/1	0/1	-	-	-
<i>A. fumigatus</i> + <i>Scedosporium</i> NOS	-	-	-	0/1	1/1	1/1	-	-	-	0/1	1/1	1/1
<i>Aspergillus</i> NOS + <i>Scedosporium</i> NOS	1/1	0/1	0/1	-	-	-	1/1	0/1	0/1	-	-	-
<i>A. terreus</i> + <i>A. niger</i>	-	-	-	0/1	1/1	0/1	-	-	-	0/1	1/1	0/1
<i>A. terreus</i> + <i>Absidia corymbifera</i>	1/1	0/1	0/1	-	-	-	1/1	0/1	0/1	-	-	-
<i>Mucor</i> NOS + <i>Fusarium</i> , NOS	-	-	-	0/1	0/1	0/1	-	-	-	0/1	0/1	0/1
Total	4/8	4/8	2/8	0/4	2/4	1/4	5/8	3/8	1/8	1/4	2/4	1/4
Galactomannan positive	16/77 (20 8)	42/74 (56 8)	28/77 (36 4)	14/67 (20 9)	36/62 (58 1)	29/67 (43 3)	21/77 (27 3)	37/75 (48 0)	24/77 (31 2)	20/67 (29 9)	29/64 (45 3)	30/67 (44 8)

NOS=not otherwise specified

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Table 62: Study ISN 9766-CL-0104 – All-cause mortality, clinical response and mycological response by baseline pathogen in PP population												
Baseline Pathogen	Day 42						Day 84					
	Isavuconazole			Voriconazole			Isavuconazole			Voriconazole		
	All-cause mortality n/N (%)	Clinical Response n/N (%)	Mycological Response n/N (%)	All-cause mortality n/N (%)	Clinical Response n/N (%)	Mycological Response n/N (%)	All-cause mortality n/N (%)	Clinical Response n/N (%)	Mycological Response n/N (%)	All-cause mortality n/N (%)	Clinical Response n/N (%)	Mycological Response n/N (%)
Single infection												
Aspergillus species												
<i>A. flavus</i>	0/3	3/3	2/3	0/7	6/7	5/7	0/3	3/3	1/3	0/7	5/7	5/7
<i>A. fumigatus</i>	2/25 (8 0)	19/25 (76 0)	10/25 (40 0)	5/16 (31 3)	9/16 (56 3)	6/16 (37 5)	4/25 (16 0)	15/25 (60 0)	9/25 (36 0)	8/16 (50 0)	8/16 (50 0)	4/16 (25 0)
<i>A. niger</i>	1/5	4/5	4/5	-	-	-	2/5	2/5	2/5	-	-	-
<i>A. sydowi</i>	0/1	1/1	1/1	-	-	-	1/1	0/1	0/1	-	-	-
<i>A. terreus</i>	0/1	1/1	1/1	0/1	1/1	1/1	0/1	1/1	0/1	0/1	1/1	0/1
<i>A. ustus</i>	-	-	-	0/1	1/1	1/1	0	0	0	0/1	1/1	0/1
<i>A. westerdijkiae</i>	0/1	0/1	0/1	-	-	-	0/1	0/1	0/1	-	-	-
<i>Aspergillus</i> NOS	-	-	-	1/3	1/3	0/3	0	0	0	2/3	1/3	0/3
<i>Aspergillus</i> species (Total)	2/36 (5 6)	28/36 (77 8)	18/36 (50 0)	6/28 (21 4)	18/28 (64 3)	13/28 (46 4)	7/36 (19 4)	21/36 (58 3)	12/36 (33 3)	10/28 (35 7)	16/28 (57 1)	9/28 (32 1)
Pathogens other than <i>Aspergillus</i> species												
<i>Rhizopus</i> NOS	1/1	0/1	0/1	-	-	-	1/1	0/1	0/1	0	0	0
<i>Fusarium</i> NOS	-	-	-	0/2	½	0/2	0	0	0	½	0/2	0/2
<i>Fusarium solani</i>	0/1	1/1	1/1	-	-	-	0/1	1/1	1/1	-	-	-
<i>Trichosporon inkin</i>	0/1	1/1	1/1	-	-	-	0/1	0/1	0/1	-	-	-
Mixed infections												
<i>A. flavus</i> + <i>A. fumigatus</i>	0/1	1/1	1/1	-	-	-	1/1	0/1	0/1	-	-	-
<i>A. flavus</i> + <i>A. niger</i>	-	-	-	0/1	0/1	0/1	-	-	-	1/1	0/1	0/1
<i>A. flavus</i> + <i>A. terreus</i>	0/1	1/1	0/1	-	-	-	0/1	1/1	0/1	-	-	-
<i>A. fumigatus</i> + <i>A. terreus</i>	0/1	1/1	0/1	-	-	-	0/1	1/1	0/1	-	-	-
<i>A. flavus</i> + <i>Absidia</i> NOS												
<i>A. fumigatus</i> + <i>Scedosporium</i> NOS	-	-	-	0/1	1/1	1/1	-	-	-	0/1	1/1	1/1
<i>Aspergillus</i> NOS + <i>Scedosporium</i> NOS	1/1	0/1	0/1	-	-	-	1/1	0/1	0/1	-	-	-
<i>A. terreus</i> + <i>A. niger</i>	-	-	-	0/1	1/1	0/1	-	-	-	0/1	1/1	0/1
<i>A. terreus</i> + <i>Absidia corymbifera</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Mucor</i> NOS + <i>Fusarium</i> , NOS	-	-	-	0/1	0/1	0/1	-	-	-	0/1	0/1	0/1
Galactomannan positive	10/57 (17 5)	33/54 (61 1)	24/57 (42 1)	9/52 (17 3)	34/47 (72 3)	29/52 (55 8)	13/57 (22 8)	30/55 (54 5)	20/57 (35 1)	13/52 (25 0)	27/49 (55 1)	28/52 (53 8)

NOS=not otherwise specified

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Table 63: Study ISN 9766-CL-0104 – All-cause mortality, clinical response and mycological response in mITT population with baseline *Aspergillus* or Mucorales infection at baseline irrespective of single or mixed infections

Baseline Pathogen	Day 42						Day 84					
	Isavuconazole			Voriconazole			Isavuconazole			Voriconazole		
	All-cause mortality n/N (%)	Clinical Response n/N (%)	Mycological Response n/N (%)	All-cause mortality n/N (%)	Clinical Response n/N (%)	Mycological Response n/N (%)	All-cause mortality n/N (%)	Clinical Response n/N (%)	Mycological Response n/N (%)	All-cause mortality n/N (%)	Clinical Response n/N (%)	Mycological Response n/N (%)
mITT												
<i>A. flavus</i>	2/11 (18.2)	9/11 (81.8)	6/11 (54.5)	1/12 (8.3)	9/12 (75.0)	7/11 (63.6)	3/11 (27.3)	8/11 (72.7)	4/11 (36.4)	2/12 (16.7)	8/12 (66.7)	7/12 (58.3)
<i>A. fumigatus</i>	3/32 (9.3)	24/32 (75.0)	13/32 (40.6)	6/22 (27.3)	12/22 (54.5)	8/22 (33.3)	6/32 (18.8)	17/32 (53.1)	10/32 (31.3)	10/22 (45.5)	10/22 (45.5)	6/22 (27.3)
<i>A. niger</i>	2/6	4/6	4/6	0/2	½	0/2	3/6	2/6	2/6	½	½	0/2
<i>A. sydowi</i>	0/1	1/1	1/1	-	-	-	1/1	0/1	0/1	-	-	-
<i>A. terreus</i>	1/5	3/5	1/5	0/2	1/2	1/2	1/4	3/4	0/4	0/2	1/2	0/2
<i>A. ustus</i>	-	-	-	0/1	1/1	1/1	-	-	-	0/1	1/1	0/1
<i>A. westerdijkiae</i>	0/1	0/1	0/1	-	-	-	0/1	0/1	0/1	-	-	-
<i>Aspergillus</i> species alone (Total)	5/50 (10.0)	39/50 (78.0)	23/50 (46.0)	8/39 (20.5)	24/39 (61.5)	16/39 (41.0)	10/50 (20.0)	28/50 (56.0)	15/50 (30.0)	14/39 (35.9)	21/39 (53.8)	12/39 (30.8)
<i>Aspergillus</i> species + other moulds (Total)	8/53 (15.1)	39/53 (73.6)	23/53 (43.4)	8/40 (20.0)	25/40 (62.5)	17/40 (42.5)	13/53 (24.5)	28/53 (52.8)	15/53 (28.3)	14/40 (35.0)	22/40 (55.0)	13/40 (32.5)
<i>Rhizopus</i> NOS	1/1	0/1	0/1	-	-	-	1/1	0/1	0/1	-	-	-
<i>Absidia corymbifera</i>	1/1	0/1	0/1	-	-	-	1/1	0/1	0/1	-	-	-
<i>Absidia</i> NOS	1/1	0/1	0/1	-	-	-	-	-	-	-	-	-
PP												
<i>A. flavus</i>	0/5	5/5	3/5	0/8	6/7	5/8	1/5	4/5	1/5	1/8	5/7	5/8
<i>A. fumigatus</i>	2/27 (7.4)	21/27 (77.8)	11/27 (40.1)	5/17 (29.4)	10/16 (62.5)	7/17 (41.2)	6/27 (22.2)	16/27 (59.3)	9/27 (33.3)	8/17 (47.1)	9/17 (52.9)	5/17 (29.4)
<i>A. niger</i>	1/5	4/5	4/5	0/2	½	0/2	2/5	2/5	2/5	1/2	1/2	0/2
<i>A. sydowi</i>	0/1	1/1	1/1	-	-	-	1/1	0/1	0/1	-	-	-
<i>A. terreus</i>	0/3	3/3	1/3	0/2	2/2	1/2	0/3	3/3	0/3	0/2	2/2	0/2
<i>A. ustus</i>	-	-	-	0/1	1/1	1/1	-	-	-	0/1	1/1	0/1
<i>A. westerdijkiae</i>	0/1	0/1	0/1	-	-	-	0/1	0/1	0/1	-	-	-
<i>Aspergillus</i> NOS	-	-	-	1/3	1/3	0/3	-	-	-	2/3	1/3	0/3
<i>Aspergillus</i> species alone (Total)	2/39 (5.1)	31/39 (79.5)	19/39 (48.7)	6/30 (20.0)	19/30 (63.3)	13/30 (43.3)	8/39 (20.5)	23/39 (59.0)	12/39 (30.8)	11/30 (36.7)	17/30 (56.7)	9/30 (30.0)
<i>Aspergillus</i> species (Total)	3/40 (7.5)	31/40 (77.5)	19/40 (47.5)	6/31 (19.4)	20/31 (64.5)	14/31 (45.2)	9/40 (22.5)	23/40 (57.5)	12/40 (30.0)	11/31 (35.5)	18/31 (58.1)	10/31 (32.3)
<i>Rhizopus</i> NOS	1/1	0/1	0/1	-	-	-	1/1	0/1	0/1	-	-	-

NOS=not otherwise specified

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Clinical fungal isolates from patients in the study with positive culture were tested for *in vitro* susceptibility at a Central Laboratory (for details see section 3.2.2.4 above); all testing was done in accordance with the CLSI M38-A2⁵ and the EUCAST⁶ methods. Isavuconazole MICs against *Aspergillus* species, ranged from 0.25 to 32 µg/mL by the CLSI method; the MIC₉₀ values for isolates from patients treated with isavuconazole or voriconazole were 4 and 2 µg/mL, respectively (Table 64). Overall, the isavuconazole MICs were similar to those of voriconazole, amphotericin B, and posaconazole. The isavuconazole MICs by the CLSI or the EUCAST methods were similar.

Table 64: Study ISN 9766-CL-0104 – MIC values for isavuconazole and other antifungal drugs for baseline fungal isolates from isavuconazole- or voriconazole-treated patients from Study 9766-CL-0104 (mITT population)

Isavuconazole treatment arm							Voriconazole treatment arm						
CLSI method							CLSI method						
Organism Genus: species (No. isolates collected)	MIC ⁺ MIC Range (mg/L)	AmB	CAS [†]	ISA	POS	VOR	Organism Genus: species (No. isolates collected)	MIC ⁺ MIC Range (mg/L)	AmB	CAS [†]	ISA	POS	VOR
<i>Aspergillus</i> spp. (51)	0.5-32	0.25-2	0.25-32	0.12-2	0.12-32		<i>Aspergillus</i> spp. (25)	0.5-8	0.25-0.5	0.25-4	0.12-1	0.25-2	
	MIC ₅₀	1	0.25	1	0.5	1		MIC ₅₀	1	0.25	1	0.5	1
	MIC ₉₀	4	1	4	1	2		MIC ₉₀	4	0.5	2	0.5	2
<i>Aspergillus flavus</i> (9)	0.5-4	0.25-1	0.25-4	0.25-1	0.5-2		<i>Aspergillus flavus</i> (7)	0.5-4	0.25	0.5-4	0.5-1	0.5-2	
	MIC Range (mg/L)	0.5-4	0.25-2	0.25-32	0.12-2	0.12-32		MIC Range (mg/L)	0.5-4	0.25	0.5-4	0.5-1	0.5-2
	MIC ₅₀	1	0.25	0.5	0.25	0.5		MIC ₅₀	1	0.25	1	0.25	1
	MIC ₉₀	4	1	2	0.5	2		MIC ₉₀	4	0.5	2	0.5	2
<i>Aspergillus fumigatus</i> (28)	0.5-4	0.25-2	0.25-32	0.12-2	0.12-32		<i>Aspergillus fumigatus</i> (17)	0.5-8	0.25-0.5	0.25-4	0.12-1	0.25-2	
	MIC Range (mg/L)	0.5-4	0.25	2-4	0.5-1	2-4		MIC Range (mg/L)	0.5-8	0.25	1	0.25	1
	MIC ₅₀	1	0.25	2	0.5	2		MIC ₅₀	1	0.25	1	0.25	1
	MIC ₉₀	4	1	2	0.5	2		MIC ₉₀	4	0.5	2	0.5	2
<i>Aspergillus niger</i> (7)	0.5-4	0.25	2-4	0.5-1	2-4		<i>Aspergillus terreus</i> (1)	1	0.25	1	0.5	1	
	MIC Range (mg/L)	1-8	0.25-2	0.25-4	0.12-0.5	0.25-16		MIC Range (mg/L)	1	0.25	1	0.5	1
	MIC ₅₀	1	0.25	2	0.5	2							
	MIC ₉₀	4	1	2	0.5	2							
<i>Aspergillus terreus</i> (6)	1-8	0.25-2	0.25-4	0.12-0.5	0.25-16								
	MIC Range (mg/L)	32	2	2	1	32							
	MIC ₅₀	32	2	2	1	32							
	MIC ₉₀	32	2	2	1	32							
<i>Aspergillus westerdijkiae</i> (1)	32	2	2	1	32								
	MIC Range (mg/L)												
	MIC ₅₀												
	MIC ₉₀												
EUCAST method							EUCAST method						
Organism Genus: species (No. isolates collected)	MIC ⁺ MIC Range (mg/L)	AmB	CAS [†]	ISA	POS	VOR	Organism Genus: species (No. isolates collected)	MIC ⁺ MIC Range (mg/L)	AmB	CAS [†]	ISA	POS	VOR
<i>Aspergillus</i> spp. (51)	0.5-32	0.12-1	0.12-1	0.25-32	0.25-1	0.25-32	<i>Aspergillus</i> spp. (25)	2-4	0.12-0.5	0.25-2	0.25-1	0.25-16	
	MIC ₅₀	4	0.12	1	0.5	1		MIC ₅₀	4	0.25	1	0.5	1
	MIC ₉₀	4	0.25	2	1	4		MIC ₉₀	4	0.25	2	0.5	2
<i>Aspergillus flavus</i> (9)	0.5-4	0.12-0.25	0.25-2	0.25-1	1-4		<i>Aspergillus flavus</i> (7)	2-4	0.12-0.25	1-2	0.5-1	1-2	
	MIC Range (mg/L)	2-4	0.12-1	0.25-32	0.25-1	0.25-32		MIC Range (mg/L)	2-4	0.12-0.5	0.25-2	0.25-0.5	0.25-16
	MIC ₅₀	4	0.25	0.5	0.5	0.5		MIC ₅₀	4	0.25	1	0.5	0.5
	MIC ₉₀	4	0.25	2	1	4		MIC ₉₀	4	0.5	1	0.5	2
<i>Aspergillus fumigatus</i> (28)	0.5-4	0.12-1	0.25-32	0.25-1	0.25-32		<i>Aspergillus fumigatus</i> (17)	2-4	0.12-0.5	0.25-2	0.25-0.5	0.25-16	
	MIC Range (mg/L)	1-2	0.12-0.25	1-4	0.5-1	0.5-4		MIC Range (mg/L)	4	0.25	1	0.5	0.5
	MIC ₅₀	1-2	0.12-0.25	1-4	0.5-1	0.5-4		MIC ₅₀	4	0.25	1	0.5	0.5
	MIC ₉₀	4	0.25	2	1	4		MIC ₉₀	4	0.5	1	0.5	2
<i>Aspergillus niger</i> (7)	1-2	0.12-0.25	1-4	0.5-1	0.5-4		<i>Aspergillus terreus</i> (1)	4	0.12	1	0.5	0.5	
	MIC Range (mg/L)	32	1	4	1	32		MIC Range (mg/L)	4	0.12	1	0.5	0.5
	MIC ₅₀	32	1	4	1	32							
	MIC ₉₀	32	1	4	1	32							
<i>Aspergillus terreus</i> (6)	0.5-8	0.12-1	0.25-2	0.25-1	0.5-32								
	MIC Range (mg/L)												
	MIC ₅₀												
	MIC ₉₀												
<i>Aspergillus terreus</i> (1)	32	1	4	1	32								
	MIC Range (mg/L)												
	MIC ₅₀												
	MIC ₉₀												

AmB: amphotericin B; CAS: caspofungin; EUCAST: European Committee for Antimicrobial Susceptibility Testing; ISA: isavuconazole; MIC: minimum inhibitory concentration; mITT: modified intent-to-treat; POS: posaconazole; ULOQ: upper limit of quantification; VOR: voriconazole. mITT: All ITT patients who have proven or probable invasive fungal disease as determined by the data review committee. If a MIC value is reported as >ULOQ, then the MIC value is imputed as single two-fold dilution above the ULOQ and used in this summary.

† The MIC₅₀ and MIC₉₀ are not reported if the number of isolates is less than 10.

‡ Sensitivities to CAS are measured as minimum effective concentrations (MEC).

For CAS, the minimal effective concentration (MEC; defined as the lowest concentration that leads to the growth of small, rounded, compact hyphal forms compared to hyphal growth seen in the control well) was used as an endpoint, as directed by the recommendations from CLSI and EUCAST.

There were a small number of isolates (n = ≤3) of filamentous fungi (*Rhizopus* species, *Fusarium* species, *Penicillium* species, *L. corymbifera*, and *Trichosporon inkin*) other than *Aspergillus* species in patients treated with either isavuconazole or voriconazole. The MICs ranged from 1 to 32 µg/mL.

The clinical and mycological responses were compared with the MIC values of baseline isolates from subjects in the mITT population. There was no correlation between MICs of baseline isolates and clinical or mycological response at any of the time points that includes end of treatment, Day 42, and Day 84 (Tables 65 to 68; Figure 42). This analysis is limited by the small number of isolates for each species.

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Table 65: Study ISN 9766-CL-0104 – Overall/clinical/mycological response at end of treatment by baseline MIC values (determined by the CLSI method) for *Aspergillus* species in mITT population

Isavuconazole:										
Genus Organism	Efficacy Assessment at EOT	MIC Values (ug/ml)								
		0.25	0.5	1	2	4	8	16	>16	
<i>Aspergillus</i> species	Overall	4/9	3/8	5/10	2/6	3/6	1/1	0	0/1	
	Success	(44.4%)	(37.5%)	(50.0%)	(33.3%)	(50.0%)	(100.0%)			
	Clinical	7/9	7/8	8/10	4/6	4/6	1/1	0	0/1	
	Success	(77.8%)	(87.5%)	(80.0%)	(66.7%)	(66.7%)	(100.0%)			
<i>Aspergillus</i> <i>flavus</i>	Overall	1/2	1/1	2/3	1/2	0/1	0	0	0	
	Success	(50.0%)	(100.0%)	(66.7%)	(50.0%)					
	Clinical	1/2	1/1	3/3	2/2	1/1	0	0	0	
	Success	(50.0%)	(100.0%)	(100.0%)	(100.0%)	(100.0%)				
<i>Aspergillus</i> <i>fumigatus</i>	Overall	4/8	3/7	3/6	1/2	0	1/1	0	0/1	
	Success	(50.0%)	(42.9%)	(50.0%)	(50.0%)		(100.0%)			
	Clinical	6/8	7/7	4/6	2/2	0	1/1	0	0/1	
	Success	(75.0%)	(100.0%)	(66.7%)	(66.7%)		(100.0%)			
<i>Aspergillus</i> <i>niger</i>	Overall	0	0	0	0/1	3/4	0	0	0	
	Success					(75.0%)				
	Clinical	0	0	0	0/1	3/4	0	0	0	
	Success					(75.0%)				
<i>Aspergillus</i> <i>terreus</i>	Overall	0/1	0/1	0/1	0/1	0/1	0	0	0	
	Success									
	Clinical	1/1	0/1	1/1	1/1	0/1	0	0	0	
	Success	(100.0%)		(100.0%)	(100.0%)					
<i>Aspergillus</i> <i>westerdijkiae</i>	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
	Success									
	Overall	0								

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Table 66: Study ISN 9766-CL-0104 – Overall/clinical/mycological response at Day 42 by baseline isavuconazole MIC values (determined by the CLSI method) for *Aspergillus* species in mITT population

Isavuconazole:

Treatment	Genus Organism	Efficacy Assessment at Day 42	MIC Values (ug/ml)							
			0.25	0.5	1	2	4	8	16	>16
ISAVUCONAZOLE	<i>Aspergillus</i> species	Overall	4/9	3/8	4/10	2/6	5/6	1/1	0	0/1
		Success	(44.4%)	(37.5%)	(40.0%)	(33.3%)	(83.3%)	(100.0%)	0	0/1
		Clinical	8/9	6/8	9/10	4/6	6/6	1/1	0	0/1
		Success	(88.9%)	(75.0%)	(90.0%)	(66.7%)	(100.0%)	(100.0%)	0	0/1
	<i>Aspergillus flavus</i>	Overall	1/2	1/1	2/3	1/2	0/1	0	0	0
		Success	(50.0%)	(100.0%)	(66.7%)	(50.0%)	0	0	0	0
		Clinical	1/2	1/1	3/3	2/2	1/1	0	0	0
		Success	(50.0%)	(100.0%)	(100.0%)	(100.0%)	(100.0%)	0	0	0
	<i>Aspergillus fumigatus</i>	Overall	4/8	3/7	2/6	1/2	0	1/1	0	0/1
		Success	(50.0%)	(42.9%)	(33.3%)	(50.0%)	0	(100.0%)	0	0/1
		Clinical	7/8	6/7	5/6	2/2	0	1/1	0	0/1
		Success	(87.5%)	(85.7%)	(83.3%)	(100.0%)	0	(100.0%)	0	0/1
<i>Aspergillus niger</i>	Overall	0	0	0	0/1	4/4	0	0	0	
	Success					(100.0%)	0	0	0	
	Clinical	0	0	0	0/1	4/4	0	0	0	
	Success					(100.0%)	0	0	0	
<i>Aspergillus terreus</i>	Overall	0/1	0/1	0/1	0/1	1/1	0	0	0	
	Success					(100.0%)	0	0	0	
	Clinical	1/1	0/1	1/1	1/1	1/1	0	0	0	
	Success	(100.0%)		(100.0%)	(100.0%)	(100.0%)	0	0	0	
<i>Aspergillus westerdijkiae</i>	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Clinical	0	0	0	0/1	0	0	0	0	
	Success					0	0	0	0	
	Overall	0	0	0	0/1	0	0	0	0	
	Success					0	0			

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Table 67: Study ISN 9766-CL-0104 – Overall/clinical/mycological response at Day 84 by baseline MIC values (determined by the CLSI method) for *Aspergillus* species in mITT population

Isavuconazole:

Treatment	Genus Organism	Efficacy Assessment at Day 84	MIC Values (ug/ml)							
			0.25	0.5	1	2	4	8	16	>16
ISAVUCONAZOLE	Aspergillus species	Overall	2/9	3/8	4/10	2/6	2/6	1/1	0	0/1
		Success	(22.2%)	(37.5%)	(40.0%)	(33.3%)	(33.3%)	(100.0%)		
		Clinical	5/9	7/8	7/10	3/6	4/6	1/1	0	0/1
	Success	(55.6%)	(87.5%)	(70.0%)	(50.0%)	(66.7%)	(100.0%)			
	Aspergillus flavus	Overall	0/2	1/1	2/3	1/2	0/1	0	0	0
		Success		(100.0%)	(66.7%)	(50.0%)				
		Clinical	0/2	1/1	3/3	2/2	1/1	0	0	0
	Success		(100.0%)	(100.0%)	(100.0%)	(100.0%)	(100.0%)			
	Aspergillus fumigatus	Overall	2/8	3/7	2/6	1/2	0	1/1	0	0/1
		Success	(25.0%)	(42.9%)	(33.3%)	(50.0%)		(100.0%)		
		Clinical	4/8	7/7	3/6	1/2	0	1/1	0	0/1
	Success	(50.0%)	(100.0%)	(50.0%)	(50.0%)		(100.0%)			
Aspergillus niger	Overall	0	0	0	0/1	2/4	0	0	0	
	Success					(50.0%)				
	Clinical	0	0	0	0/1	2/4	0	0	0	
Success					(50.0%)					
Aspergillus terreus	Overall	0/1	0/1	0/1	0/1	0/1	0	0	0	
	Success									
	Clinical	1/1	0/1	1/1	1/1	1/1	0	0	0	
Success	(100.0%)		(100.0%)	(100.0%)	(100.0%)					
Aspergillus westerdijkiae	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
Success										
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
Success										
	Overall	0	0	0	0/1	0	0	0	0	
	Success									
	Clinical	0	0	0	0/1	0	0	0	0	
Success										

Voriconazole:

Treatment	Genus Organism	Efficacy Assessment at Day 84	MIC Values (ug/ml)							
			0.25	0.5	1	2	4	8	16	>16
	Aspergillus species	Overall	0/2	1/5	7/12	1/5	0	0	0	0
		Success		(20.0%)	(58.3%)	(20.0%)				
		Clinical	0/2	2/5	9/12	2/5	0	0	0	0
		Success		(40.0%)	(75.0%)	(40.0%)				
		Mycological	0/2	1/5	8/12	1/5	0	0	0	0
Eradication		(20.0%)	(66.7%)	(20.0%)						
	Aspergillus flavus	Overall	0	0/1	4/4	1/2	0	0	0	0
		Success			(100.0%)	(50.0%)				
		Clinical	0	1/1	3/4	1/2	0	0	0	0
		Success		(100.0%)	(75.0%)	(50.0%)				
		Mycological	0	0/1	4/4	1/2	0	0	0	0
Eradication			(100.0%)	(50.0%)						
	Aspergillus fumigatus	Overall	0/2	1/4	3/7	0/3	0	0	0	0
		Success		(25.0%)	(42.9%)					
		Clinical	0/2	1/4	5/7	1/3	0	0	0	0
		Success		(25.0%)	(71.4%)	(33.3%)				
		Mycological	0/2	1/4	4/7	0/3	0	0	0	0
Eradication		(25.0%)	(57.1%)							
	Aspergillus terreus	Overall	0	0	0/1	0	0	0	0	0
		Success								
	Aspergillus terreus	Clinical	0	0	1/1	0	0	0	0	0
		Success			(100.0%)					
		Mycological	0	0	0/1	0	0	0	0	0
Eradication										

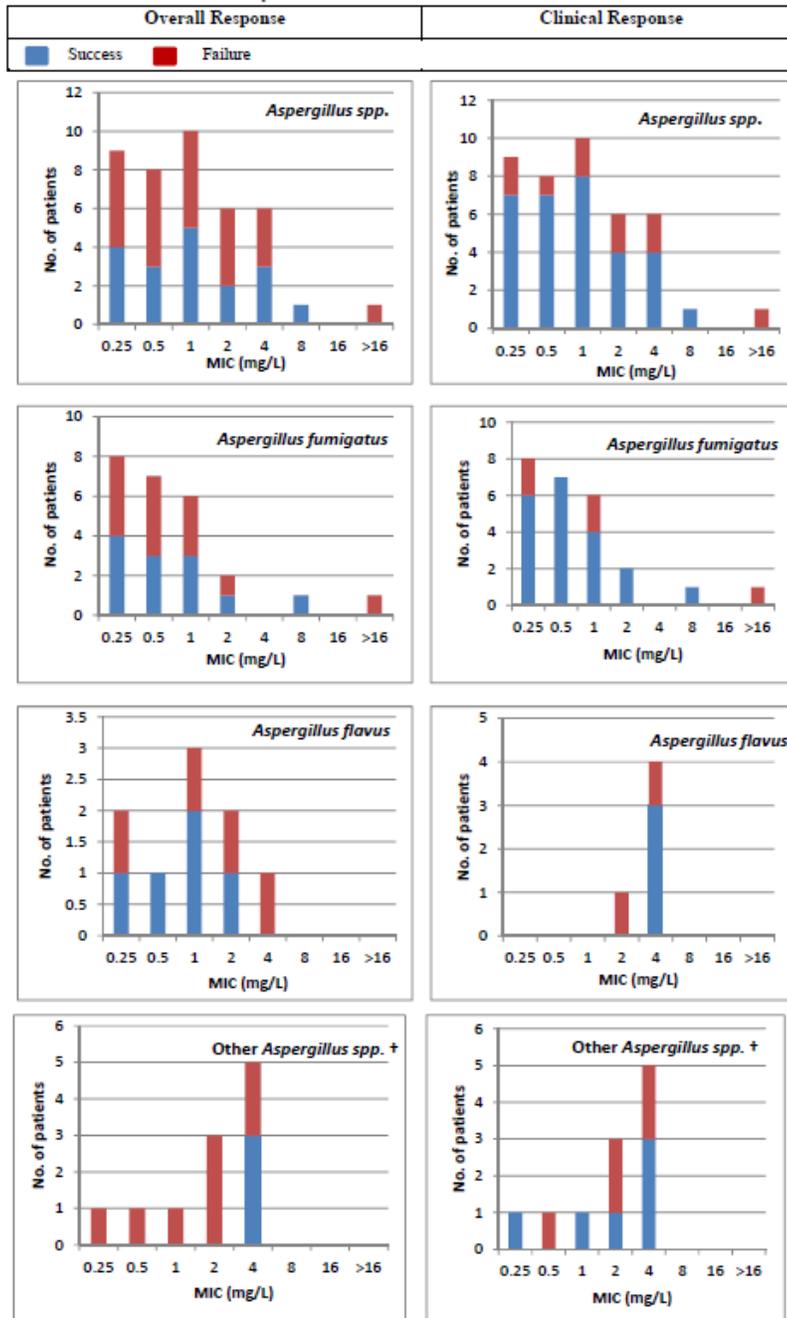
MIC: Minimum inhibitory concentration.
 Only samples collected between Day -28 and Day 7 are included in this analysis.
 Only results reported at 100% inhibition are included for this analysis.
 Source: Applicant's submission [2 patients in the voriconazole arm (*A. flavus* and *A. fumigatus* as the baseline pathogen) were considered non-evaluable by the applicant and as failure by the FDA].

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Figure 42: Study 9766-CL-0104 – Overall and clinical response at the end of treatment by isavuconazole MIC values by the CLSI method



MIC: minimum inhibitory concentration; spp: species

mITT: All ITT patients who have proven or probable invasive fungal disease as determined by the data review committee.

† Other *Aspergillus* spp. includes *terreus*, *niger*, *westerdijkiae*

Source: [9766-CL-0104 Table 12.3.6.2.1]

Source: Applicant's submission [2 patients in the voriconazole arm (*A. flavus* and *A. fumigatus* as the baseline pathogen) were considered non-evaluable by the applicant and as failure by the FDA].

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Table 68: Study ISN 9766-CL-0104 – Overall/clinical/mycological response at end of treatment, Day 42 and Day 84 by baseline MIC values (determined by the CLSI method) for *Mucor* species in modified intent to treat population

Isavuconazole									
Genus Organism	Efficacy Assessment at EOT	MIC Values (ug/ml)							
		0.25	0.5	1	2	4	8	16	>16
<i>Lictheimia</i> species	Overall	0	0	0	0	0	0	0/1	0
	Success								
	Clinical	0	0	0	0	0	0	0/1	0
	Success								
	Mycological	0	0	0	0	0	0	0/1	0
	Eradication								
<i>Lictheimia</i> corymbifera	Overall	0	0	0	0	0	0	0/1	0
	Success								
	Clinical	0	0	0	0	0	0	0/1	0
	Success								
	Mycological	0	0	0	0	0	0	0/1	0
	Eradication								

Voriconazole: No results
MIC: Minimum Inhibitory Concentration.
Only samples collected between Day -28 and Day 7 are included in this analysis.
For Isavuconazole arm, only results reported at 100% inhibition are included for this analysis.

Post-baseline (after day 7) isolates from 15 patients were tested for *in vitro* susceptibility and MICs determined. Of the 15 isolates, 12 were from isavuconazole treated patients and three from voriconazole treated patients (Table 69). For five patients (four in the isavuconazole arm and one in the voriconazole arm), there were no baseline isolates collected. The applicant stated that of the small number of patients that had repeat cultures tested for *in vitro* susceptibility to isavuconazole and voriconazole, no increase in MIC suggestive of resistance during therapy was demonstrated.” The number of isolates tested is very small. Additionally, of the 15 patients with the post-baseline isolates tested for *in vitro* susceptibility, only five were tested at Day 42 or later (three in the isavuconazole arm and two in the voriconazole arm).

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Table 69: Study ISN 9766-CL-0104 - Summary of post-treatment isolates			
Patient ID	Baseline	Post –treatment	
	Species (Day; MIC ~ µg/mL)	Species (Day; MIC ~ µg/mL)	Clinical response/Comments
Isavuconazole treatment arm – isavuconazole MICs			
200201	<i>A. flavus</i> (Day-3; 4)	<i>A. flavus</i> (Day10; 4) <i>A. flavus</i> (Day14; 2)	Resolution of all clinical symptoms at Day 42, 84, EOT
320107	<i>A. fumigatus</i> (Day-3; 1)	<i>A. fumigatus</i> (Days 7, 16, 19, and 23; 1)	Resolution of all clinical symptoms at Day 42, 84, EOT
660208	<i>A. fumigatus</i> (Day-4; 0.3)	<i>A. fumigatus</i> (Days 25 and 39; 1)	Resolution of all clinical symptoms at Day 42, 84, EOT
910502	<i>A. flavus</i> (Day-23; 1)	<i>A. flavus</i> (Day 84; 1)	No IFD; no invasive mould infection
911404	<i>A. fumigatus</i> (Day 2; 1)	<i>A. fumigatus</i> (Day 11; 1)	No resolution of any attributable clinical symptom
070606	<i>A. fumigatus</i> (Day 1; 0.25)	<i>A. fumigatus</i> (Day 70; 1)	No resolution of any attributable clinical symptom
660207	<i>A. fumigatus</i> (Day -6 and 2; 1)	<i>A. fumigatus</i> (Day 9, 43; 0.25 and 0.125, respectively) (By EUCAST method, MIC of Day 9 increased 5-fold; however Day 43 showed no change) (b) (6)	Resolution of all attributable clinical symptom
330401	No isolate	<i>A. flavus</i> (b) (6)	Patient died; failure
320444	No isolate	<i>A. fumigatus</i> (Day 20; 0.25) <i>squalene, calciferol, zymosterol, ergosterol, obstusifolial present</i>	No resolution of any attributable clinical symptom
320105	No isolate	<i>A. fumigatus</i> (Day 28; 1)	Partial resolution of any attributable clinical symptom
011805	No isolate	<i>A. fumigatus</i> (Day 45; 2)	Resolution of any attributable clinical symptom
330121	<ul style="list-style-type: none"> • <i>A. terreus</i> (Day-3; MIC 0.5) • <i>A. westerdijkiae</i> (Day 6; 2) • <i>Lichtheimia corymbifera</i> (Day 6; 16) 	<ul style="list-style-type: none"> • <i>L. corymbifera</i> (Day 13; 8) 	No resolution of any attributable clinical symptom
Voriconazole treatment arm/voriconazole MICs			
320402*	<i>A. niger</i> (Day-47; 2)*	<i>A. fumigatus</i> (Day 54; 0.5)*	Partial resolution of any attributable clinical symptom
330114	No isolate	<i>A. fumigatus</i> (Day 14; 1)	Resolution of any attributable clinical symptom
550701	<i>Fusarium fujikuroi</i> (Day -1; 4)	<i>Rhizopus oryzae</i> (Day 42; > 32) ↑ <i>squalene and calciferol, no difference in efflux pump function</i>	No resolution of any attributable clinical symptom

*Subject 320402 was listed as part of Study 103 (Day -47; MIC 2) and 104 (Day 54; MIC 0.5) in the MIC datasets

Comments

- *The study suggests that isavuconazole was effective in reducing all-cause mortality and improving clinical and microbiological responses in patients with invasive aspergillosis.*
- *The efficacy of isavuconazole was better or similar to voriconazole in patients with infections due to Aspergillus species.*
- *The number of isolates of Aspergillus species other than A. fumigatus and A. flavus identified at baseline was small for analysis.*
- *There was no correlation between the MICs or the resistance mechanism and clinical or microbiological response. This could be due to the small number of baseline isolates tested from patients enrolled in the trial.*

5.2. Study ISN 9766-CL-0103

This was a phase 3, open-label, multicenter (34 centers in the US, European Union, South America, Asia, and the Middle East), non-randomized trial of isavuconazole for the treatment of patients with aspergillosis and renal impairment or of patients with IFD caused by rare moulds, yeasts or dimorphic fungi.

Primary objective

The primary objective was to evaluate the efficacy of isavuconazole in the treatment of IA in patients with renal impairment or in patients with IFD caused by rare moulds, yeasts, or dimorphic fungi.

Secondary objectives

The secondary objectives were to characterize the safety and tolerability while assessing additional efficacy.

Exploratory objectives

- To summarize the concentration-time profiles of study drug and metabolite(s) if warranted in patients from the PK sub-study.
- To characterize PK trough values of study drug and metabolite(s) if warranted.

Study design

Inclusion criteria

Some of the inclusion criteria such as age, inclusion of non-lactating females at no risk of pregnancy, informed consent were similar as for Study ISN 9766-CL-0104. Other inclusion criteria were as follows:

- Patients in one of the following subgroups:
 - Patients with proven, probable or possible IA who had renal impairment (including dialysis), defined as calculated CLCr < 50 mL/min at enrollment who required primary therapy.
Note: Patients fulfilling the criteria for possible IA and had renal impairment were eligible for enrollment; however, diagnostic tests to confirm the IA as probable or proven by culture, histology/cytology or GM antigen were completed within 7 days after the first administration of study drug.
 - Patients meeting EORTC/MSG definition of proven or culture positive probable IFD caused by rare moulds, yeasts, or dimorphic fungi (i.e., fungal pathogens other than *A. fumigatus* or *Candida* species) whether renally impaired (RI) or not (including dialysis) who required primary therapy for their IFD at the time of enrollment.
 - Patients who had proven or probable zygomycosis, whether RI or not (including dialysis), who required primary therapy. Zygomycosis was documented by culture or histology/cytology.
 - Patients meeting EORTC/MSG definition of proven or culture positive probable IFD caused by rare moulds, yeasts, or dimorphic fungi (i.e., fungal pathogens other than *A. fumigatus* or *Candida* species), whether RI or not (including dialysis), who were refractory to current treatment defined as,
 - Clear documentation of progression of disease.
Note: radiological progression only in association with white blood cell (WBC) count recovery was not acceptable.
 - Failure to improve clinically despite receiving at least 7 days of standard antifungal regimen.
Prior to enrolling patients who fell into this category, the Medical Monitor was contacted for approval.
 - Patients meeting EORTC/MSG definition of proven or culture positive probable IFD caused by rare moulds, yeasts, or dimorphic fungi (i.e., fungal pathogens other than *A.*

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fumigatus or *Candida* species), whether RI or not (including dialysis), who were intolerant to current treatment for example:

- Doubling of serum creatinine value to higher than the upper limit of normal (ULN) within 48 hours.
- Serum creatinine > 2.0 mg/mL and current treatment with polyene or IV voriconazole.
- Other significant drug-related adverse reaction(s) to the current antifungal agent, resulting in discontinuation of the treatment, e.g., persistence of visual disturbance, allergic reaction, phototoxicity or severe infusion reaction (hypertensive crisis, severe chills or shock).
- Documented inability to achieve adequate blood levels of posaconazole, voriconazole, or itraconazole.

Details regarding the criteria used to assess if a patient had proven or probable IFD, including diagnostic tests, the presence of host factors, radiological/clinical features and mycological evidence, as well as characterization of patients as proven, probable or possible invasive fungal infections were according to EORTC/MSG guidelines and similar to that summarized above for Study ISN 9766-CL-0104.

Exclusion criteria

Some of the inclusion criteria were similar as for the Study ISN 9766-CL-0104 and include exclusion of pregnant or breast feeding women, history of allergy or hypersensitivity to azoles, patients at risk of QT prolongation, evidence of hepatic dysfunction, concomitant use of other drugs, patients with either chronic IA, aspergilloma or allergic bronchopulmonary IA, microbiological findings suggestive of different etiology, any known or suspected condition of the patient that may jeopardize adherence to the protocol requirements or impede the accurate measurement of efficacy, concomitant medical condition that were an unacceptable additional risk to the patient, treatment with any investigational drug in any clinical trial 30 days prior to the first administration of study drug, patients who were unlikely to survive 30 days, as well as patients with a BW < 40 kg. Other inclusion criteria were as follows:

- Advanced human immunodeficiency virus (HIV) infection with CD4 count < 50 or uncontrolled acquired immunodeficiency syndrome-defining condition suggesting a more sicker population compared to the patients enrolled in Study ISN 9766-CL-0104 where a CD4 count < 200 was an exclusion criterion.
- Patients who needed primary therapy for IA who have been administered more than 4 cumulative days of itraconazole, voriconazole, or posaconazole for any reason, within the 7 days prior to the first administration of study drug.
- Patients with applicable host factors who developed new evidence of IFD while on prophylactic therapy, for at least 14 days, with either an amphotericin B product or an echinocandin, were eligible for enrollment.

Although one of the exclusion criteria was enrollment of patients previously enrolled in a phase 3 isavuconazole study, the applicant stated that patients were transferred from study WSA-CS-004 if mycological testing identified zygomycetes which was not expected to be susceptible to voriconazole. One patient (add subject ID 320402 was enrolled in both studies)

Drug administration and assessments

Patients were administered a loading dose (200 mg q8h for 2 days) of isavuconazole followed by a maintenance dose (200 mg q24h) from Day 3 to EOT which is same as for the Study ISN

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9766-CL-0104 summarized above. Some changes were made in the protocol over the period of the study. For example, patients enrolled under Amendment 1 (6 patients) were treated up to a maximum of 84 days. All patients enrolled under Amendments 3 and 5 were eligible to receive treatment for a maximum of 180 days. Country-specific Amendment 4 allowed patients that derived clinical benefit to continue on treatment beyond 180 days. Minimum infusion duration of 1 hour was recommended to minimize the risk of local intolerance at the injection site.

All patients were assessed for clinical and radiological [by computed tomography scan (high resolution computed tomography if available) or magnetic resonance imaging] responses, laboratory parameters including mycological response at different time intervals (Table 70).

In amendment 3, various study procedures in the schedule of assessments were amended, clarified and added. From example, BAL fluid GM was clarified as mycological criteria for enrollment of patients with IA. The protocol was amended to classify these patients as possible versus probable cases of IFD. Additional follow-up criteria for enrollment of these patients were also added.

For all patients, the follow-up visit took place 4 weeks after the last administration of study drug, and occurred before or after Day 42 or Day 84 (i.e., the follow-up visit was not necessarily the end of study visit for a given patient). An additional follow-up visit 8 weeks after end of the EOT was made if abnormalities (e.g., adverse events) were still ongoing at the 4-week follow-up visit.

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Table 70: Study ISN 9766-CL-0103 - Schedule of assessments

Period	Screen	Study Treatment										Post-treatment		
		D -5 to -1	D1	D2	D3	D7 + 1 d	D14 ± 2 d	D28 ± 2 d	D42 ± 7 d	D84 ± 7 d	Every 4 Weeks until EOT ¹⁶ ± 7 d	D180/ EOT ¹ ± 3 d	FU1 4 Weeks after EOT ± 7 d	FU2 ⁹ 8 Weeks after EOT ± 7 d
GENERAL														
Written informed consent	X													
Inclusion/exclusion	X													
Demographics/height	X													
Body weight	X							X	X	X ¹⁸	X ¹⁴			
Pregnancy test (if applicable) ²	X						X	X	X ¹⁶	X ¹⁴		X ⁶	X ⁶	
Underlying disease or condition	X													
Infectious disease history	X													
Medical history	X													
Enrollment	X ³													
Prior and Concomitant medication	X									← ongoing →				
Non-medication procedures	X									← ongoing →				
Study drug ²⁰										← ongoing →				
Drug accountability										← ongoing →				
Hospitalization status										← ongoing →				
EFFICACY														
Investigator's assessment of overall response								X4	X4		X			
Assessment of clinical symptoms and physical findings of IFD										← ongoing →				
Mycological assessments ⁵	X			X ⁶				X ⁴	X ⁴	X	X			
Serum galactomannan antigen ⁷	X	X	X			X	X	X	X	X ¹⁰	X			
Survival Status								X	X		X ⁴	X		
Radiologic assessment of IFD ¹⁵	X ¹⁷			X ¹⁷		X ⁶	X ⁶	X ⁴	X ⁴	X ⁶	X	X ⁶		
Bronchoscopic assessment of IFD ^{8, 6}										← ongoing →				
Neutropenic status/ANC										← ongoing →				
SAFETY/OTHER														
Adverse events										← ongoing →				
Laboratory tests														
Hematology	X				X	X	X	X ¹⁶	X ¹⁶	X ¹⁶	X ¹⁴	X	X ⁹	
Biochemistry	X				X	X	X	X ¹⁶	X ¹⁶	X ¹⁶	X ¹⁴	X	X ⁹	
Urinalysis	X				X	X	X	X ¹⁶	X ¹⁶	X ¹⁶	X ¹⁴	X	X ⁹	
Whole blood sample for optional genotype analysis ¹⁹		X												
Vital signs (SBP/DBP, PR, BT) ¹⁰	X	X	X	X	X	X	X	X ¹⁶	X ¹⁶	X ¹⁶	X ¹⁴	X	X ⁹	
12-lead ECG ¹¹	X	X				X		X ¹⁶	X ¹⁶	X ¹⁶	X ¹⁴	X	X ⁹	
Physical examination	X								X6		X ¹⁴	X	X ⁹	
PHARMACOKINETIC														
Blood sampling for trough levels ¹²					X	X	X	X ¹⁶	X ¹⁶	X ¹⁶	X ^{13, 14}	X		
PK sub-study (Day 7 or 14) ¹³					X	X								

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Schedule of events is taken from the final SAP Amendment 1 (February 05, 2014)

ANC: absolute neutrophil count; BT: body temperature; CT: computed tomography; D: Study day; d: day(s); DBP: diastolic blood pressure; ECG: electrocardiogram; EOT: end of treatment; FU: follow-up; HRCT: high resolution computed tomography; IFD: invasive fungal disease; LRTD: lower respiratory tract disease; MRI: magnetic resonance imaging; PK: pharmacokinetic; PR: pulse rate; SBP: systolic blood pressure

1. EOT procedures were to be performed in all patients, including patients who withdrew.
2. Women of childbearing potential.
3. Enrollment may have occurred up to and including day 1 prior to first dosing.
4. Efficacy assessments were not required at day 42 or day 84 for patients who had stopped treatment prior to day 42 or 84 because of an unsuccessful outcome.
5. Complete mycological characterization were to be collected and reported from 4 weeks prior to study drug administration. In case of fungemia, must have documented clearance of bloodstream (two sequential negative blood cultures obtained on two separate days). If patient was suspected to have LRTD, and was able to provide sputum, sample was to be sent for culture and histology/cytology.
6. Only performed was clinically indicated.
7. Serum GM antigen (Bio-Rad Platelia™) were to be drawn at Screening and on days 1 and 2 for those patients suspected to have invasive aspergillosis. For patients with invasive aspergillosis, additional serum GM were to be done while patient was on study drug. A single value of ≥ 0.7 or 2 consecutive values of $\geq 0.5 - < 0.7$ were considered a positive result except in patients receiving concomitant amoxicillin-clavulanate, or Plasma-Lyte™ (Baxter) within 7 days prior to sampling. If Screening and day 1 occurred on the same day, 2 samples were to be obtained pre-dose at least 1 hour apart. At centers where GM testing can be performed locally, samples were to be split into two aliquots with one sample being tested locally and one sample being sent to the central laboratory. A minimum of 3 mL of serum was required for testing at the central laboratory.
8. Bronchoscopic assessments performed only as clinically indicated. During all bronchoscopic procedures if BAL specimen was sent for GM antigen detection, results were to be recorded in addition to specimen for histopathology and specimen for fungal culture. At centers where GM testing could be performed locally, BAL specimens were to be split into 2 aliquots with one sample being sent to the local lab where it was to be further aliquoted so that the local lab runs BAL GM twice with reporting in the source documents as well as in the electronic case report form, and one sample being sent to the central laboratory. A minimum of 3 mL of BAL fluid was required for testing. For BAL GM of ≥ 1.0 (2 aliquots of the same sample should be tested) was considered a probable invasive aspergillosis infection. Plasma-Lyte™ (Baxter) may not have been used as lavage fluid.
9. Only in patients with abnormalities observed at previous visit.
10. During treatment with IV study drug on days 1-3, vital signs were to be measured within 1 hour before and 1 hour after study drug infusion. From day 4 onwards of IV treatment and during inpatient treatment with oral study drug, vital signs were to be measured once prior to study drug administration.
11. ECG was to be performed 15 minutes prior to the end of the first infusion of the day or approximately 3 hours post first oral dose. If Screening and day 1 occurred on the same day, one ECG was to be performed prior to dosing and an additional performed either 15 minutes prior to the end of the first infusion of the day or approximately 3 hours post oral dose.
12. Trough samples were to ideally be taken immediately prior to dosing but must be taken no earlier than one hour before dosing.
13. For EOT, blood sample was to be prior (trough) to the last dose or if not collected as trough, obtained 24 hours after the last dose.
14. Required at EOT only.
15. Patients with fungemia would not require radiology, however patient with LRTD, CNS infection or invasive sinonasal infection would require radiology. Subsequent radiological examination of the site of IFD were to utilize the same CT/HRCT/or MRI modality as the baseline radiologic exam.
16. Only required for patients who were currently receiving study drug.
17. If plain x-ray was used as evidence of disease it was to be confirmed by CT scan (or HRCT) or MRI within 7 days after the first administration of study drug.
18. Only required at first 4-week visit for patients continuing on study drug after day 84.
19. A separate patient consent was required (see Protocol Sections 8.2.4.2 and 8.2.4.3 for further instruction).
20. Oral medication was supplied in kits sufficient for 1 week of dosing. During the informed consent process the Investigator should discuss with the patient the need to return to the study center between visits for oral drug re-supply. Patients were to be given an oral drug dosing log to take home and complete. This log was to be reviewed by center staff at each oral drug dispensing visit.

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• ***Mycological Assessments***

Mycological assessments were same as for the Study ISN 9766-CL-0104 and included

- fungal culture, histology/cytology.
- GM antigen detection by Bio-Rad Platelia™ *Aspergillus* EIA in serum as well as BAL fluid.
- *in vitro* susceptibility testing.

The central laboratories used for testing clinical specimens were also same as for the Study ISN 9766-CL-0104 (Table 55).

• ***Definition of clinical, radiological, and mycological response***

Complete and partial overall responses were classified as a successful overall outcome (same as for the Study ISN 9766-CL-0104). Like for the Study ISN 9766-CL-0104, DRC was established to adjudicate (independently from the Applicant and the Investigator) the categorization of each patient's IFD at enrollment (including data up to Day 7 as relevant) and to evaluate clinical, mycological, radiological and overall response at EOT, Day 42, and Day 84, as well as to assess attributable mortality.

Results

Of the 149 patients enrolled in the study, 146 patients (98.0%) received at least 1 dose of isavuconazole and were included in the ITT population; 140 of the 146 subjects were in the mITT population (3 patients (2.1%) were assessed by the DRC as having no IFD and 3 patients (2.1%) were assessed by the DRC as having possible IFD (Table 71). *Aspergillus* species was identified in 24 patients (16.1%) and Mucorales in 37 (24.8%) patients.

Table 71: Study ISN 9766-CL-0103 - Patient Disposition and Analysis Set RI

	RI (n = 59)	NRI (n = 90)	Total (n = 149)
Signed informed consent			149
Enrolled	59 (100.0%)	90 (100.0%)	149 (100.0%)
Intent-to-Treat (ITT)	59 (100.0%)	87 (96.7%)	146 (98.0%)
Modified Intent-to-Treat (mITT)	54 (91.5%)	86 (95.6%)	140 (94.0%)
mITT-Mucorales	11 (18.6%)	26 (28.9%)	37 (24.8%)
mITT- <i>Aspergillus</i>	20 (33.9%)	4 (4.4%)	24 (16.1%)
mITT-Other filamentous fungi (not <i>Aspergillus</i> or Mucorales)	9 (15.3%)	8 (8.9%)	17 (11.4%)
mITT-Mold species not otherwise specified	5 (8.5%)	2 (2.2%)	7 (4.7%)
mITT-Dimorphic fungi	2 (3.4%)	27 (30.0%)	29 (19.5%)
mITT- <i>Non-Candida</i> yeast	4 (6.8%)	7 (7.8%)	11 (7.4%)
mITT-Mixed infection	3 (5.1%)	12 (13.3%)	15 (10.1%)
Safety (SAF)	59 (100.0%)	87 (96.7%)	146 (98.0%)
Pharmacokinetics (PKAS)	54 (91.5%)	84 (93.3%)	138 (92.6%)

Renal impairment was defined at baseline as eGFR < 60 mL/min/1.73 m² by the MDRD formula.

eGFR: estimated glomerular filtration rate; ITT: intent-to-treat; MDRD: Modification of Diet in Renal Disease; mITT: modified ITT; NRI: not renally impaired; PKAS: All ITT patients who have at least one isavuconazole plasma concentration; RI: renally impaired; SAF: Safety Analysis Set.

ITT = Intent-to-treat population consisted of patients who received at least one dose of trial medication.

mITT = Modified ITT population consisted of a subset of the ITT population of patients who had confirmation of definite or probable diagnosis (based on applicant's criteria and FDA's criteria of mycological findings serum or BAL GM findings as the sole microbiological criteria) of invasive aspergillosis.

In addition to the 149 patients enrolled, 1 patient was entered in the study and took study drug; however at the time the patient was enrolled, the site did not have local EC approval. The EC requested that this patient be removed from the study database.

DRC assessments

***Aspergillus* infection:**

DRC assessed 24 patients as having only *Aspergillus* infection; 9 patients (37.5%) were proven and 15 patients (62.5%) probable invasive aspergillosis. In addition to these 24 patients, 11 patients had *Aspergillus* infection in combination with an additional fungal pathogen. *A. fumigatus* was the most common pathogen identified (Table 72). There were 3 subjects in which no pathogen was identified but were GM positive. All-cause mortality through Day 42 and Day 84 was 12.5% and 25%, respectively (Table 72).

Table 72: Study ISN 9766-CL-0103 – (A) DRC assessment of pathogen causing IFD at baseline and (B) all-cause mortality (mITT- <i>Aspergillus</i> population)			
A: DRC assessment of pathogen causing IFD at baseline			
	RI (n = 20)	NRI (n = 4)	Total (n = 24)
Pathogen			
<i>Aspergillus fumigatus</i>	9 (45.0%)	1 (25.0%)	10 (41.7%)
<i>Aspergillus flavus</i>	4 (20.0%)	1 (25.0%)	5 (20.8%)
<i>Aspergillus</i> NOS	3 (15.0%)	0	3 (12.5%)
<i>Aspergillus niger</i>	0	1 (25.0%)	1 (4.2%)
<i>Aspergillus terreus</i>	0	1 (25.0%)	1 (4.2%)
<i>Aspergillus versicolor</i>	1 (5.0%)	0	1 (4.2%)
No Pathogen Identified	3 (15.0%)	0	3 (12.5%)
Serum GM Positive only	2 (10.0%)	0	2 (8.3%)
BAL GM Positive only	1 (5.0%)	0	1 (4.2%)
Renal impairment was defined at baseline as eGFR < 60 mL/min/1.73 m ² by the MDRD formula. BAL: bronchoalveolar lavage; DRC: Data Review Committee; eGFR: estimated glomerular filtration rate; GM: galactomannan; MDRD: Modification of Diet in Renal Disease; mITT: modified intent-to-treat; NOS: not otherwise specified; NRI: not renally impaired; RI: renally impaired.			
B: All-cause mortality through Day 42 and Day 84			
	RI (n = 20)	NRI (n = 4)	Total (n = 24)
Outcome			
All-cause Mortality Through Day 42[†]	3 (15.0%)	0	3 (12.5%)
Deaths	3 (15.0%)	0	3 (12.5%)
All-cause Mortality Through Day 84[†]	5 (25.0%)	1 (25.0%)	6 (25.0%)
Deaths	5 (25.0%)	1 (25.0%)	6 (25.0%)
Renal impairment was defined at baseline as eGFR < 60 mL/min/1.73 m ² by the MDRD formula. eGFR: estimated glomerular filtration rate; MDRD: Modification of Diet in Renal Disease; mITT: modified intent-to-treat; NRI: not renally impaired; RI: renally impaired. [†] A patient with a last known survival status before day 42 or before day 84, or missing, with the last assessment day before day 42 or before day 84, was counted as a death.			

• ***Mucorales* infection**

DRC assessed 37 patients as having a proven or probable *Mucorales* infection, with 32 patients (86.5%) having proven and 5 patients (13.5%) probable invasive mucormycosis. Of the 37 patients, 21 were stated to be primary therapy, 11 refractory, and 5 intolerant (Table 73). The number of patients with any *Mucorales* species was small for analysis (Table 73A). All-cause mortality through Day 42 in the mITT-*Mucorales* population occurred in 14 patients (38%) and at Day 84 16 patients (43%); all-cause mortality for primary therapy patients occurred in 7 patients (33%) at Day 42 and 9 patients (43%) at Day 84 in the mITT-*Mucorales* population (Table 73B).

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Table 73: Study ISN 9766-CL-0103 – (A) DRC assessment of pathogen causing IFD at baseline and (B) all-cause mortality through Days 42 and 84 (mITT-Mucorales population)

A: DRC assessment

	Primary Therapy (n = 21)	Refractory (n = 11)	Intolerant (n = 5)	Total (n = 37)
Pathogen				
<i>Mucormycetes</i> NOS	6 (28.6%)	5 (45.5%)	2 (40.0%)	13 (35.1%)
<i>Mucor</i> NOS	7 (33.3%)	0	0	7 (18.9%)
<i>Rhizopus oryzae</i>	4 (19.0%)	3 (27.3%)	0	7 (18.9%)
<i>Rhizomucor spp.</i>	2 (9.5%)	2 (18.2%)	1 (20.0%)	5 (13.5%)
<i>Absidia corymbifera</i>	2 (9.5%)	0	0	2 (5.4%)
<i>Rhizopus</i> NOS	0	1 (9.1%)	1 (20.0%)	2 (5.4%)
<i>Cunninghamella spp.</i>	0	0	1 (20.0%)	1 (2.7%)

DRC: Data Review Committee; mITT: modified intent-to-treat; NOS: not otherwise specified.

B: All-cause mortality through Day 42 and Day 84

	Primary Therapy (n = 21)	Refractory (n = 11)	Intolerant (n = 5)	Total (n = 37)
Outcome				
All-cause Mortality Through Day 42†	7 (33.3%)	5 (45.5%)	2 (40.0%)	14 (37.8%)
Deaths	7 (33.3%)	4 (36.4%)	2 (40.0%)	13 (35.1%)
Unknown Survival Status	0	1 (9.1%)	0	1 (2.7%)
All-cause Mortality Through Day 84†	9 (42.9%)	5 (45.5%)	2 (40.0%)	16 (43.2%)
Deaths	9 (42.9%)	4 (36.4%)	2 (40.0%)	15 (40.5%)
Unknown Survival Status	0	1 (9.1%)	0	1 (2.7%)

† A patient with the last known survival status was before day 42 or before day 84 or missing and the last assessment day was before day 42 or before day 84 was counted as death

• **Clinical and microbiological response by baseline pathogen**

A. fumigatus, *R. oryzae*, and *Mucormycetes* (species not identified) were the most common pathogens identified (Tables 74 and 75). The number of patients with other filamentous fungi were small (<10) for analysis. All-cause mortality rate at Day 42 and Day 84 were higher in patients with *Aspergillus* species as a baseline pathogen in this study compared to subjects treated with isavuconazole in Study ISN 9766-CL-0104 (Tables 75 and 63). Similarly, clinical response was lower in this study compared to isavuconazole treated subjects in the mITT population in Study ISN 9766-CL-0104 (Tables 75 and 63). Such differences could be due to sicker patient population in Study ISN 9766-CL-0103 compared to Study ISN 9766-CL-0104.

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Table 74: Study ISN 9766-CL-0103 – All-cause mortality, clinical response and mycological response by baseline pathogen in mITT population

Baseline Pathogen	Day 42			Day 84		
	All-cause mortality n/N (%)	Clinical Response n/N (%)	Mycological Response n/N (%)	All-cause mortality n/N (%)	Clinical Response n/N (%)	Mycological Response n/N (%)
Single infection						
Aspergillus species						
<i>A. flavus</i>	0/5	4/5	3/5	1/5	3/5	3/5
<i>A. fumigatus</i>	2/10 (20%)	5/10 (50%)	3/10 (30%)	3/10 (30%)	5/10 (50%)	3/10 (30%)
<i>A. niger</i>	0/1	1/1	1/1	1/1	0/1	0/1
<i>A. terreus</i>	0/1	1/1	1/1	0/1	0/1	0/1
<i>A. versicolor</i>	0/1	1/1	1/1	0/1	0/1	0/1
<i>Aspergillus</i> NOS	1/3	0/3	0/3	1/3	0/3	0/3
<i>Aspergillus</i> species (Total)	3/21 (14.3)	12/21 (57.1)	9/21 (42.9)	6/21 (28.6)	8/21 (38.1)	6/21 (28.6)
Mucorales						
<i>Lichtheimia corymbifera</i>	1/2	1/2	0/2	2/2	0/2	0/2
<i>Mucor amphibiorum</i>	0/1	0/1	0/1	0/1	0/1	0/1
<i>Mucor</i> NOS	1/5	4/5	0/5	1/5	4/5	0/5
Mucormycetes NOS	3/13 (23.1)	6/13 (46.2)	1/13 (7.7)	4/13 (30.8)	5/13 (41.7)	2/13 (15.4)
<i>Rhizomucor pusillus</i>	3/4	1/4	0/4	3/4	0/4	0/4
<i>Rhizomucor</i>	1/1	0/1	0/1	1/1	0/1	0/1
<i>R. azygosporus</i>	0/1	0/1	0/1	0/1	0/1	1/1
<i>R. microsporus</i>	0/1	1/1	0/1	0/1	1/1	1/1
<i>R. oryzae</i>	5/7	1/7	0/7	5/7	1/7	2/7
Mucorales (Total)	14/35 (40.0)	14/35 (40.0)	1/35 (2.9)	16/35 (45.7)	11/35 (31.4)	6/35 (17.1)
Mixed infection						
<i>A. flavus</i> + <i>Fusarium</i> NOS + <i>A. fumigatus</i>	0/1	0/1	0/1	0/1	0/1	0/1
<i>A. flavus</i> + <i>Lichtheimia corymbifera</i>	0/1	1/1	0/1	1/1	0/1	0/1
<i>A. flavus</i> + <i>Candida glabrata</i>	1/1	0/1	0/1	1/1	0/1	0/1
<i>A. fumigatus</i> + <i>Phaeoacremonium</i> NOS	0/1	0/1	0/1	0/1	0/1	0/1
<i>A. fumigatus</i> + <i>Verticillium tricorpus</i>	0/1	0/1	0/1	1/1	0/1	0/1
<i>A. niger</i> + <i>Fonsecaea monophora</i>	0/1	1/1	0/1	0/1	1/1	0/1
<i>A. niger</i> + <i>Mucormycetes</i> NOS	0/1	1/1	0/1	0/1	1/1	0/1
<i>A. niger</i> + <i>R. oryzae</i>	0/2	2/2	1/2	0/2	2/2	1/2
<i>A. terreus</i> + <i>R. oryzae</i>	1/1	0/1	0/1	1/1	0/1	0/1
<i>Mucor circinelloides</i> + <i>Fusarium solani</i>	1/1	0/1	0/1	1/1	0/1	0/1
<i>Aspergillus</i> NOS + <i>Mucormycetes</i> NOS	0/1	0/1	0/1	0/1	0/1	0/1
<i>Rhizopus</i> NOS + <i>Scedosporium prolificans</i>	0/1	1/1	1/1	0/1	1/1	1/1
NOS=not otherwise specified						

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Table 75: Study ISN 9766-CL-0103 – All-cause mortality, clinical response and mycological response by baseline pathogen (irrespective of single or mixed infections) in mITT population with baseline <i>Aspergillus</i> or Mucorales infection						
Baseline Pathogen	Day 42			Day 84		
	All-cause mortality n/N (%)	Clinical Response n/N (%)	Mycological Response n/N (%)	All-cause mortality n/N (%)	Clinical Response n/N (%)	Mycological Response n/N (%)
Aspergillus species						
<i>A. flavus</i>	1/8	5/8	3/8	3/8	3/8	3/8
<i>A. fumigatus</i>	2/13 (15.4)	5/13 (38.5)	3/13 (23.1)	4/13 (30.8)	5/13 (38.5)	3/13 (23.1)
<i>A. niger</i>	0/5	5/5	2/5	1/5	4/5	1/5
<i>A. terreus</i>	1/2	1/2	1/2	1/2	0/2	0/2
<i>A. versicolor</i>	0/1	1/1	1/1	0/1	0/1	0/1
<i>Aspergillus</i> NOS	1/4	0/4	0/4	1/4	0/4	0/4
Aspergillus species (Total)	5/33 (15.2)	17/33 (51.5)	10/33 (30.3)	10/33 (30.3)	12/33 (36.4)	7/33 (21.2)
Mucorales						
<i>Lichtheimia corymbifera</i>	1/3	2/3	0/3	3/3	0/3	0/3
<i>Mucor amphibiorum</i>	0/1	0/1	0/1	0/1	0/1	0/1
<i>Mucor circinelloides</i>	1/1	0/1	0/1	1/1	0/1	0/1
<i>Mucor</i> NOS	1/5	4/5	0/5	1/5	4/5	0/5
Mucormycetes NOS	3/15 (20.0)	7/15 (46.7)	1/15 (6.7)	4/15 (26.7)	5/15 (33.3)	2/15 (13.3)
<i>Rhizomucor pusillus</i>	3/4	1/4	0/4	3/4	0/4	0/4
<i>Rhizomucor</i>	1/1	0/1	0/1	1/1	0/1	0/1
<i>R. azygosporus</i>	0/1	0/1	0/1	0/1	0/1	1/1
<i>R. microsporus</i>	0/1	1/1	0/1	0/1	1/1	1/1
<i>R. oryzae</i>	6/10	3/10	1/10	6/10	3/10	3/10
<i>Rhizopus</i> NOS	0/1	1/1	1/1	0/1	1/1	1/1
Mucorales (Total)	16/43 (37.2)	19/43 (44.2)	3/43 (7.0)	19/43 (44.2)	14/43 (32.6)	8/43 (18.7)
NOS=not otherwise specified						

- In vitro* susceptibility of baseline pathogens and clinical and microbiological response by MIC of the baseline pathogen**

Clinical fungal isolates from patients in the study with positive culture were tested for *in vitro* susceptibility at a Central Laboratory (for details see section 3.2.2.4. above); all testing was performed in accordance with the CLSI M38-A2⁵ and the EUCAST⁶ methods. Isavuconazole MICs against *Aspergillus* species ranged from 0.25 to 8 µg/mL by the CLSI method and 0.12 to 32 µg/mL by the EUCAST method (Table 76). Against Mucorales, the isavuconazole MICs ranged from 0.25 to 32 µg/mL by both the CLSI method EUCAST methods (Table 76). Overall, the isavuconazole MICs were in the same range as for Study ISN 9766-CL-0104.

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Table 76: Study ISN 9766-CL-0103 - MIC Values for fungal isolates of *Aspergillus* and Mucorales species by the CLSI and EUCAST methods (mITT-*Aspergillus* Population)

Aspergillus species

Organism Genus (No. isolates)		AmB	CAS	ISA	POSA	VRC
CLSI Standard						
<i>Aspergillus</i> spp. (26)	MIC Range (µg/mL)	0.5, 4	0.25, 1	0.25, 8	0.03, 32	0.03, 8
	MIC50	1	0.5	1	0.5	1
	MIC90	4	0.5	8	1	4
<i>Aspergillus flavus</i> (8)	MIC Range (µg/mL)	1, 4	0.25, 1	0.5, 2	0.25, 2	0.06, 4
	<i>Aspergillus fumigatus</i> (13)	MIC Range (µg/mL)	0.5, 4	0.25, 1	0.25, 2	0.06, 1
	MIC50	1	0.5	1	0.5	1
	MIC90	4	0.5	1	0.5	2
<i>Aspergillus niger</i> (5)	MIC Range (µg/mL)	0.5, 1	0.25, 0.5	0.25, 8	0.03, 32	0.03, 8
EUCAST Standard						
<i>Aspergillus</i> spp. (26)	MIC Range (µg/mL)	1, 4	0.12, 0.25	0.12, 32	0.06, 1	0.25, 32
	MIC50	4	0.25	1	0.5	2
	MIC90	4	0.25	4	1	8
<i>Aspergillus flavus</i> (8)	MIC Range (µg/mL)	2, 4	0.12, 0.25	0.25, 32	0.5, 1	1, 8
<i>Aspergillus fumigatus</i> (13)	MIC Range (µg/mL)	1, 4	0.12, 0.25	0.12, 2	0.25, 1	0.5, 32
	MIC50	4	0.25	0.5	0.5	1
	MIC90	4	0.25	2	1	8
<i>Aspergillus niger</i> (5)	MIC Range (µg/mL)	1, 2	0.12, 0.25	0.12, 4	0.06, 1	0.25, 8

Mucorales

Organism Genus (No. isolates)		AmB	CAS	ISA	POSA	VRC
CLSI Standard						
<i>Mucor circinelloides</i> (1)	MIC Range (µg/mL)	0.5	128	32	32	16
<i>Actinomucor elegans</i> (1)	MIC Range (µg/mL)	0.5	128	0.25	0.25	8
<i>Lichtheimia (Absidia) corymbifera</i> (3)	MIC Range (µg/mL)	0.5, 1	32, 128	8, 16	0.5, 1	32, 64
	<i>Rhizomucor pusillus</i> (5)	MIC Range (µg/mL)	0.25, 8	64	8, 32	0.5, 1
<i>Rhizopus azygosporus</i> (1)	MIC Range (µg/mL)	1	0.12	1	1	4
<i>Rhizopus microsporus</i> (1)	MIC Range (µg/mL)	4	128	16	32	32
<i>Rhizopus oryzae</i> (10)	MIC Range (µg/mL)	0.5, 4	32, 128	0.5, 32	0.25, 32	4, 32
	MIC50	0.5	128	2	1	16
	MIC90	2	128	32	32	32
EUCAST Standard						
<i>Mucor circinelloides</i> (1)	MIC Range (µg/mL)	2	64	32	32	16
<i>Actinomucor elegans</i> (1)	MIC Range (µg/mL)	2	128	4	0.5	32
<i>Lichtheimia (Absidia) corymbifera</i> (3)	MIC Range (µg/mL)	0.25, 2	32, 128	8, 16	0.5, 1	32, 64
	<i>Rhizomucor pusillus</i> (5)	MIC Range (µg/mL)	0.25, 1	32, 64	4, 32	1
<i>Rhizopus azygosporus</i> (1)	MIC Range (µg/mL)	2	1	0.5	1	2
<i>Rhizopus microsporus</i> (1)	MIC Range (µg/mL)	2	128	4	8	16
<i>Rhizopus oryzae</i> (10)	MIC Range (µg/mL)	0.25, 4	32, 128	0.25, 32	0.5, 32	16, 32
	MIC50	2	128	4	2	32
	MIC90	4	128	32	32	32

In this summary, if an MIC value was reported as > ULOQ, then the MIC value was imputed as 2 times the ULOQ (i.e., one 2-fold dilution higher). If an MIC value is reported as ≥ ULOQ or ≤ LLOQ, then the MIC value was imputed as the ULOQ or LLOQ.

MIC₅₀ and MIC₉₀ values were not calculated when the number of isolates is < 10.

AmB: amphotericin B; CAS: caspofungin; CLSI: Clinical Laboratory Standards Institute; EUCAST: European Committee for Antimicrobial Susceptibility Testing; ISA: isavuconazole; ITT: intent-to-treat; LLOQ: lower limit of quantitation; MIC: minimum inhibitory concentration; VRC: voriconazole; POSA: posaconazole; ULOQ: upper limit of quantitation.

The clinical and mycological responses were compared with the MIC values of baseline isolates from subjects in the mITT population. There was no correlation between MICs of baseline isolates and clinical or mycological response at any of the time points that includes end of treatment, Day 42, and Day 84 (Table 77; Figure 43). This analysis is limited by the small number of isolates for each species.

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Table 77: Study ISN 9766-CL-0103 - Overall/clinical/mycological response at end of treatment by baseline MIC values (determined by the CLSI) for *Aspergillus* species method in mITT population

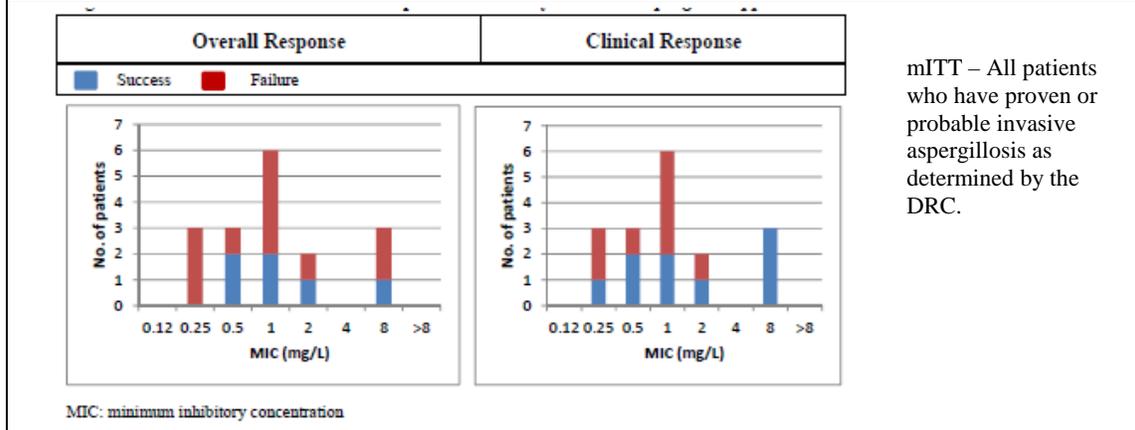
<i>Aspergillus</i> species		Efficacy Assessment at EOT	MIC Values (ug/ml)						
Genus Organism	<=0.12		0.25	0.5	1	2	4	8	>8
<i>Aspergillus</i> species	Overall	0	0/3	2/3 (66.7%)	2/6 (33.3%)	1/2 (50.0%)	0	1/3 (33.3%)	0
	Success								
	Clinical	0	1/3 (33.3%)	2/3 (66.7%)	2/6 (33.3%)	1/2 (50.0%)	0	3/3 (100.0%)	0
	Success								
<i>Aspergillus flavus</i>	Overall	0	0	1/2 (50.0%)	2/3 (66.7%)	0/1	0	0	0
	Success								
	Clinical	0	0	1/2 (50.0%)	2/3 (66.7%)	0/1	0	0	0
	Success								
<i>Aspergillus fumigatus</i>	Overall	0	0/2	1/1 (100.0%)	0/3	1/1 (100.0%)	0	0	0
	Success								
	Clinical	0	1/2 (50.0%)	1/1 (100.0%)	0/3	1/1 (100.0%)	0	0	0
	Success								
<i>Aspergillus niger</i>	Overall	0	0/1	0	0	0	0	1/3 (33.3%)	0
	Success								
	Clinical	0	0/1	0	0	0	0	3/3 (100.0%)	0
	Success								
	Overall	0	0/1	0	0	0	0	1/3 (33.3%)	0
	Success								
	Clinical	0	0/1	0	0	0	0	3/3 (100.0%)	0
	Success								
	Overall	0	0/1	0	0	0	0	1/3 (33.3%)	0
	Success								
	Clinical	0	0/1	0	0	0	0	3/3 (100.0%)	0
	Success								

Mucorales		Efficacy Assessment at EOT	MIC Values (ug/ml)						
Genus Organism	<=0.12		0.25	0.5	1	2	4	8	>8
<i>Lichtheimia</i> species	Overall	0	0	0	0	0	0	1/2 (50.0%)	0/1
	Success								
	Clinical	0	0	0	0	0	0	1/2 (50.0%)	0/1
	Success								
<i>Lichtheimia (absidia) corymbifera</i>	Overall	0	0	0	0	0	0	1/2 (50.0%)	0/1
	Success								
	Clinical	0	0	0	0	0	0	1/2 (50.0%)	0/1
	Success								
<i>Mucor</i> species	Overall	0	0	0	0	0	0	0	0/1
	Success								
	Clinical	0	0	0	0	0	0	0	0/1
	Success								
<i>Mucor circinelloides</i>	Overall	0	0	0	0	0	0	0	0/1
	Success								
	Clinical	0	0	0	0	0	0	0	0/1
	Success								
<i>Rhizomucor</i> species	Overall	0	0	0	0	0	0	0/2	0/1
	Success								
	Clinical	0	0	0	0	0	0	1/2 (50.0%)	0/1
	Success								
<i>Rhizomucor pusillus</i>	Overall	0	0	0	0	0	0	0/2	0/1
	Success								
	Clinical	0	0	0	0	0	0	1/2 (50.0%)	0/1
	Success								
<i>Rhizopus</i> species	Overall	0	0	0/1	1/2 (50.0%)	0/2	0/1	0	1/5 (20.0%)
	Success								
	Clinical	0	0	0/1	0/2	0/2	0/1	0	1/5 (20.0%)
	Success								
<i>Rhizopus azygosporus</i>	Overall	0	0	0	1/1 (100.0%)	0	0	0	0
	Success								
	Clinical	0	0	0	0/1	0	0	0	0
	Success								
<i>Rhizopus microsporus</i>	Overall	0	0	0	0	0	0	0	0/1
	Success								
	Clinical	0	0	0	0	0	0	0	1/1 (100.0%)
	Success								
<i>Rhizopus oryzae</i>	Overall	0	0	0/1	0/1	0/2	0/1	0	1/4 (25.0%)
	Success								
	Clinical	0	0	0/1	0/1	0/2	0/1	0	0/4
	Success								
	Overall	0	0	0/1	0/1	0/2	0/1	0	1/4 (25.0%)
	Success								
	Clinical	0	0	0/1	0/1	0/2	0/1	0	0/4
	Success								

MIC: Minimum Inhibitory Concentration.
Only samples collected on or prior to Day 7 were considered as baseline and included in this analysis.

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Figure 43: Study 9766-CL-0103 - Overall and clinical response at EOT by MIC for *Aspergillus* species



Aspergillus or Mucorales isolates from 5 patients were collected between Days 8 and 26 post-treatment (Table 78). No baseline isolate was collected from 2 of the 5 patients. The number of isolates was too small to evaluate development of resistance post-treatment.

Patient ID	Baseline	Post –treatment	
	Species (Day; MIC ~ µg/mL)	Species (Day; MIC ~ µg/mL)	Clinical response/Comments
970903	<i>R. oryzae</i> (-6; 0.5)	<i>A. niger</i> (26; 2)	Stable - failure
320405	-	<i>A. fumigatus</i> (9; 1)	Stable - failure
	-	<i>A. fumigatus</i> (9; 1)	
110506	-	<i>R. pusillus</i> (9; 16)	Progression - Failure
070202	<i>A. flavus</i> (-1; 0.5)	<i>A. flavus</i> (21; 2)	Failure – patient died by (b) (6)
	<i>Lichtheimia corymbifera</i> (-1; 8)		
070201	<i>R. pusillus</i> (-6; 8) ↑ calciferol	<i>R. pusillus</i> (14; >16)	Failure – patient died by (b) (6)

*Subject 320402 was listed as part of Study ISN 9766-CL-0104 and was treated with voriconazole. In Study ISN 9766-CL-103 (Day -47; MIC 2) and 104 (Day 54; MIC 0.5) in the MIC datasets

Comments:

- The study suggests that isavuconazole was effective in reducing all-cause mortality and improving clinical and microbiological response in patients with invasive aspergillosis or Mucorales infections. The all-cause mortality in patients with invasive aspergillosis was higher and clinical response lower in Study ISN 9766-CL-0103 compared to Study ISN 9766-CL-0104. This could be due to sicker patients enrolled in the clinical trial as one of the exclusion criteria for Study ISN 9766-CL-0103 was CD4 cell count < 50; for Study ISN 9766-CL-0104, the exclusion criteria was CD4 cell count < 200. However, the actual CD4 cell count was not included in the submission.
- The number of isolates of *Aspergillus* or Mucorales species identified at baseline was too small for analysis.
- There was no correlation between the MICs and clinical or microbiological response. This could be due to the small number of baseline isolates tested from patients enrolled in the trial.
- The numbers of post-treatment isolates collected were small and insufficient to evaluate resistance.

6. INTERPRETIVE CRITERIA/BREAKPOINTS

6.1. *Aspergillus* species

The applicant proposed clinical interpretive criteria / breakpoints for isavuconazole MICs (by the CLSI-M38A2 method⁵) for *A. fumigatus* species only (Table 79).

Pathogen	Broth Microdilution at 48 hours (MIC in µg/mL)		
	Susceptible (S)	Intermediate (I)	Resistant (R)
<i>Aspergillus fumigatus</i>	≤ 1	2	≥ 4

This is based on

- an evaluation of the epidemiological cut-off values based on *in vitro* susceptibility testing,
- PK-PD modeling ~ *in vitro* and in animal models,
- an assessment of patient outcome by MIC,
- efficacy in isolates with resistant mechanism identified ~ clinical response
- isavuconazole population PK analysis, and
- exposure-response analysis for patients in phase 3 studies with *Aspergillus* infection.

6.1.1. Epidemiological cut-off values

As discussed in section 3.2.4.1. above, the ECV for *A. fumigatus* is 1 µg/mL. Against *A. flavus*, *A. niger*, and *A. terreus*, ECVs were 1 µg/mL, 4 µg/mL, and 1 µg/mL, respectively.

6.1.2. Pharmacokinetic/pharmacodynamic modeling ~ *in vitro* and in animal models

Using the population PK analysis, Monte Carlo simulations were performed by using mean population estimates from the best 2-compartment model with covariates, for three studies that include an *in vitro* dynamic model study, non-neutropenic disseminated aspergillosis murine model study, and neutropenic pulmonary aspergillosis murine model study.

In the *in vitro* dynamic model study,²⁶ strains included for testing were two *cyp51A* mutant strains [F/16216 (L98H substitution and isavuconazole MIC mode = 2 µg/mL) and strain F/11628 (G138C substitution and isavuconazole MIC mode = 4 µg/mL)] of *A. fumigatus*, and 2 WT strains with isavuconazole mode MIC of 1 µg/mL. Suppression of GM was achieved for the two WT strains as well as strain F/16216 at the highest dose tested which was within clinically relevant systemic exposures. However, sufficient GM index suppression, in such an early infection model, was not maintained for the strain F/11628 mutant with an MIC of 4 µg/mL (for details see section 3.3.1. above). The % target attainment based on AUC:MIC that represented 90% effective PD index was 11.2 (Table 80).

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Table 80: Probability of target attainment – MICs by the CLSI method

MIC (mg/L)	% Target Attainment (PD Target EI ₉₀ : AUC/MIC - 11.2)†	% Target Attainment (PD Target EI ₅₀ : AUC/MIC - 50.48)‡	% Target Attainment (PD Target Net Stasis: AUC/MIC - 503)§
0.03	100%	100%	100%
0.06	100%	100%	99.80%
0.125	100%	100%	87.44%
0.25	100%	100%	27.32%
0.5	100%	99.98%	0.58%
1	100%	95.42%	0%
2	100%	49.66%	0%
4	97.5%	2.92%	0%
8	74.16%	0.04%	0%
16	5.86%	0%	0%
32	0.06%	0%	0%

PD: pharmacodynamic

The EI₉₀ and EI₅₀ represent the 90% and 50% effective pharmacodynamic index (EI).

† Box H *et.al.* (2014)²⁶

‡ Seyedmousavi S. (2014)³³

§ Lepak AJ *et al.* (2013)³⁶

It appears that total drug concentrations were used for analysis. Since the drug is 99% protein bound and only 0.01% is free, the AUC should be multiplied by 0.01.

Source: 9766-PK-0005, Table 5.

In the non-neutropenic disseminated aspergillosis murine model study,³³ 3 *cyp51A* mutants of *A. fumigatus* were studied:

- G54W (isavuconazole MIC 0.25 µg/mL),
- M220I (isavuconazole MIC 2 µg/mL), and
- TR34/L98H (isavuconazole MIC 8 µg/mL).

For the G54W and M220I mutant strains, 100% survival rate was observed in mice treated with isavuconazole at doses of 128 and 256 mg/kg/day, respectively. For the TR34/L98H mutant, survival rate at the highest dose (256 mg/kg/day) tested was 27.27% (for details see section 3.5.1.1.2. above). The AUCs at these doses were 107.4 and 146.7 mg·h/L, respectively, the former being consistent with mean clinical exposure at the phase 3 dosing regimen and the latter being at the upper end of the clinical exposure range. The % target attainment based on AUC:MIC that represented 50% effective PD index was 50.48 (Table 80).

In the neutropenic pulmonary aspergillosis murine model study,³⁶ nine *A. fumigatus* strains were tested:

- 3 WT strains (AF41, AF293 and DPL with isavuconazole MICs of ≤1 µg/mL), and one echinocandin mutant (strain EMFR S678 with isavuconazole MIC 0.25 µg/mL), and
- 6 *cyp51A* mutants [Strain F11628 (G138C mutation; isavuconazole MIC 8 µg/mL), Strain F14403 (G54R mutations; isavuconazole MIC 0.125 µg/mL), Strain F16216 (TR34/L98H mutation; isavuconazole MIC 8 µg/mL), Strain AF72 (G54E mutation; isavuconazole MIC 2 µg/mL), Strain F14532 (M220T mutation; isavuconazole MIC 1 µg/mL), and Strain F13737 (G54C mutation; isavuconazole MIC 4 µg/mL)].

A net stasis endpoint was achieved for all the mutations with MICs less than 2 µg/mL and a 1-log kill endpoint was achieved for the one mutant with an MIC in the WT range (Strain F14403 with G54R mutation and isavuconazole MIC 0.125 µg/mL). For all strains where net stasis was achieved the median static dose total drug AUC/MIC was 503 (Table 80; for more details see section 3.5.1.2.1. above). However, such a target attainment would result in breakpoints well below the ECV which inappropriately will split the WT population MIC distributions.

Comments:

The studies suggest that outcome may depend on the MIC of the baseline pathogen. However, the activity may vary with the experimental conditions used for testing. For example, using an in vitro model and GM levels as an endpoint, strains with MICs of ≤ 4 $\mu\text{g/mL}$ are likely to predict a response; however, the limitation is that such a target attainment may be applicable to early infection only.

Based on studies in non-neutropenic mice with disseminated aspergillosis or immunosuppressed mice with pulmonary aspergillosis (more closely mimics human infection), a strain with MIC ≤ 1 $\mu\text{g/mL}$ or ≤ 0.06 $\mu\text{g/mL}$, respectively, is likely to predict response.

The applicant used the non-neutropenic model using survival as the PD target for assessing exposure response relationship in humans. The applicant states that at clinically relevant exposures, MICs of 1 $\mu\text{g/mL}$ or less is like to predict response. One of the limitations of the studies in mice is the short (1-5 hours) terminal half-life ($t_{1/2}$) of isavuconazole; in such a situation, drug exposure may not be adequate at the end of the dosing period, allowing residual fungal organisms time and opportunity to regrow.

Each model utilized different efficacy endpoints to estimate PD target required for the exposure-response relationship. In the non-neutropenic model, 14-day survival was used as an endpoint; whereas in the neutropenic mouse model, a decrease in lung fungal burden was used as an endpoint. In the in vitro model suppression of GM was evaluated. It remains unclear which model and PD endpoint will be appropriate for predicting clinical response.

6.1.3. Effect of MIC on DRC-adjudicated outcome for Study 9766-CL-0104 and Study 9766-CL-0103

The all-cause mortality as well as clinical and microbiological responses as assessed by the DRC were evaluated by the baseline MIC values (by the CLSI method) for the mITT population; there was no correlation between isavuconazole MICs and clinical or microbiological response (for details see sections 5.1.above). If the data is analyzed based on the proposed cut-offs, approximately 80% of the subjects with *A. fumigatus* were considered a success at Day 42 and 63% at Day 84 (Table 81). This analysis is limited by the small number of isolates.

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Table 81: Study ISN 9766-CL-0104 and Study ISN 9766-CL-0103 - Clinical and microbiological response by MIC at the proposed cut-off									
Pathogen	MIC ≤1 µg/mL			MIC 2 µg/mL			MIC ≥4 µg/mL		
	All-cause mortality n/N (%)	Clinical success n/N (%)	Mycological eradication n/N (%)	All-cause mortality n/N (%)	Clinical success n/N (%)	Mycological eradication n/N (%)	All-cause mortality n/N (%)	Clinical success n/N (%)	Mycological eradication n/N (%)
mITT – Day 42									
<i>A. flavus</i>	2/11 (18.2)	9/11 (81.8)	6/11 (54.5)	0/3	2/3	1/3	0/1	1/1	0/1
<i>A. fumigatus</i>	3/25 (12.0)	20/25 (80.0)	10/25 (40.0)	0/3	3/3	2/3	1/2	1/2	1/2
<i>A. nidulans</i>	-	-	-	-	-	-	-	-	-
<i>A. niger</i>	0/1	1/1	1/1	1/1	0/1	0/1	0/7	7/7	5/7
<i>A. terreus</i>	1/3	2/3	0/3	0/1	1/1	0/1	0/1	1/1	1/1
<i>A. westerdijkiae</i>	-	-	-	1/1	0/1	0/1	-	-	-
mITT – Day 84									
<i>A. flavus</i>	4/11 (36.4)	7/11 (63.6)	6/11 (54.5)	1/3	2/3	1/3	0/1	1/1	0/1
<i>A. fumigatus</i>	5/25 (20.0)	15/25 (60.0)	9/25 (36)	1/3	2/3	2/3	1/2	1/2	1/2
<i>A. nidulans</i>									
<i>A. niger</i>	0/1	1/1	1/1	1/1	0/1	0/1	2/7	4/7	2/7
<i>A. terreus</i>	1/3	2/3	0/3	0/1	1/1	0/1	0/1	1/1	0/1
<i>A. westerdijkiae</i>	-	-	-	1/1	0/1	0/1	-	-	-

6.1.4. Efficacy in isolates with resistant mechanism identified ~ clinical response

An attempt was made to correlate MICs and resistance mechanism with clinical response. A very small number of *Aspergillus* species isolates were tested (three in Study 9766-CL-0104 and four in Study 9766-CL-0103). There was no threshold for response based on MIC (Tables 82 and 83). The number of isolates tested was small for analysis.

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Table 82: Study 9766-CL-0104 - List of selected fungal isolates, patient identification, isavuconazole MIC value and DRC assessed overall response at EOT.

Organism†	Laboratory Accession Number	Patient ID	Treatment Group	ISA MIC(µg/ml) (100% inhibition)	Role of Sterol Composition	Efflux Pumps Functional Analysis	Efflux Pumps Transcriptional Analysis	Last Dose Day	DRC Assessed Overall Response at EOT
<i>Rhizopus oryzae</i>‡	27710	003015001	ISA	1	↑ ergosterol		NC	24	complete - success
<i>Rhizopus oryzae</i>	17825	004550701	VOR	>16	↑squalene; ↓calciferol	No diff	NC	42	progression - failure
<i>Fusarium subglutinans</i>‡	17828	004011804	ISA	>16	↑ obtusifolial; ↑ calciferol		NC	14	progression - failure
<i>Fusarium solani</i>	18749	004970402	VOR	>16	↑squalene; ↓obtusifolial	↑efflux activity	NC	62	partial - success
<i>Fusarium oxysporum</i>	27718	004970411	ISA	>16	↑squalene; ↓obtusifolial	↑efflux activity	NC	77	complete - success
<i>Aspergillus fumigatus</i>‡	27722	004320444	ISA	0.25	squalene; calciferol; zymosterol; ergosterol; obtusifolial are present	NA		8	progression - failure
<i>Aspergillus fumigatus</i>	20438	004970911	ISA	8	↑squalene; ↓ergosterol; ↓zymosterol; ↓obtusifolial; ↓lanosterol	↑efflux activity	↑expression of <i>MDR2</i> ; no differences in <i>MDR3</i> or <i>CYP51</i>	45	partial - success
<i>Aspergillus fumigatus</i>	28500	004320455	ISA	>16	↓zymosterol; ↓lanosterol	↑efflux activity		10	progression - failure

DRC: Data Review Committee; EOT: end of therapy; ISA: isavuconazole; MIC: minimum inhibitory concentration; NA: not available; NC: transcriptional analysis not conducted due to gene-specific probes unavailable MIC values obtained using CLSI methodology.

† All isolates obtained at baseline (up to day 7) except isolate ID 3018266-775-366-1 (patient 004320444; obtained on day 20).

‡ Bolded isolates represent the 3 WT isolates with ISA MIC < 8 mg/L except for *Fusarium subglutinans*. Isolate laboratory accession number 27710 (*Rhizopus oryzae*) was obtained from clinical study 9766-CL-0103.

Source: [9766-PH-0127, 9766-CL-0103 and 9766-CL-0104]

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Table 83: Study 9766-CL-0103 -List of selected fungal isolates, patient identification, isavuconazole MIC value and DRC assessed overall response at EOT.

Organism†	Laboratory Accession Number	Patient ID	Treatment Group	ISA MIC (µg/ml) (100% inhibition)	Role of Sterol Composition	Efflux Pumps Functional Analysis	Efflux Pumps Transcriptional Analysis	Last Dose Day	DRC Assessed Overall Response at EOT
<i>Rhizopus oryzae</i> ‡	27710	003015001	ISA	1	↑ ergosterol		NC	24	complete - success
<i>Rhizopus oryzae</i>	19447	003970401	ISA	>16	↑squalene; ↑calciferol	No diff	NC	15	progression - failure
<i>Rhizopus oryzae</i>	19448	003490401	ISA	16	↑squalene; ↑zymosterol	No diff	NC	84	partial - success
<i>Rhizopus oryzae</i>	19446	003491001	ISA	>16	↑squalene; ↑obtusifolial	No diff	NC	2	progression - failure
<i>Rhizopus oryzae</i>	28399	003011803	ISA	>16	↑squalene; ↑calciferol; ↑4, 14-dimethylzymosterol	No diff	NC	6	progression - failure
<i>Mucor circinelloides</i>	19445	003970301	ISA	>16	↑squalene; ↑calciferol; ↑obtusifolial	No diff	NC	15	progression - failure
<i>Rhizomucor pusillus</i>	28404	003070201	ISA	8	↑calciferol	No diff	NC	18	progression - failure
<i>Aspergillus niger</i>‡	28402	003970304	ISA	0.25	squalene; calciferol; ergosterol are present		NC	229	progression - failure
<i>Aspergillus niger</i>	27709	003320402	ISA	8	↑squalene; ↓calciferol; ↓ergosterol	↑efflux activity	NC	182	stable - failure
<i>Aspergillus niger</i>	27713	003550301	ISA	8	↑squalene; ↓calciferol	↑efflux activity	NC	181	stable - failure
<i>Aspergillus niger</i>	27715	003970406	ISA	8	↑squalene; ↓calciferol	↑efflux activity	NC	50	partial - success

DRC: Data Review Committee; EOT = end of therapy; ISA: isavuconazole; MIC: minimum inhibitory concentration; NC = transcriptional analysis not conducted due to gene-specific probes unavailable. MIC values obtained using CLSI methodology.

†All isolates obtained at baseline (up to day 7).

‡ Bolded isolates represent the 3 WT isolates with ISA MIC < 8 mg/L except for *Fusarium subglutinans*

Source: [9766-PH-0127 and 9766-CL-0103]

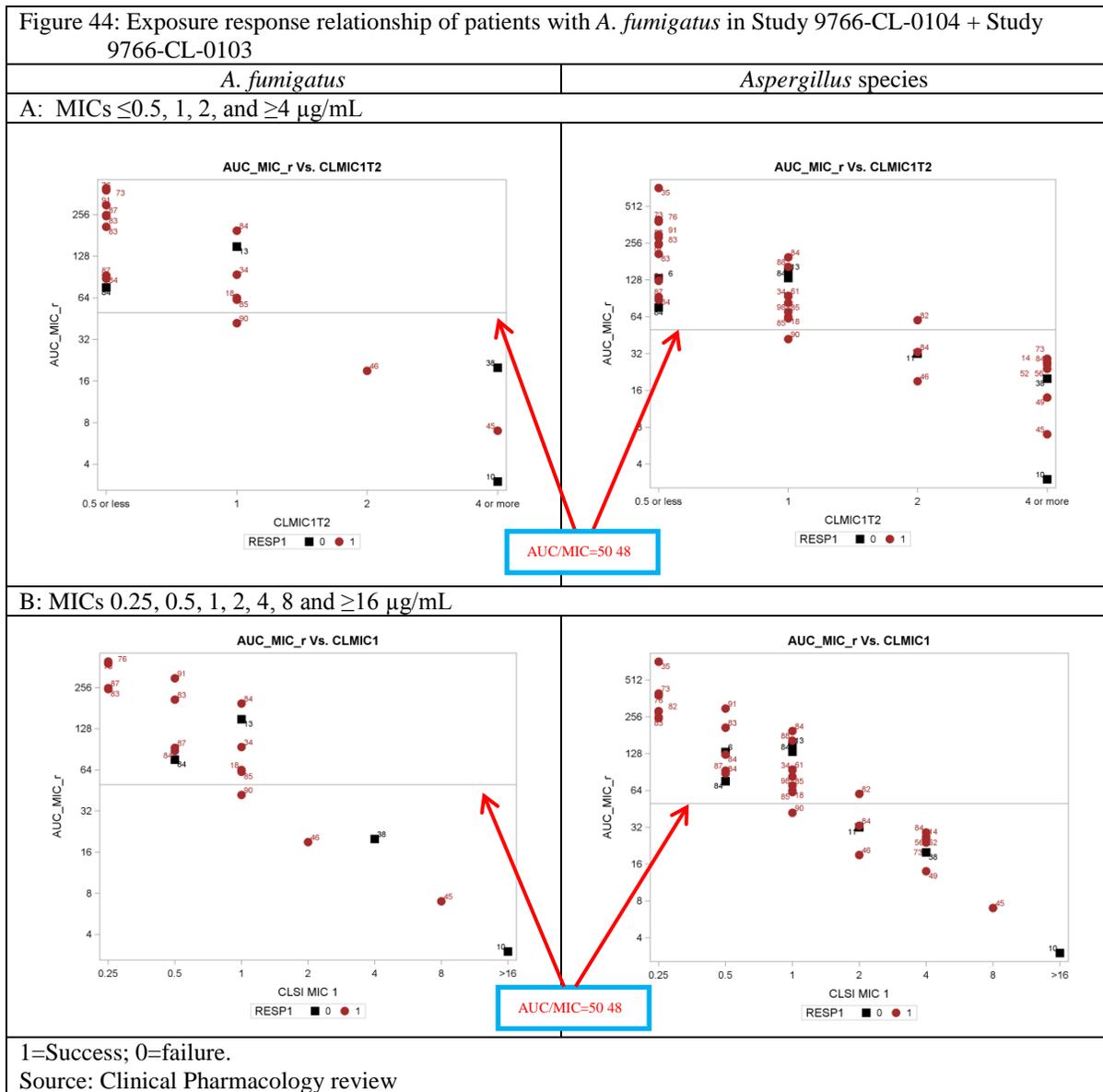
6.1.5. Isavuconazole population pharmacokinetic analysis

Based on a population PK model, the half-life was 135 hours and AUC 2495 µg·hr/mL (see section 4.1 above: Table 53). For more details see Clinical Pharmacology review.

6.1.6. Exposure-response analysis for patients in phase 3 studies with *Aspergillus* infection

There was no relationship between any of the clinical response variables tested and the PK parameters.

Using 50.48 as target attainment (based on a nonclinical study in disseminated non-neutropenic murine model), a comparison of exposure by response with MIC as well as clinical response showed a trend towards failure in patients with *A. fumigatus* as the baseline pathogen with MIC ≥ 4 µg/mL (Figure 44). No trend was observed if patients with all *Aspergillus* species were combined. The sample size was small (for details see Clinical Pharmacology review).



Comments:

The applicant tested target attainment in three different models: in vitro dynamic model, non-neutropenic murine model of disseminated aspergillosis and immunocompromised murine model of pulmonary aspergillosis. Each model utilized different endpoints to estimate PD target required for the exposure-response relationship. In the in vitro model, a reduction in GM was used as the PD endpoint. In the non-neutropenic model 14-day survival was used as an endpoint; whereas in the immunocompromised mouse model, a 1-log decrease in lung fungal burden on Day 7, by PCR, was used as an endpoint. It is unclear which model and PD endpoint will be appropriate for predicting clinical response. Additionally, the murine models are limited by the short half-life of isavuconazole as the half-life in humans is longer. The shorter half-life in mice would possibly enable fungal regrowth in the latter parts of the dosing interval as drug concentrations progressively decline.

The applicant based breakpoint analysis on the target attainment of 50.48 from the non-neutropenic A. fumigatus disseminated aspergillosis murine model using 14 day survival as the endpoint.

Exposure-response analysis showed no correlation between either MIC of the baseline pathogen or exposure-response and clinical response based on patients enrolled in the clinical trial. (b) (4)

Although the use of PK-PD to guide antibacterial development and establish interpretive criteria/breakpoints is well established, the utility of such an approach in establishing breakpoints for anti-fungal drugs especially for filamentous fungi is not known.

6.2. Mucorales Species

The number of patients with mucormycosis was small. No ECVs were established for Mucorales. There was no correlation between MIC and clinical response nor were any studies performed to evaluate PK-PD analysis in animal models of mucormycosis.

Comments:

There was insufficient information to establish clinical interpretive criteria/breakpoints for any of the Mucorales species.

7. THE LABELING

7.1. Applicant's version of the microbiology section of the labeling

12.1 Mechanism of action

[see Clinical Pharmacology (12.4)].

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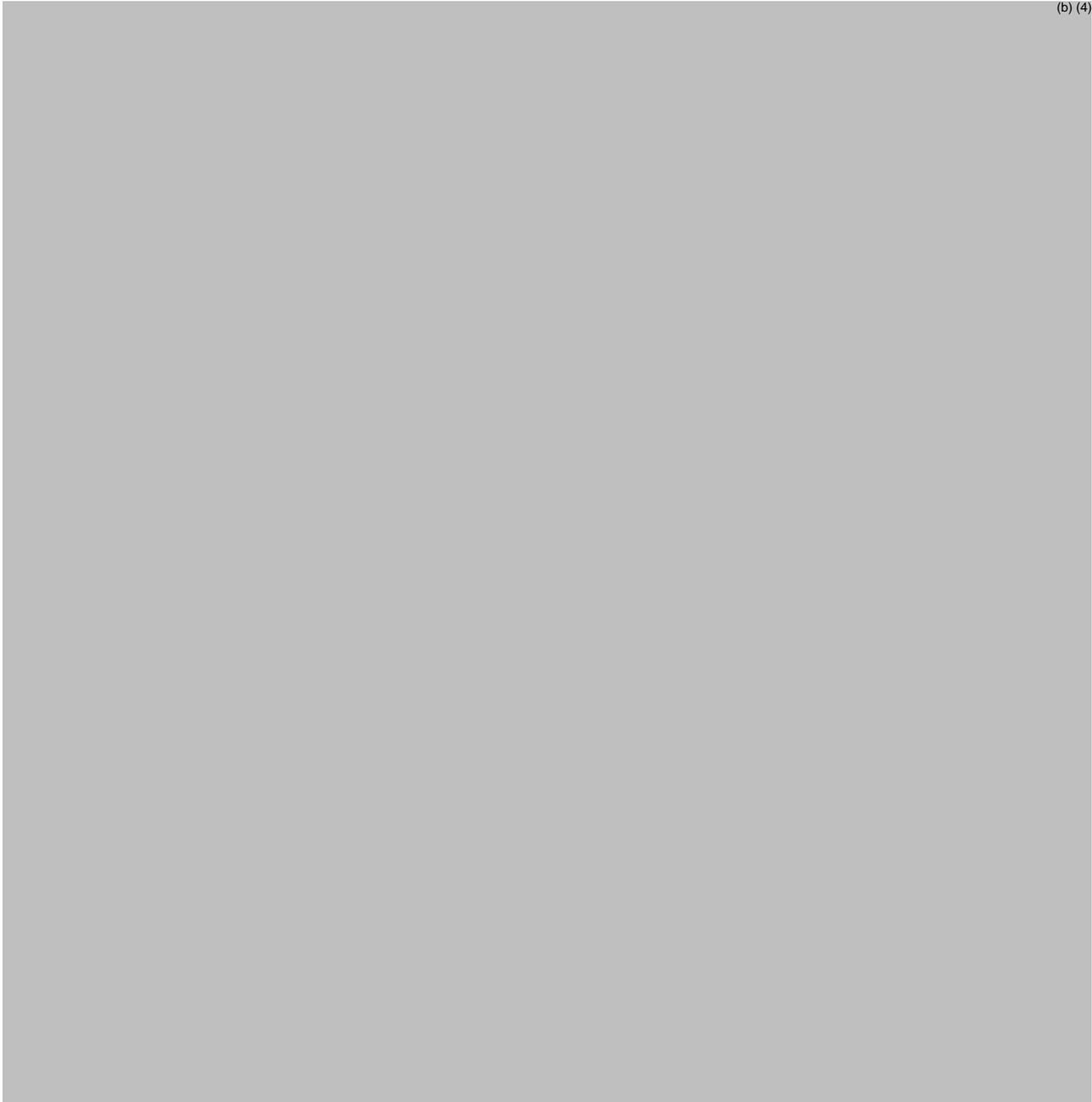
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12.4 Microbiology

Mechanism of Action

(b) (4) the synthesis of ergosterol, a key component of the fungal cell membrane, through the inhibition of cytochrome P-450 dependent enzyme lanosterol 14-alpha-demethylase responsible for the conversion of lanosterol to ergosterol. (b) (4) an accumulation of methylated sterol precursors and a depletion of ergosterol within the fungal cell membrane (b) (4) weaken (b) (4) the membrane structure and function.

(b) (4)
Isavuconazole has (b) (4) activity against most strains of the following microorganisms, both *in vitro* and in clinical infections: *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, (b) (4), *Rhizopus oryzae*, (b) (4)



(b) (4)



Drug Resistance

(b) (4) the (b) (4) mechanism of resistance to isavuconazole is (b) (4) (b) (4) substitutions (b) (4) the target protein CYP51. (b) (4)

he relevance of cross-resistance to clinical outcome has not been fully characterized. (b) (4) may require alternative antifungal therapy.

7.2. Comments

- *The information stated in the 'Mechanism of action' section is appropriate. However, minor edits are recommended for clarity and suggest adding information on activity of isavuconazole against mammalian cells.*
- *Under the subheading 'Activity in vitro and in clinical infections' (b) (4) Data for a minimum of 10 patients should be available for listing in this section of the labeling.*



However, the pathogens identified in patients enrolled in the clinical trial may be added in section 14 (Clinical Studies).

- Under the subheading 'Drug resistance' it should be stated that there is a potential for development of resistance to isavuconazole. The applicant has proposed to state (b) (4)
Since changes in sterol profile and elevated efflux pump activity were also observed, it will be useful to add such information in the labeling.

Minor edits are recommended for clarity and accuracy.

7.3. FDA's version of the labeling

12.1 Mechanism of action

(b) (4)

[see Clinical Pharmacology, Microbiology (12.4)].

(b) (4)

12.4 Microbiology

Mechanism of Action

Isavuconazole, (b) (4) inhibits (b) (4) the synthesis of ergosterol, a key component of the fungal cell membrane, through the inhibition of cytochrome P-450 dependent enzyme lanosterol 14-alpha-demethylase. This enzyme is responsible for the conversion of lanosterol to ergosterol. (b) (4) An accumulation of methylated sterol precursors and a depletion of ergosterol within the fungal cell membrane (b) (4) weakens (b) (4) the membrane structure and function. Mammalian cell demethylation is less sensitive to isavuconazole inhibition.

Activity in vitro and (b) (4) -in Clinical Infections:

Isavuconazole has (b) (4) activity against most strains of the following microorganisms, both *in vitro* and in clinical infections: *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, and Mucorales such as (b) (4) *Rhizopus oryzae*, and Mucormycetes species (b) (4)

[see Clinical Studies (14)].

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Drug Resistance

There is a potential for development of resistance to isavuconazole.

(b) (4) The (b) (4) mechanism of resistance to isavuconazole, like other (b) (4) is likely due to multiple mechanisms that include (b) (4) substitutions (b) (4) in the target (b) (4) gene *cyp51*. Changes in sterol profile and elevated efflux pump activity were observed.

In vitro and animal studies suggest (b) (4) -cross-resistance between isavuconazole and other azoles- (b) (4). The relevance of cross-resistance to clinical outcome has not been fully characterized. However, patients failing prior azole therapy (b) (4) may require alternative antifungal therapy.

The following information should be added to Section 14 Clinical studies:

14.1 Treatment of Invasive Aspergillosis

At least one *Aspergillus* species was identified in 30% of the subjects; *A. fumigatus* and *A. flavus* were the most common pathogens identified. There were (b) (4) patients with other *Aspergillus* species (*A. niger*, *A. sydowi*, *A. terreus*, and *A. westerdijkiae*).

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14.2 Treatment of Invasive Mucormycosis

There were less than 6 patients with other Mucorales (b) (4) *Lichtheimia corymbifera*, *Mucor amphibiorum*, *Mucor circinelloides*, *Rhizomucor pusillus*, *Rhizopus azygosporus*, and *Rhizopus microsporus*.

(b) (4)

[See appended electronic signature page]

Shukal Bala, Ph.D.

Microbiologist, DAIP

CONCURRENCE:

DAIP/Microbiology Team Leader/ Kerry Snow MS, MT (ASCP)

CC:

NDA # 207500 and 207501

DAIP/PM/Alison Rodgers

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Appendix-1

Sources of raw MIC results entered on Isavuconazole MIC Database

DB Ref	Data source used for Isavuconazole MIC Database	Subsequent / related publication
1	Warn PA, Sharp A, Denning DW. 2004. <i>In vitro</i> activity of a new triazole BAL4815, the active component of BAL8557 (the water-soluble prodrug) against <i>Aspergillus</i> spp. 44 th Interscience Conference on Antimicrobial Agents and Chemotherapy, Poster F-842, Washington, DC, USA.	Warn PA, Sharp A, Denning DW. 2006a. <i>In vitro</i> activity of a new triazole BAL4815, the active component of BAL8557 (the water-soluble prodrug), against <i>Aspergillus</i> spp. J Antimicrob Chemother. 57(1):135-138.
2	Warn P, Denning D, Heep M, Isham N, Ghannoum M. 2005a. <i>In vitro</i> activity of a new triazole BAL4815 against <i>Candida</i> isolates with decreased fluconazole susceptibility. 45 th Interscience Conference on Antimicrobial Agents and Chemotherapy, Poster M-1620, Washington, DC, USA.	
5	Ghannoum M and Isham N. 2005b. Antifungal activity of BAL4815, a novel azole against dermatophytes and emerging non-dermatophyte fungi including Zygomycetes. 45 th Interscience Conference on Antimicrobial Agents and Chemotherapy, Poster M-1623, Washington, DC, USA.	
7	Mouton JW, Verweij PE, Warn P, Denning D, Heep M, Isham N, Ghannoum M. 2005. <i>In vitro</i> activity of a new triazole BAL4815 against <i>Candida</i> isolates with decreased fluconazole susceptibility. 2 nd Trends in Medical Mycology, Poster P-021, Berlin, Germany.	
10	Nweze EI, Curfs-Breuker I, Janssen BGJ, De Hoog GS, Meis JF. 2006. <i>In vitro</i> activity of posaconazole compared with seven antifungal agents against 80 clinical and 15 environmental isolates of <i>Exophiala dermatitidis</i> . 46 th Interscience Conference on Antimicrobial Agents and Chemotherapy, Poster M-1588, San Francisco, CA, USA.	Badali H, de Hoog GS, Sudhadham M, Meis JF. 2011. Microdilution <i>in vitro</i> antifungal susceptibility of <i>Exophiala dermatitidis</i> , a systemic opportunist. Med Mycol. 49(8):819-824.
11	Seifert H, Aurbach U, Stefanik D, Cornely O. 2006. <i>In vitro</i> activity of the new azole BAL4815 and six other antifungal agents against <i>Candida</i> bloodstream isolates. 46 th Interscience Conference on Antimicrobial Agents and Chemotherapy, Poster M-1589, San Francisco, CA, USA.	Seifert H, Aurbach U, Stefanik D, Cornely O. 2007. <i>In vitro</i> activities of isavuconazole and other antifungal agents against <i>Candida</i> bloodstream isolates. Antimicrob Agents Chemother. 51(5):1818-1821.
13	González GM and Heep M. 2006. <i>In vitro</i> activity of BAL4815, a new water-soluble broad-spectrum triazole, against opportunistic filamentous and dimorphic fungi. 16 th International Society for Human and Animal Mycology Congress, Poster P-0081, Paris, France.	González GM. 2009. <i>In vitro</i> activities of isavuconazole against opportunistic filamentous and dimorphic fungi. Med Mycol. 47(1):71-76.
13	Wheat LJ, Connolly P, Smedema M, Durkin M, Goldman M. 2006. <i>In vitro</i> activity of a new triazole, BAL8557, against <i>Histoplasma capsulatum</i> isolates from patients who failed fluconazole therapy. 16 th International Society for Human and Animal Mycology Congress, Oral Presentation A-957, Paris, France.	
14	Warn P, Sharp A, Denning D. 2006c. <i>In vitro</i> activity of BAL4815 against zygomycetes. 16 th International Society for Human and Animal Mycology Congress, Poster P-0158, Paris, France.	
15	Martin de la Escalera C, Aller AI, López-Oviedo E, Martos AI, Romero A, Castro C, Cantón E, Martín-Mazuelos E. 2006a. <i>In vitro</i> activity of BAL4815 (a new azole) against filamentous fungi. 16 th International Society for Human and Animal Mycology Congress, Poster P-0554, Paris, France.	
17	Heep M, Grover P, Brown NP, Sahn D, Jones ME. 2007c. Evaluation of isavuconazole (BAL8557/BAL4815) Etest compared to broth microdilution antifungal susceptibility testing against quality control strains and fluconazole susceptible clinical <i>Candida</i> isolates. 17 th European Congress of Clinical Microbiology and Infectious Diseases, Poster P-1676, Munich, Germany.	
18	Hagen F, Illnait-Zaragozi MT, Meis JF, Chew WH, Curfs-Breuker I, Mouton JW, Hoepelman AI, Spanjaard L, Verweij PE, Kampinga GA, Kuijper EJ, Boekhout T, Klaassen CH. 2012. Extensive genetic diversity within the Dutch clinical <i>Cryptococcus neoformans</i> population. J Clin Microbiol. 50(6):1918-1926.	
18	Curfs-Breuker IM, Mouton JW, Janssen BGJ, Illnait-Zaragozi MT, Hagen F, Spanjaard L, Boekhout T, Meis JF. 2007b. <i>In vitro</i> activity of the new azole isavuconazole (BAL 4815) compared with six other antifungal agents against 180 <i>Cryptococcus neoformans</i> meningitis isolates from The Netherlands. 17 th European Congress of Clinical Microbiology and Infectious Diseases, Poster P-1977, Munich, Germany.	
19	Curfs-Breuker IM, Mouton JW, Debets-Ossenkopp YJ, Endtz HP, Verweij PE, Meis JF. 2007a. <i>In vitro</i> activity of isavuconazole (BAL4815) compared with	

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DB Ref	Data source used for Isavuconazole MIC Database	Subsequent / related publication
	seven other antifungal agents against 309 prospectively collected clinical <i>Candida</i> isolates from The Netherlands. 17 th European Congress of Clinical Microbiology and Infectious Diseases, Poster P-1978, Munich, Germany.	
20	Guinea J, Peláez T, Recio S, Torres-Narbona M, Bouza E. 2008a. <i>In vitro</i> antifungal activities of isavuconazole (BAL4815), voriconazole, and fluconazole against 1,007 isolates of zygomycete, <i>Candida</i> , <i>Aspergillus</i> , <i>Fusarium</i> , and <i>Scedosporium</i> species. <i>Antimicrob Agents Chemother.</i> 52(4):1396-1400.	
20	Guinea J, Recio S, Peláez T, Torres-Narbona M, Bouza E. 2007. <i>In vitro</i> activity of isavuconazole (BAL4815) and voriconazole against 702 isolates of <i>Aspergillus</i> spp. 3 rd Trends in Medical Mycology, Poster P-026, Torino, Italy.	
22	Sanglard D, Ischer F, Coste A, Ferrari S. 2008. Comparison between isavuconazole (ISA) and other azoles against characterised clinical isolates and yeast model systems. 18 th European Congress of Clinical Microbiology and Infectious Diseases, Poster P-2176, Barcelona, Spain.	
23	Guinea J, Peláez T, Tahoune H, Recio S, Torres-Narbona M, Hagen F, Boekhout T, Bouza E. 2008b. Antifungal activity of old and new antifungal agents against 94 clinical isolates of <i>Cryptococcus neoformans</i> var. <i>grubii</i> and var. <i>neoformans</i> : Are there differences between the varieties? 48 th Interscience Conference on Antimicrobial Agents and Chemotherapy, Poster M-1525, Washington, DC, USA.	Guinea J, Hagen F, Peláez T, Boekhout T, Tahoune H, Torres-Narbona M, Bouza E. 2010a. Antifungal susceptibility, serotyping, and genotyping of clinical <i>Cryptococcus neoformans</i> isolates collected during 18 years in a single institution in Madrid, Spain. <i>Med Mycol.</i> 48(7):942-948.
24	Perkhofer S, Lechner V, Lass-Flörl C. 2008. The <i>in vitro</i> activity of isavuconazole against <i>Aspergillus</i> species and zygomycetes according to EUCAST methodology. 48 th Interscience Conference on Antimicrobial Agents and Chemotherapy, Poster M-1526, Washington, DC, USA.	
25	Thompson III GR, Fothergill AW, Vallor AC, Wiederhold NP, Wickes BL, Patterson TF. 2008. Antifungal susceptibility among different serotypes of <i>Cryptococcus gattii</i> and <i>Cryptococcus neoformans</i> . 48 th Interscience Conference on Antimicrobial Agents and Chemotherapy, Poster M-1531, Washington, DC, USA.	Thompson III GR, Wiederhold NP, Fothergill AW, Vallor AC, Wickes BL, Patterson TF. 2009a. Antifungal susceptibilities among different serotypes of <i>Cryptococcus gattii</i> and <i>Cryptococcus neoformans</i> . <i>Antimicrob Agents Chemother.</i> 53(1):309-311.
26	Curfs-Breuker IM, Debets-Ossenkopp YJ, Endtz HP, Verweij PE, Meis JF; Dutch Caspo Study Group. 2008. <i>In vitro</i> activity compared with six other antifungal agents against 239 prospectively collected clinical <i>Aspergillus</i> isolates from The Netherlands. 3rd Advances Against Aspergillosis, Poster 37, Miami, FL, USA.	
27	Badali H, de Hoog GS, Curfs-Breuker I, Andersen B, Meis JF. 2009a. <i>In vitro</i> activities of eight antifungal drugs against 70 clinical and environmental isolates of <i>Alternaria</i> species. 17 th International Society for Human and Animal Mycology Congress, Poster PP-03-24, Tokyo, Japan.	Badali H, De Hoog GS, Curfs-Breuker I, Andersen B, Meis JF. 2009b. <i>In vitro</i> activities of eight antifungal drugs against 70 clinical and environmental isolates of <i>Alternaria</i> species. <i>J Antimicrob Chemother.</i> 63(6):1295-1297.
29	Verweij PE, González G, Wiederhold N, Lass-Flörl C, Warn P, Heep M, Ghannoum M, Guinea J. 2009a. <i>In vitro</i> antifungal activity of isavuconazole against 345 mucorales isolates collected at eight study centers worldwide. 19 th European Congress of Clinical Microbiology and Infectious Diseases, Poster P-1298, Helsinki, Finland.	Verweij PE, González GM, Wiederhold NP, Lass-Flörl C, Warn P, Heep M, Ghannoum MA, Guinea J. 2009b. <i>In vitro</i> antifungal activity of isavuconazole against 345 mucorales isolates collected at study centers in eight countries. <i>J Chemother.</i> 21(3):272-281.
30	Peláez T, Guinea J, Gama B, Flores R, Recio S, Torres-Narbona M, Munoz O, Bouza E. 2009a. Is <i>Aspergillus nidulans</i> susceptible to all antifungal agents? <i>In vitro</i> activity of an updated panel of antifungal agents against 63 clinical isolates. 19 th European Congress of Clinical Microbiology and Infectious Diseases, Poster P-1297, Helsinki, Finland.	
31	Thompson III GR, Wiederhold NP, Sutton DA, Fothergill A, Patterson TF. 2009b. <i>In vitro</i> activity of isavuconazole against <i>Trichosporon</i> . 17 th International Society for Human and Animal Mycology Congress, Poster PP-03-11, Tokyo, Japan.	Thompson III GR, Wiederhold NP, Sutton DA, Fothergill A, Patterson TF. 2009c. <i>In vitro</i> activity of isavuconazole against <i>Trichosporon</i> , <i>Rhodotorula</i> , <i>Geotrichum</i> , <i>Saccharomyces</i> and <i>Pichia</i> species. <i>J Antimicrob Chemother.</i> 64(1):79-83.
32	Verweij PE, van der Lee HAL, Rijs AJMM. 2007. Isavuconazole is active against zygomycetes <i>in vitro</i> . 3 rd Trends in Medical Mycology, Poster P-051, Torino, Italy.	
34	Peláez T, Gama B, Guinea J, Sanchez-Cambronero L, Martin-Rabadan P, Flores R, Alcalá L, Munoz P, Bouza E. 2009b. Assessment of the antifungal susceptibility of <i>Aspergillus terreus</i> over an 18-year period in a general hospital. 49 th Interscience Conference on Antimicrobial Agents and Chemotherapy, Poster M-1714, San Francisco, CA, USA.	

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DB Ref	Data source used for Isavuconazole MIC Database	Subsequent / related publication
35	Meis JF, Badali H, de Hoog GS, Breuker-Curfs I, Heep M. 2009. <i>In vitro</i> activities of conventional and new antifungal drugs against <i>Rhinocladiella mackenziei</i> an agent of cerebral phaeoophomycosis. 17 th International Society for Human and Animal Mycology Congress, Poster PP-03-23, Tokyo, Japan.	Badali H, de Hoog GS, Curfs-Breuker I, Meis JF. 2010d. <i>In vitro</i> activities of antifungal drugs against <i>Rhinocladiella mackenziei</i> , an agent of fatal brain infection. <i>J Antimicrob Chemother.</i> 65(1):175-177.
36	Viljoen JJ, Mitha I, Heep M, Ghannoum M. 2005. Efficacy, safety, and tolerability of three different dosing regimens of BAL8557 vs. fluconazole in a double-blind, randomized, multicenter trial for the treatment of esophageal candidiasis (EC) in immunocompromised adults. 45 th Interscience Conference on Antimicrobial Agents and Chemotherapy, Poster LB2-32, Washington, DC, USA.	
39	Meis JF. 2008. 20081129 Trichosporon results.xls. Unpublished lab data. Micron DB Study 39 (x3).	
41	Heep M. 2007a. MH1-2007070713 Zygo final MICs for poster (aspergillus).xls. Unpublished lab data. Micron DB Study 41.	
42	Odabasi Z. 2007a. Odabasi Isavuconazole Odd Moulds Test 1 raw data.xls. Unpublished lab data. Micron DB Study 42.	
43	Odabasi Z. 2007b. Odabasi ISAVUC Aspergillus raw data.xls. Unpublished lab data. Micron DB Study 43 (x7).	
44	Kappe R. 2004. Reinhard Kappe 06-2004-first BAL 3815-Gesamt.xls. Unpublished lab data. Micron DB Study 44 (x8).	
46	Najafzadeh MJ, Badali H, Illnait-Zaragozi MT, De Hoog GS, Meis JF. 2010. <i>In vitro</i> activities of eight antifungal drugs against 55 clinical isolates of <i>Fonsecaea</i> spp. <i>Antimicrob Agents Chemother.</i> 54(4):1636-1638.	
48	Meis JF. 2009. Cladophialophora carrionii data.xls. Unpublished lab data. Micron DB Study 48 (x10).	Badali H, de Hoog GS, Curfs-Breuker I, Klaassen CH, Meis JF. 2010a. Use of amplified fragment length polymorphism to identify 42 <i>Cladophialophora</i> strains related to cerebral phaeoophomycosis with <i>in vitro</i> antifungal susceptibility. <i>J Clin Microbiol.</i> 48(7):2350-2356.
49	Illnait-Zaragozi MT, Curfs-Breuker I, Martinez GF, Fernandez CM, Boekhout T, Meis JF. 2006. <i>In vitro</i> activity of the new azole isavuconazole BAL 4815 compared with six other antifungal agents against 155 <i>Cryptococcus neoformans</i> isolates from Cuba. 46 th Interscience Conference on Antimicrobial Agents and Chemotherapy, Poster M-1587, San Francisco, CA, USA.	Illnait-Zaragozi MT, Martinez GF, Curfs-Breuker I, Fernández CM, Boekhout T, Meis JF. 2008. <i>In vitro</i> activity of the new azole isavuconazole (BAL4815) compared with six other antifungal agents against 162 <i>Cryptococcus neoformans</i> isolates from Cuba. <i>Antimicrob Agents Chemother.</i> 52(4):1580-1582
49	Meis JF. 2010a. Data_Asian_Cgrubbi.xls. Unpublished lab data. Micron DB Study 49 (x11).	
50	Meis JF. 2010b. PMarneffi susceptibility.xls. Unpublished lab data. Micron DB Study 50 (x12).	
51	Heep M. MH1-2007070713 Zygo final MICs for poster (aspergillus).xls. Unpublished lab data. Micron DB Study 51	
52	Castanheira M, Pfäller MA, Jones RN. 2012. International antifungal surveillance program for isavuconazole using the SENTRY antimicrobial surveillance program platform for 2011 (11-AST-05). Internal Report. JMI Laboratories, IA, USA.	
53	Meis JF, Mouton J, Klaassen C, Geertsen E. 2012. <i>In vitro</i> activity of eight antifungal drugs against 237 <i>Aspergillus terreus</i> isolates. Internal report.	
54	Pan W, Khayhan K, Hagen F, Wahyuningsih R, Chakrabarti A, Chowdhary A, Ikeda R, Taj-Aldeen SJ, Khan Z, Inuran D, Sjam R, Sriburee P, Liao W, Chaicumpar K, Ingviya N, Mouton JW, Curfs-Breuker I, Boekhout T, Meis JF, Klaassen CH. 2012. Resistance of Asian <i>Cryptococcus neoformans</i> serotype A is confined to few microsatellite genotypes. <i>PLoS One.</i> 7(3):e32868	
55	Hagen F, Illnait-Zaragozi MT, Bartlett KH, Swinne D, Geertsen E, Klaassen CH, Boekhout T, Meis JF. 2010. <i>In vitro</i> antifungal susceptibilities and amplified fragment length polymorphism genotyping of a worldwide collection of 350 clinical, veterinary, and environmental <i>Cryptococcus gattii</i> isolates. <i>Antimicrob Agents Chemother.</i> 54(12):5139-5145.	
56	Badali H, de Hoog S, Curfs-Breuker I, Meis JF. 2009c. Antifungal activity of isavuconazole and comparators against chromoblastomycotic <i>Cladophialophora</i> spp. 4 th Trends in Medical Mycology, Poster P-248, Athens, Greece.	
57	Ostrosky-Zeichner L, Inurria N, Rodriguez J, Chen E, Paetznick V. 2009. Comparative <i>in vitro</i> activity of isavuconazole (ISA) against medically important yeasts and moulds. 49 th Interscience Conference on Antimicrobial Agents and Chemotherapy, Poster M-1707/476, San Francisco, CA, USA.	
58	Ostrosky-Zeichner L. 2012b. <i>In vitro</i> activity of isavuconazole against fluconazole-resistant <i>Candida</i> spp. Internal report.	
58	Ostrosky-Zeichner L. 2012a. <i>In vitro</i> activity of isavuconazole against <i>Aspergillus</i> spp. Internal report.	
59	Howard SJ, Lass-Flörl C, Cuenca-Estrella M, Gomez-Lopez A, Arendrup MC. 2013a. Isavuconazole susceptibility of <i>Aspergillus</i> and <i>Candida</i> species by EUCAST method. Internal report.	Howard SJ, Lass-Flörl C, Cuenca-Estrella M, Gomez-Lopez A, Arendrup MC. 2013. Determination of isavuconazole susceptibility of <i>Aspergillus</i> and <i>Candida</i> species by the EUCAST method. <i>Antimicrob Agents Chemother.</i> 57(11):5426-5431.
59	Howard SJ. 2013b. <i>In vitro</i> susceptibility of <i>Aspergillus fumigatus</i> to isavuconazole: correlation with itraconazole, voriconazole and posaconazole. Internal report.	Gregson L, Goodwin J, Johnson A, McEntree L, Moore CB, Richardson M, Hope WW, Howard SJ. 2013. <i>In vitro</i> susceptibility of <i>Aspergillus fumigatus</i> to isavuconazole: correlation with itraconazole, voriconazole and posaconazole. <i>Antimicrob Agents Chemother.</i> 57(11):5778-5780.

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/s/

SHUKAL BALA
12/09/2014

KERRY SNOW
12/09/2014

Product Quality Microbiology Review

November 18, 2014

NDA: 207501

Drug Product Name

Proprietary: Cresemba® for Injection

Non-proprietary: Isavuconazonium Sulfate, 200 mg/vial

Review Number: 1

Dates of Submission(s) Covered by this Review

Submit	Received	Review Request	Assigned to Reviewer
July 8, 2014	August 13, 2014	October 2, 2014	October 3, 2014

Submission History (for 2nd Reviews or higher) – N/A

Applicant/Sponsor

Name: Astellas Pharma Inc.

Address: 1 Astellas Way, Northbrook, IL 60062.

Representative: Robert M. Reed, Sr. Director, Regulatory Affairs
TEL: (224)-205-8985

Name of Reviewer: Vinayak B. Pawar, Ph.D.

Conclusion: Recommend Approval

Product Quality Microbiology Data Sheet

- A. 1. **TYPE OF SUBMISSION:** Original NDA
2. **SUBMISSION PROVIDES FOR:** Lyophilized Isavuconazonium Sulfate powder for intravenous infusion.
3. **MANUFACTURING SITE:** [REDACTED] (b) (4)
4. **DOSAGE FORM, ROUTE OF ADMINISTRATION AND STRENGTH/POTENCY:** [REDACTED] (b) (4) Injection for intravenous administration.
5. **METHOD(S) OF STERILIZATION:** [REDACTED] (b) (4)
6. **PHARMACOLOGICAL CATEGORY:** Antimicrobial agent.
- B. **SUPPORTING/RELATED DOCUMENTS:** DMF [REDACTED] (b) (4).
- C. **REMARKS:** Astellas Pharma Global Development Inc. submits an original New Drug Application (NDA 207501) for isavuconazonium sulfate, a water-soluble triazole prodrug with the proprietary name, CRESEMBA®, a sterile lyophilized powder for intravenous infusion. Reference is made to [REDACTED] (b) (4) DMF [REDACTED] (b) (4) for manufacturing and sterilization process validation information. Adequate information pertaining to the sterilization qualification/re-qualification of containers, closures, components and manufacturing equipment were provided in the DMF with the exception of the [REDACTED] (b) (4). This missing information was requested in an IR dated October 16, 2014. This is an electronic submission.

filename: N207501R1

Executive Summary

I. Recommendations

- A. Recommendation on Approvability** – Recommend Approval.
- B. Recommendations on Phase 4 Commitments and/or Agreements, if Approvable** – N/A

II. Summary of Microbiology Assessments

- A. Brief Description of the Manufacturing Processes that relate to Product Quality Microbiology** – (b) (4)
- B. Brief Description of Microbiology Deficiencies** – None.
- C. Assessment of Risk Due to Microbiology Deficiencies** – N/A
- D. Contains Potential Precedent Decision(s)** - Yes No

III. Administrative

- A. Reviewer's Signature** _____
Vinayak B. Pawar, Ph.D., Sr. Review Microbiologist, OPS/CDER
- B. Endorsement Block** _____
Stephen E. Langille, Ph.D., Sr. Review Microbiologist,
OPS/CDER
- C. CC Block**
N/A

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/s/

VINAYAK B PAWAR
11/21/2014

STEPHEN E LANGILLE
11/21/2014

NDA Number: 207500 and
207501

Applicant: Astellas Pharma Global
Development, Inc. **Stamp Date:** 07/08/2014

Drug Name: CRESEMBA®
(Isavuconazole)

NDA Type: NME

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?	X		
2	Is the microbiology information (preclinical/nonclinical and clinical) indexed, paginated and/or linked in a manner to allow substantive review to begin?	X		
3	Is the microbiology information (preclinical/nonclinical and clinical) legible so that substantive review can begin?	X		
4	On its face, has the applicant <u>submitted</u> <i>in vitro</i> 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?	X		
5	Has the applicant <u>submitted</u> any required animal model studies necessary for approvability of the product based on the submitted draft labeling?	X		
6	Has the applicant <u>submitted</u> all special/critical studies/data requested by the Division during pre-submission discussions?	X		
7	Has the applicant <u>submitted</u> the clinical microbiology datasets in a format which intends to correlate baseline pathogen with clinical and microbiologic outcome?	X		
8	Has the applicant <u>submitted</u> 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?	X		
9	Has the applicant <u>submitted</u> a clinical microbiology dataset in an appropriate/standardized format which intends to determine resistance development by correlating changes in the phenotype (such as <i>in vitro</i> susceptibility) and/or genotype (such as mutations) of the baseline pathogen with clinical and microbiologic outcome?	X		

	Content Parameter	Yes	No	Comments
10	Has the applicant used standardized or non-standardized methods for measuring microbiologic outcome? If non-standardized 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?			The applicant has used standardized tests.
11	Has the applicant <u>submitted</u> draft labeling consistent with current regulation, divisional and Center policy, and the design of the development package?	X		
12	Has the applicant <u>submitted</u> annotated microbiology draft labeling consistent with current divisional policy, and the design of the development package?	X		
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?	X		
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?		X	

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.

N/A

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

May request some summary Tables.

<i>Shukal Bala</i>	<i>08/27/2014</i>
_____ Reviewing Microbiologist, DAIP	_____ Date
<i>Kerry Snow</i>	<i>08/27/2014</i>
_____ Microbiology Team Leader, DAIP	_____ Date

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/s/

SHUKAL BALA
08/27/2014

KERRY SNOW
08/27/2014

MEMORANDUM



DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

DATE: August 4, 2014

TO: NDA 207500

FROM: Vinayak B. Pawar, Ph.D., Senior Review Microbiologist, OPS/CDER

THROUGH: Stephen E. Langille, Ph.D., Senior Review Microbiologist, OPS/CDER

cc: Alison Rodgers, Sr. Regulatory Project Manager, OMPT/CDER/OND

SUBJECT: Product Quality Microbiology assessment of Microbial Limits for “Isavuconazonium sulfate” [Submission Date: July 8 2014]

The Microbial Limits specification for “Isavuconazonium sulfate” is acceptable from a Product Quality Microbiology perspective. Therefore, this submission is recommended for approval from the standpoint of product quality microbiology.

“Isavuconazonium sulfate” is a “Capsule” for oral administration with the dosage strength of 186.3 mg isavuconazonium sulfate corresponding to 100 mg of isavuconazole.

The drug product was tested for Microbial Limits at release and at initial stability time points using a method consistent with USP Chapter <61> (Microbiological Examination of Non-sterile Products: Microbial Enumeration Tests) and <62> (Microbiological Examination of Non-sterile Products: Tests for Specified Microorganisms). The Microbial Limits acceptance criteria are consistent with USP Chapter <1111> (Microbiological Examination of Non-sterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use). Specifications are provided in Table 1 (reproduced from Table 1, Section 3.2.P.5.1).

Table 1. Specifications for Isavuconazonium Capsules

Attributes	Method	Acceptance Criteria
Microbial Limits	USP <61>, USP <62>	TAMC: (b) (4) cfu/g TYMC: (b) (4) cfu/g <i>E. coli.</i> : Absent/g

TAMC - Total Aerobic Microbial Count, TYMC - Total Combined Yeast & Mold Count

The Microbial Limits test methods were verified to be appropriate for use with the drug product following procedures consistent with those in USP Chapter <61> and <62>.

MEMORANDUM

The drug product will also be tested for Microbial Limits annually as part of the post-approval stability protocol.

ADEQUATE

Reviewer Comments – The microbiological quality of the drug product is controlled via a suitable testing protocol.

END

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/s/

VINAYAK B PAWAR
08/05/2014

STEPHEN E LANGILLE
08/08/2014

PRODUCT QUALITY MICROBIOLOGY FILING CHECKLIST

NDA Number: 207501

Applicant: Astellas Pharma
Global Development Inc.

Letter Date: July 8, 2014

Drug Name: Isavuconazonium
Sulfate

NDA Type: Original NDA

Stamp Date: July 23, 2014

The following are necessary to initiate a review of the NDA application:

	Content Parameter	Yes	No	Comments
1	Is the product quality microbiology information described in the NDA and organized in a manner to allow substantive review to begin? Is it legible, indexed, and/or paginated adequately?	X		
2	Has the applicant submitted an overall description of the manufacturing processes and microbiological controls used in the manufacture of the drug product?	X		Manufacturing Process & Controls: Submission Section 3.2.P.3.3.
3	Has the applicant submitted protocols and results of validation studies concerning microbiological control processes used in the manufacture of the drug product?	X		Submission Section 3.2.P.3.5.
4	Are any study reports or published articles in a foreign language? If yes, has the translated version been included in the submission for review?		X	
5	Has the applicant submitted preservative effectiveness studies (if applicable) and container-closure integrity studies?	X		(b) (4) Val. Report VL10009018
6	Has the applicant submitted microbiological specifications for the drug product and a description of the test methods?	X		Submission Section 3.2.P.5.1
7	Has the applicant submitted the results of analytical method verification studies?	X		BET per USP <85> (Section 5.3.5), Sterility Test per USP <71> (Section 5.3.6)
8	Has the applicant submitted all special/critical studies/data requested during pre-submission meetings and/or discussions?		X	
9	If sterile, are extended post-constitution and/or post-dilution hold times in the draft labeling supported by microbiological data?		X	
10	Is this NDA fileable? If not, then describe why.	X		

Additional Comments: None

Vinayak B. Pawar, Ph.D., Senior Review Microbiologist

Date

Stephen E. Langille, Ph.D., Senior Review Microbiologist

Date

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/s/

VINAYAK B PAWAR
08/08/2014

STEPHEN E LANGILLE
08/08/2014