Are *In Vitro* Susceptibilities to Azole Antifungals Predictive of Clinical Outcome in the Treatment of Candidemia?

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ABSTRACT The purpose of this review is to critically analyze published data evaluating the impact of azole pharmacokinetic and pharmacodynamic parameters, MICs, and *Candida* species on clinical outcomes in patients with candidemia. Clinical breakpoints (CBPs) for fluconazole and voriconazole, which are used to determine susceptibility, have been defined by the Clinical and Laboratory Standards Institute (CLSI) for *Candida* species. Studies evaluating the relationship between treatment efficacy and *in vitro* susceptibility, as well as the pharmacodynamic targets, have been conducted in patients treated with fluconazole for candidemia; however, for species other than *Candida albicans* and *Candida glabrata*, and for other forms of invasive candidiasis, data remain limited and randomized trials are not available. Limited data evaluating these relationships with voriconazole are available. While pharmacodynamic targets for posaconazole and isavuconazole have been proposed based upon studies conducted in murine models, CBPs have not been established by CLSI. Fluconazole remains an important antifungal agent for the treatment of candidemia, and data supporting its use based on *in vitro* susceptibility are growing, particularly for *C. albicans* and *C. glabrata*. Further investigation is needed to establish the roles of voriconazole, posaconazole, and isavuconazole in the treatment of candidemia and for all agents in the treatment of other forms of invasive candidiasis.

KEYWORDS *Candida*, antifungal, azole, clinical breakpoints, outcomes, pharmacodynamics, pharmacokinetics, susceptibility testing

In 2016, the Infectious Diseases Society of America (IDSA) published updated guidelines for the management of candidiasis, which recommend initial echinocandin therapy for most patients with candidemia, while stating that fluconazole may be considered for initial therapy in hemodynamically stable patients without previous exposure to azole antifungals. Fluconazole was also deemed appropriate as stepdown therapy from an echinocandin in stable patients with microbiological clearance and susceptible isolates. Routine susceptibility testing was recommended for all clinically relevant *Candida* isolates, given the changing epidemiology and increased threat of emerging resistance to commonly used antifungals (1).

Echinocandin resistance appears to reasonably predict treatment failure and is most concerning in *Candida glabrata*, with some centers reporting up to 12% resistance in bloodstream isolates (2). In the United States, fluconazole resistance is rare ($\leq 3\%$) in bloodstream isolates of *Candida albicans*, *Candida tropicalis*, and *Candida parapsilosis*. However, an estimated 10 to 30% of *C. glabrata* isolates are resistant (MIC ≥ 64 mg/liter), and the less commonly observed *Candida krusei* isolates are intrinsically resistant (2, 3). Unfortunately, the correlation between susceptibility of *Candida* species and treatment success for fluconazole is not straightforward. Given that fluconazole remains an important component of the antifungal armamentarium, it is imperative that clinicians understand relationships between azole dose, MIC, *Candida* species, and

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TABLE 1 Clinical breakpoints and epidemiologic cutoff values for *Candida* species and azole antifungals^{a,b}

Drug ^c	Species	CLSI CBPs (mg/liter)				EUCAST CBPs (mg/liter)		ECV (mg/liter)
		S	I	SDD ^d	R	S	R	
Fluconazole	<i>C. albicans</i>	≤2	—	4	≥8	≤2	>4	0.5
	<i>C. glabrata</i>	—	—	≤32	≥64	≤0.002	>32	8
	<i>C. krusei</i>	—	—	—	—	—	—	—
	<i>C. parapsilosis</i>	≤2	—	4	≥8	≤2	>4	1
	<i>C. tropicalis</i>	≤2	—	4	≥8	≤2	>4	1
Voriconazole	<i>C. albicans</i>	≤0.12	0.25–0.5	—	≥1	≤0.064	>0.25	0.03
	<i>C. glabrata</i>	—	—	—	—	—	—	0.25
	<i>C. krusei</i>	≤0.5	1	—	≥2	—	—	0.5
	<i>C. parapsilosis</i>	≤0.12	0.25–0.5	—	≥1	≤0.125	>0.25	0.03
	<i>C. tropicalis</i>	≤0.12	0.25–0.5	—	≥1	≤0.125	>0.25	0.12
Posaconazole	<i>C. albicans</i>	—	—	—	—	≤0.064	>0.064	0.06
	<i>C. glabrata</i>	—	—	—	—	—	—	2
	<i>C. krusei</i>	—	—	—	—	—	—	0.5
	<i>C. parapsilosis</i>	—	—	—	—	≤0.064	>0.064	0.25
	<i>C. tropicalis</i>	—	—	—	—	≤0.064	>0.064	0.12

^aSee references 4 to 6 and 8. CLSI, Clinical and Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing; CBPs, clinical breakpoints; ECVs, epidemiologic cutoff values; S, susceptible; I, intermediate; SDD, susceptible-dose dependent; R, resistant; —, not defined or not applicable.

^bECVs were determined using an iterative statistical method that establishes the true wild-type distribution, encompassing 97.5% of the modeled wild-type population.

^cCBPs and ECVs for isavuconazole have not been established by CLSI.

^dCLSI notes that fluconazole susceptibility is dependent on achieving maximum possible blood concentrations, which may require higher than standard (6 mg/kg/day) dosing in adult patients with normal renal function and body size.

outcome (3). In this review, we investigate these relationships, incorporating fluconazole pharmacokinetic and pharmacodynamic data when available. In addition, we will discuss whether similar relationships are available for the newer triazole antifungals voriconazole, posaconazole, and isavuconazole.

ANTIFUNGAL SUSCEPTIBILITY TESTING: CLINICAL BREAKPOINTS VERSUS EPIDEMIOLOGIC CUT-OFF VALUES

Clinical breakpoints (CBPs) should differentiate isolates for which there is a high likelihood of treatment success from those for which treatment is more likely to fail. A myriad of data are used to determine CBPs, including pharmacokinetic-pharmacodynamic relationships, clinical outcome data, and wild-type MIC distributions (3). In 2012, the Clinical and Laboratory Standards Institute (CLSI) updated CBPs for *Candida* species and fluconazole and voriconazole (CLSI document M27-S4) (4). Voriconazole CBPs were further updated in 2017 (CLSI document M60) (5). The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has also defined CBPs for *Candida* species and azole antifungals, which largely align with those of the CLSI (3, 6). Epidemiologic cutoff values (ECVs) can be used to distinguish wild-type strains (i.e., those without resistance mutations) from non-wild-type strains. ECVs are determined by evaluation of species-specific MIC distributions. The ECV is set at the upper limit of the wild-type distribution, such that an organism with an MIC above the ECV is presumed to express resistance mutations that may impact treatment response. Thus, although not a direct predictor of clinical efficacy, the ECV can provide useful information about the isolate, especially when a CBP has not been established (3, 7). Further information regarding ECVs, as well as an example distribution, are available in a review by Eschenauer and Carver (3). Table 1 provides an overview of established CBPs and ECVs (4, 5, 6, 8).

FLUCONAZOLE SUSCEPTIBILITY AND TREATMENT SUCCESS

In vitro and *in vivo* models have demonstrated that fluconazole exhibits fungistatic activity against *Candida* species. In neutropenic murine models of candidemia and candidiasis due to *C. albicans*, there is generally a good correlation between fluconazole

dose, MIC, and outcome (9). For example, among *C. albicans* isolates with fluconazole MICs of 0.5 to 32 mg/liter, a fluconazole free-drug 24 h AUC to MIC ratio (fAUC/MIC) of 12 to 25 is associated with efficacy. Given the low degree of fluconazole protein binding, total drug exposure is essentially equivalent to free drug exposure (9). In healthy adults with a normal body weight and renal function, the fluconazole 24-hour AUC is virtually equivalent to daily dose (3, 10). As such, dose/MIC can serve as a surrogate for AUC/MIC, and fluconazole doses of 400 to 800 mg should achieve pharmacodynamic targets for *Candida* MICs up to 32 mg/liter (9). Furthermore, in 1997, Rex and colleagues (11) published composite data for 519 *Candida* isolates (the majority of which were *C. albicans*), showing that clinical success for fluconazole and various forms of candidiasis (oropharyngeal, bloodstream, or visceral) was dependent on MIC and dose. For infections caused by *Candida* isolates with MICs of ≤ 8 mg/liter, success was achieved in $>90\%$ of patients, even with low (100 mg/day) doses of fluconazole (11). However, in infections due to *Candida* isolates with MICs of 16 to 32 mg/liter, more aggressive dosing was required to improve outcomes, with treatment success ranging from 78% in patients receiving 100 mg/day to 86% in patients receiving a median dose of 400 mg/day. In patients infected with isolates with MICs of ≥ 64 mg/liter, outcomes were poor ($<60\%$ success) (11). These findings provided support for the initial selection of CBPs in 1997 by the NCCLS (now CLSI) for fluconazole and *Candida* species, which designated isolates with MICs of ≤ 8 mg/liter as susceptible, 16 to 32 mg/liter as susceptible dose-dependent (SDD, noting that doses of ≥ 400 mg/day should be utilized), and ≥ 64 as resistant (12).

These initial breakpoints came under scrutiny for several reasons. First, $\sim 80\%$ of the outcomes data were obtained from patients with mucosal rather than invasive disease (11). Although the association of an increasing fluconazole dose being necessary to successfully treat recurrent oropharyngeal candidiasis due to *C. albicans* with progressively increasing fluconazole MICs in AIDS patients was well known, the relationship between MIC, fluconazole dose, and clinical outcome in invasive infections such as candidemia was less evident (12). Second, $>70\%$ of the clinical outcomes data were derived from infections due to isolates with MICs of ≤ 4 mg/liter, limiting confidence in the efficacy of fluconazole in the treatment of infections caused by isolates with elevated MICs, especially those in the SDD category. Finally, the *in vitro*, *in vivo*, and clinical data were largely comprised of *C. albicans*, leaving uncertainty regarding the utility of the breakpoints in treating infections caused by *Candida* species other than *C. albicans*; in particular, those caused by *C. glabrata*, which accounted for only 13% of the Rex et al. data set (11).

In an attempt to validate these initial breakpoints, several studies categorized outcomes by fluconazole susceptibility, as reviewed in reference 12. While these studies allow more robust examination of patients with invasive infections, they continued to suffer from limitations similar to those of the Rex et al. data set, that is, they included small ($<15\%$) numbers of isolates with MICs in the SDD category, they grouped all *Candida* species together (thus precluding analysis of individual species, such as *C. glabrata*, for example), and they included patients treated with a wide range of doses (12). Similarly, to explore the role of azole pharmacodynamics, several investigators have investigated correlations between AUC/MIC (or dose/MIC) and outcome in patients with candidemia (Table 2) (10, 13–20). These studies were unable to identify a definitive AUC/MIC or dose/MIC threshold that correlates with treatment success for all patients. Notably, few studies used adjusted analyses to account for differences in severity of illness (18–20). This is an important distinction, as candidemia generally occurs in patients with multiple comorbidities that can significantly influence patient outcomes (21). In a study by Brosh-Nissimov and colleagues, which used a Cox proportional hazards model applied to the entire cohort, AUC/MIC thresholds were not associated with 30-day mortality. Furthermore, use of a classification and regression tree (CART) analysis was unable to determine a significant AUC/MIC or dose/MIC breakpoint associated with mortality (18). Similarly, in a study by Fernandez-Ruiz et al.,

TABLE 2 Studies evaluating the relationship between fluconazole pharmacodynamic targets and clinical outcomes in the treatment of candidemia^{a,c}

Study(ies), yr(s) (reference(s))	Sample size (n) ^a	Candida spp. (no.)	FLUC exposure	Outcome definition	Correlation of FLUC dose/MIC or AUC/MIC ratio with outcome	Adjusted for severity of illness?	Does paper allow PD analysis specific to species?	Comment(s)
Rex et al., 1995 (13)	68	<i>albicans</i> , <i>glabrata</i> , <i>parapsilosis</i> , <i>tropicalis</i> (no. not specified)	Entire course of therapy (treatment continued until 14 days after last positive blood culture)	Persistence of candidemia at end of therapy	Not assessed, since an inverse correlation ($P = 0.05$) was found between MIC and outcome.	NA	No	Patients were administered renally adjusted FLUC dosages equivalent to ~6 mg/kg daily.
Clancy et al., 2005 (14)	19	<i>albicans</i> (9), <i>glabrata</i> (3), <i>parapsilosis</i> (3), <i>tropicalis</i> (3), <i>lusitanae</i> (1)	≥3 days	Persistence of candidemia despite 3 days of therapy	Not performed, due to exclusions (see comments).	No	Yes	Results exclude breakthrough infections and those due to <i>C. krusei</i> , and thus the analysis performed in the study is not included. Small numbers preclude ability to reliably determine an optimal dose/ MIC threshold.
Pai et al., 2007 (15)	77	<i>albicans</i> (49), <i>glabrata</i> (11), <i>parapsilosis</i> (6), <i>tropicalis</i> (5), <i>lusitanae</i> (3), other (3)	NR	Mortality at hospital discharge	AUC/MIC ratio breakpoint of 55.2 identified by CART analysis ($P = 0.008$).	No	No	FLUC dose (expressed in mg/kg)/MIC breakpoint of 12 identified by CART analysis ($P = 0.007$). AUC calculated by dividing FLUC dose by estimated FLUC CL. CL _{FLUC} calculated using equation that models CL _{FLUC} as a function of CL _{CR} . High overall response (113/126; 90%) precludes ability to reliably discriminate an optimal dose/MIC threshold.
Rodriguez-Tudela et al., 2007 (10) ^a	126	<i>albicans</i> (73), <i>parapsilosis</i> (27), <i>tropicalis</i> (12), <i>glabrata</i> (9), other (5)	NR	Persistence of candidemia despite at least 4 days of FLUC therapy	Patients with a dose/MIC ratio of ≥100 were associated with 92% (109/118) success vs 50% (4/8) for those with a dose/MIC ratio of ≤100. Patients with a dose/MIC ratio of ≥50 were associated with 91% success (110/121) vs 60% (3/5) for those with a dose/MIC ratio of <50.	No	No	AUC calculated by using FLUC daily dose, estimate for CL _{FLUC} (0.96 ml/min), and protein binding (12%).
Baddley et al., 2008 (16)	84	<i>albicans</i> (37), <i>parapsilosis</i> (17), <i>glabrata</i> (17), <i>tropicalis</i> (10), <i>krusei</i> (2), other (1)	Initial monotherapy for ≥3 days	All-cause mortality 6 weeks after first positive culture	Dose/MIC breakpoint of 12.5 (corresponds to AUC/MIC of 11.5) identified by CART analysis ($P = 0.09$). 74% of patients survived above this threshold vs 43% below.	No	No	Higher avg FLUC dose (OR, 1.006; 95% CI, 1.001–1.010; $P = 0.008$), avg FLUC dose of ≥400 mg (OR, 3.965; 95% CI, 1.509– 10.418; $P = 0.005$), and higher FLUC dose on day 1 of therapy (OR, 1.007; 95% CI, 1.002–1.011; $P = 0.002$) were independent predictors of survival on multivariable analysis. In dose/MIC calculations, dose was doubled if estimated CL _{CR} was <50 ml/min. AUC was calculated by dividing FLUC dose by estimated CL _{FLUC} . CL _{FLUC} was calculated using equation that models CL _{FLUC} as a function of CL _{CR} .
Eschenauer et al., 2013 (17), and Eschenauer et al., 2018 (20)	127	<i>glabrata</i>	≥5 consecutive days as first antifungal after collection of index blood culture	Clinical response: composite of clinical success, microbiological success and survival at day 14. Mortality: All-cause at day 28.	(i) Unadjusted analysis. Clinical response: Dose/MIC ratio of >12.5 associated with 49% (50/102) clinical response vs 20% (4/20) if ratio was ≤12.5 ($P = 0.025$). In ICU patients, 43% (13/30) response vs 0% (0/9) ($P = 0.018$), respectively, at same threshold. Survival: 82% (84/102) at >12.5 vs 65% (13/20) at ≤12.5 ($P =$ 0.079). (ii) Adjusted analysis. No AUC/MIC thresholds significantly associated with mortality in multivariable analysis.	Yes (in reference 20)	Yes	

(Continued on next page)

TABLE 2 (Continued)

Study(ies), yr(s) (reference(s))	Sample size (n) ^a	Candida spp. (no.)	FLUC exposure	Outcome definition	Correlation of FLUC dose/MIC or AUC/MIC ratio with outcome	Adjusted for severity of illness?	Does paper allow PD analysis specific to species?	Comment(s)
Brosh-Nissimov and Ben-Ami, 2015 (18)	75	<i>albicans</i> (36), <i>tropicalis</i> (17), <i>parapsilosis</i> (13), <i>glabrata</i> (8), <i>guilliermondii</i> (1)	Initial monotherapy with FLUC for ≥ 48 h	30-day mortality	CART analysis could not identify a dose/ MIC or AUC/MIC breakpoint for the entire cohort. For <i>C. albicans</i> , FLUC dose/MIC and AUC/MIC ratios of ≤ 400 were associated with 30-day mortality (HR, 9.72; 95% CI 1.75–54.0; $P = 0.009$; and HR, 7.94; 95% CI 1.09–57.7; $P =$ 0.04, respectively) in the Cox proportional hazards model.	No (CART); yes (Cox)	Yes, for <i>C. albicans</i>	For entire cohort, FLUC dose of ≤ 200 mg/ day (HR, 3.25; 95% CI, 1.28–8.27; $P =$ 0.013) was associated with 30-day mortality. For <i>C. albicans</i> , a FLUC dose of ≤ 200 mg/day (HR, 4.7; 95% CI, 1.45– 15.4; $P = 0.010$) associated with 30-day mortality in the Cox proportional hazards model. AUC was calculated by dividing FLUC dose by estimated CL_{FLUC} . FLUC clearance was calculated using equation that models CL_{FLUC} as a function of CL_{CR} .
Fernandez-Ruiz et al., 2017 (19)	249	<i>albicans</i> (138), <i>parapsilosis</i> (54), <i>tropicalis</i> (23), <i>glabrata</i> (23), other (11)	Received FLUC as initial therapy for ≥ 72 h	Clinical failure: all-cause mortality between days 3–30 from index culture and/or persistent candidemia ≥ 72 h after initiation of therapy.	No AUC/MIC thresholds were significantly associated with failure in multivariate analyses.	Yes	No	AUC calculated using a published formula ($0.99 \times$ daily dose in mg $- 9.2$). FLUC dose calculated by taking avg daily dose over the first 3 days of treatment. Dose halved in patients receiving continuous venovenous hemodiafiltration.

^aNumber of episodes of candidemia evaluated in the study. Rodriguez-Tudela et al. (10) also evaluated 110 episodes of oropharyngeal candidiasis; results reported in the table refer to the 126 patients with candidemia.

^bAll AUCs in this table represent 24-hour AUCs.

^cAUC, area under the concentration-time curve (total drug exposure over a defined period); CART, classification and regression tree; CL, clearance; CL_{CR} , creatinine clearance; CL_{FLUC} , fluconazole clearance; CI, confidence interval; FLUC, fluconazole; HR, hazard ratio; NA, not applicable; OR, odds ratio; and PD, pharmacodynamic(s), NR, not reported.

AUC/MIC thresholds were not significantly associated with fluconazole failure (30-day mortality and/or persistent candidemia) in a multivariate logistic regression model (19).

The failure of these studies to identify a consistent pharmacodynamic target perhaps suggests that a singular approach to establishing CBPs for fluconazole applicable to all *Candida* species may not be optimal. The argument for species-specific breakpoints is strongest for *C. glabrata*. *C. glabrata*, while in the same clade as *C. albicans*, is actually more closely related to *Saccharomyces cerevisiae* than to *C. albicans*. *C. albicans* is diploid, while *C. glabrata* is haploid, and the 2 species differ in many aspects of host invasion and in their interactions with the host immune system (22). While drug efflux is the most common mechanism of resistance in both species, molecular mechanisms involved in the development of resistance may differ, and some mechanisms of resistance may result in differential effects on *C. albicans* versus *C. glabrata*. Whether these differences are related to or cause the pronounced difference in susceptibility between these species (e.g., the 16-fold higher ECV of fluconazole for *C. glabrata* than *C. albicans*; Table 1) is unclear (23). In 2012, the CLSI published revised breakpoints for *Candida* species and fluconazole, with substantially lower breakpoints for *C. albicans*, *C. parapsilosis*, and *C. tropicalis* compared to those for *C. glabrata*, and eliminated the "susceptible" category for *C. glabrata* (Table 1) (4). These revisions were made to be consistent with EUCAST breakpoint recommendations, as well as to better align susceptibility thresholds with ECVs rather than basing them purely on clinical data. In fact, no relationship was found between fluconazole MICs for *C. tropicalis* and outcome, nor were any clinical data supporting the *C. parapsilosis* breakpoints provided (24). As shown in Table 2, only two studies have assessed the relationships between pharmacodynamic parameters and outcomes stratified by specific *Candida* species.

For infections caused by *C. glabrata*, 2016 IDSA treatment guidelines for candidemia recommend transitioning from an echinocandin to high-dose fluconazole in stable patients with microbiological clearance when the isolate is fluconazole-susceptible (1). This recommendation has resulted in confusion among clinicians, since no *C. glabrata* fluconazole MICs are designated by CLSI as susceptible (4). However, data by Eschenauer et al. suggest that isolates designated SDD (those isolates with an MIC of ≤ 32 mg/liter) may be treated successfully when fluconazole is aggressively dosed. In their study of 122 patients with candidemia due to *C. glabrata* (56, 49, and 17 isolates with MICs of ≤ 8 mg/liter, 16 to 32 mg/liter, and ≥ 64 mg/liter, respectively) that were treated with fluconazole, complete response (defined as a composite of clinical success, microbiological success, and survival) at day 14 was attained in 48% (50/105) of patients infected with an isolate with an MIC of ≤ 32 mg/liter compared to 24% (4/17) for patients infected with an isolate with an MIC of > 32 mg/liter ($P = 0.07$). This 48% response rate was similar to the 52% response rate seen in patients treated with an echinocandin, although echinocandin-treated patients tended to be more ill, both at baseline and at the time of fungemia (17). Regarding fluconazole dose optimization, while a dose/MIC of > 12.5 was significantly associated with improved clinical response in an unadjusted analysis (49% versus 20%; $P = 0.025$) (17), no significant correlations were found between fluconazole AUC, AUC/MIC, or MIC and survival in a subsequent adjusted analysis. On multivariable logistic regression, higher average fluconazole dose (odds ratio [OR], 1.006; 95% confidence interval [CI], 1.001 to 1.010; $P = 0.008$), average fluconazole dose of ≥ 400 mg (OR, 3.965; 95% CI, 1.509 to 10.418; $P = 0.005$), and higher fluconazole dose on day 1 of therapy (OR, 1.007; 95% CI, 1.002 to 1.011; $P = 0.002$) were identified as independent predictors of 28-day survival, with doses of ≥ 400 mg/day associated with 28-day survival of 87.7% (20). In a recent study from Korea of 65 patients with *C. glabrata* fungemia treated with "standard doses" (dosing not elucidated further) of fluconazole for at least 48 h, a fluconazole MIC of 32 mg/liter was independently associated with mortality. However, only 3 fluconazole-treated patients were infected with isolates with MICs of 32 mg/liter, limiting the rigor of this conclusion (25). As such, the current CLSI CBP of ≤ 32 mg/liter may be appropriate, if fluconazole dosing of at least 400 mg/day is utilized.

Regarding *C. albicans*, in the study by Brosh-Nissimov et al., in an adjusted analysis,

a dose/MIC ratio of ≤ 400 was associated with 30-day mortality (HR, 9.72; 95% CI, 1.75 to 54.0; $P = 0.009$) in the subgroup of 36 patients with *C. albicans* fungemia. However, there were only two isolates with an MIC of > 2 mg/liter (i.e., nonsusceptible) (18). A dose/MIC of > 400 may be difficult to safely achieve for *C. albicans* with MICs in the upper range of CLSI susceptible or susceptible dose-dependent breakpoints (≤ 2 and 4 mg/liter, respectively) (4, 18). In accordance with these findings, a study of 217 patients with *C. albicans* fungemia (including 8 isolates with MICs of > 2 mg/liter) who received treatment with fluconazole monotherapy (with $\sim 70\%$ receiving doses of ≥ 400 mg/day) found an MIC of ≥ 2 mg/liter to be independently associated with infection-related mortality by multivariate analysis (OR, 8.2; 95% CI, 2.3 to 29.7; $P = 0.001$) (26). Both studies suggest that a breakpoint of ≤ 1 mg/liter (or perhaps ≤ 0.5 mg/liter) may be more appropriate for infections due to *C. albicans*. An analysis of $> 8,000$ clinical isolates of *C. albicans* found 98.1% of isolates to have fluconazole MICs of ≤ 0.5 mg/liter, with 1.1% having MICs of 1 to 2 mg/liter; thus, the vast majority of infections due to *C. albicans* can be treated with standard fluconazole dosing (400 mg/day) (24).

Currently, there are insufficient data to propose CBPs for other *Candida* species. As stated above, in the absence of such data, ECVs may be used to identify isolates with possible acquired resistance that may impact treatment outcomes. Finally, given that guidelines largely resign fluconazole to stepdown therapy after initial echinocandin treatment, further research is needed to assess optimal dosing in this scenario, as the available data largely consist of patients treated with fluconazole as initial therapy (1).

NEWER TRIAZOLES

Voriconazole. In a neutropenic murine candidiasis model, a mean free-drug 24-h AUC/MIC ratio of 24 was necessary to achieve 50% maximal effect against 10 isolates of *C. albicans*. Human pharmacokinetic data suggest that a free drug 24-h AUC of 8.4 mg $\cdot$ h/liter may be obtained following administration of voriconazole at an oral dose of 200 mg twice daily (27). Thus, pharmacodynamic targets may be expected to be achieved for infections due to isolates of *C. albicans* with MICs of ≤ 0.25 mg/liter. Importantly, voriconazole displays significant interpatient variability (see below), and thus individual patient exposure is difficult to predict (3, 28).

Relative to fluconazole, a paucity of data exists exploring the relationship between voriconazole susceptibility or pharmacodynamic parameters and clinical outcomes. Pfaller and colleagues published phase III clinical trial data from 249 patients treated with voriconazole for *Candida* (96 *C. albicans*, 51 *C. tropicalis*, 47 *C. glabrata*, 34 *C. parapsilosis*, 12 *Candida* spp., and 9 *C. krusei* isolates) collected from blood and normally sterile body sites. They identified a significant correlation ($P = 0.021$) between voriconazole MIC and treatment success (29). Subsequently, the EUCAST Antifungal Subcommittee conducted a classification and regression tree (CART) analysis of these data but was not able to identify a breakpoint with low relative error. A clinical response of 76% was seen among patients with infections caused by wild-type *C. albicans*, *C. tropicalis*, and *C. parapsilosis* isolates, which EUCAST identified as those with MICs of ≤ 0.125 mg/liter. Thus, this breakpoint was adopted as a CBP for those three species by EUCAST in 2010. There were too few isolates of *C. krusei* ($n = 9$) to conduct a meaningful analysis, and there was no relationship identified between *C. glabrata* MIC and response (30). In 2017, EUCAST changed the *C. albicans* CBP to ≤ 0.064 mg/liter, presumably because an updated MIC distribution identified an ECV of 0.032 mg/liter (28). These data, although limited, represent the only available analyses evaluating the relationship between voriconazole *in vitro* susceptibility and clinical outcome. As such, CBPs for *C. albicans*, *C. tropicalis*, and *C. parapsilosis*, as listed in Table 1, may be reasonable but should be viewed as preliminary. The CLSI-defined CBP for *C. krusei* is not supported by data and should be viewed with caution (4, 6, 8).

Voriconazole is licensed for the treatment of candidemia. Due in part to genetic polymorphisms in the CYP2C19 gene, voriconazole exposure exhibits significant interpatient variability, necessitating therapeutic drug monitoring (TDM) of trough (lowest concentration prior to the next administered dose) serum concentrations (31). Data

evaluating the relationship between voriconazole trough and efficacy in patients with infections due to *Candida* species is limited to a single study that simulated voriconazole pharmacokinetics and pooled *Candida* species with molds (32). Since most fluconazole-resistant *Candida* species display cross-resistance to voriconazole (e.g., 98.8% of fluconazole-resistant *C. glabrata* isolates have MICs of >0.5 mg/liter to voriconazole), it does not provide a reliable alternative to fluconazole (33). As such, notwithstanding the limited data to support CBPs, voriconazole appears to have a limited place in the therapy of invasive candidiasis.

Posaconazole. Posaconazole is a broad-spectrum triazole with limited clinical data in the treatment of candidemia or invasive candidiasis. A neutropenic murine model of disseminated candidiasis utilizing 12 isolates of *C. albicans* identified a mean free-drug 24-h AUC/MIC ratio of 16.9 to achieve 50% maximal effect (34). However, this translates to a total drug 24-h AUC/MIC of 845 due to posaconazole's high (98%) protein binding (35). The recent introduction of the delayed-release tablet formulation of posaconazole provides improved exposure compared to that of the suspension formulation, achieving a total 24-h AUC of $37.9 \text{ mg} \cdot \text{hr/liter}$ following administration of 300 mg twice daily on day 1 followed by 300 mg once daily, which is similar to the exposure achieved with equivalent dosing of intravenous posaconazole (35). Thus, pharmacodynamic goals may be achieved for infections caused by *C. albicans* with posaconazole MICs up to 0.04 mg/liter. However, the optimal AUC/MIC ratio may be different for non-*albicans* species of *Candida*. In addition, no human data exist evaluating posaconazole pharmacodynamic targets in the treatment of candidiasis or candidemia. Furthermore, there are no data evaluating the relationship between posaconazole MIC and clinical outcomes. Therefore, while ECVs for posaconazole and *Candida* species are provided in Table 1, CLSI CBPs are not available (8). In summary, there are currently insufficient clinical data to establish CBPs or evaluate pharmacodynamic targets for posaconazole in the treatment of invasive candidiasis. In rare scenarios where the use of posaconazole may be necessary for treatment, ECVs may be utilized to delineate isolates that may express resistance mutations.

Isavuconazole. Similar to other triazole antifungals, neutropenic murine models of invasive candidiasis identified the AUC/MIC ratio as the pharmacodynamic parameter most predictive of isavuconazole efficacy. Mean free-drug AUC/MIC ratios of 50.5, 1.6, and 6.2 were necessary to achieve 50% maximal effect in *C. albicans*, *C. glabrata*, and *C. tropicalis* models, respectively (36). Using the identified targets and an expected free-drug 24-h AUC of $\sim 1.8 \text{ mg} \cdot \text{hr/liter}$ (based on a pharmacokinetic study conducted in healthy subjects utilizing dosing of 200 mg per day of isavuconazole), pharmacodynamic targets would likely be achieved for infections caused by *C. albicans* with isavuconazole MICs of ≤ 0.04 mg/liter, *C. glabrata* with MICs of ≤ 1.125 mg/liter, and *C. tropicalis* with MICs of ≤ 0.3 mg/liter (36). Currently, there are no clinical data evaluating the impact of the identified pharmacodynamic target on outcomes in humans, and as such, these recommendations are preliminary, CBPs are not available, and ECVs cannot be evaluated.

The role of isavuconazole for the treatment of candidemia or invasive candidiasis remains undefined, as the results of a phase III clinical trial for the treatment of candidemia and other invasive *Candida* infections in which the efficacy of isavuconazole was compared to caspofungin remain unpublished (37).

CONCLUSIONS

This review describes the relationships between *in vitro* susceptibility, pharmacodynamic targets, and clinical outcome for azole antifungals. Based on our analysis of these data, a fluconazole susceptible CBP of ≤ 1 mg/liter for *C. albicans* and susceptible dose-dependent CBP of ≤ 32 mg/liter (using aggressive fluconazole dosing of ≥ 400 mg daily) for *C. glabrata* may be warranted. Evaluations that are more robust are needed to establish CBPs for voriconazole, posaconazole, and isavuconazole; thus, the role of these agents in the treatment of invasive candidiasis remains uncertain. When use of these agents is necessary, ECVs may be used to identify isolates with acquired azole

resistance that may impair treatment efficacy. For all agents, additional, robust species-specific evaluations are needed, as are studies evaluating outcomes in patients with infections due to isolates with higher MICs. Lastly, as the majority of existing data are for patients with candidemia, studies exploring the impact of azole susceptibility and pharmacodynamic parameters on outcomes in patients with other types of invasive candidiasis are warranted.

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