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Activity of a long-acting echinocandin, CD101, determined using CLSI and EUCAST reference methods, against *Candida* and *Aspergillus* spp., including echinocandin- and azole-resistant isolates

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Objectives: The objective of this study was to evaluate the *in vitro* activity of CD101, a novel echinocandin with a long serum elimination half-life, and comparator (anidulafungin and caspofungin) antifungal agents against a collection of *Candida* and *Aspergillus* spp. isolates.

Methods: CD101 and comparator agents were tested against 106 *Candida* spp. and 67 *Aspergillus* spp. isolates, including 27 isolates of *Candida* harbouring *fks* hotspot mutations and 12 itraconazole non-WT *Aspergillus*, using CLSI and EUCAST reference susceptibility broth microdilution (BMD) methods.

Results: Against WT and *fks* mutant *Candida albicans*, *Candida glabrata* and *Candida tropicalis*, the activity of CD101 [MIC_{90} = 0.06, 0.12 and 0.03 mg/L, respectively (CLSI method values)] was comparable to that of anidulafungin (MIC_{90} = 0.03, 0.12 and 0.03 mg/L, respectively) and caspofungin (MIC_{90} = 0.12, 0.25 and 0.12 mg/L, respectively). WT *Candida krusei* isolates were very susceptible to CD101 (MIC = 0.06 mg/L). CD101 activity ($MIC_{50/90}$ = 1/2 mg/L) was comparable to that of anidulafungin ($MIC_{50/90}$ = 2/2 mg/L) against *Candida parapsilosis*. CD101 (MIC mode = 0.06 mg/L for *C. glabrata*) was 2- to 4-fold more active against *fks* hotspot mutants than caspofungin (MIC mode = 0.5 mg/L). CD101 was active against *Aspergillus fumigatus*, *Aspergillus terreus*, *Aspergillus niger* and *Aspergillus flavus* (MEC_{90} range = ≤0.008–0.03 mg/L). The essential agreement between CLSI and EUCAST methods for CD101 was 92.0%–100.0% among *Candida* spp. and 95.0%–100.0% among *Aspergillus* spp.

Conclusions: The activity of CD101 is comparable to that of other members of the echinocandin class for the prevention and treatment of serious fungal infections. Similar results for CD101 activity versus *Candida* and *Aspergillus* spp. may be obtained with either CLSI or EUCAST BMD methods.

Introduction

Accumulated *in vitro* and clinical experience with the available echinocandin antifungal agents (anidulafungin, caspofungin and micafungin) strongly supports recommendations that this class of antifungal agents be used as primary therapy for candidaemia and other forms of invasive candidiasis (IC; infections involving normally sterile sites and tissues).[1–5] The fact that these agents also have activity against *Aspergillus* spp. makes them attractive agents for empirical therapy in patients who are at risk of both IC and aspergillosis.[6] The members of the echinocandin class share a common mechanism of action [i.e. inhibition of glucan synthase (GS)], must be administered intravenously (iv) and generally are well tolerated.[6] Despite the broad utilization of echinocandins to treat IC in critically ill hospitalized patients, clinical resistance to these agents remains uncommon, although both breakthrough infections and acquired resistance mutations in certain species of *Candida* have been reported.[3,7–12]

Although the currently available echinocandins are highly efficacious and relatively easy to use in the treatment of invasive fungal infections,[1,6] they must be administered by daily iv infusion, potentially prolonging hospital stay of patients undergoing echinocandin therapy and limiting their use to the hospital inpatient setting. The availability of an echinocandin antifungal agent with *in vitro* activity that is comparable to that of echinocandins presently in use, but with improved pharmacokinetics and utility, may expand the benefits of echinocandin therapy and improve clinical outcomes.

CD101 (formerly SP3025; Cidara Therapeutics) is a novel echinocandin antifungal agent being developed for high-exposure,

once-weekly iv administration for the treatment of IC and for topical treatment of acute and recurrent vulvovaginal candidiasis. CD101 displays chemical stability in plasma, aqueous solution and at elevated temperature,[13] a long half-life[14,15] and *in vivo* efficacy against *Candida* and *Aspergillus* spp.[16–18] The pharmacokinetic profile of CD101 enables once-weekly iv administration and front-loaded antimicrobial plasma exposure.[17] This approach maximizes drug effect early in the course of therapy when the density of the pathogen is greatest, in order to increase the rate and extent of pathogen killing, reduce spontaneous mutations and eliminate pre-existing drug-resistant subpopulations. The less frequent dosing of CD101, compared with currently available echinocandins, should facilitate shorter and less expensive hospital stays, improve compliance for outpatients and provide more

Table 1. *In vitro* susceptibilities of 173 *Candida* and *Aspergillus* spp. to CD101, anidulafungin and caspofungin as determined by the CLSI BMD method[a]

	No. of isolates (no. of mutants[b]) at MIC or MEC (mg/L)										
Organism (no. tested)/antifungal agent	≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	≥8
C. albicans (25)											
CD101	1	7	3	4	1 (1)	5 (5)	2 (2)	2 (2)			
anidulafungin	4	6	5		2 (2)	4 (4)	1 (1)	2 (2)	1 (1)		
caspofungin			2	5	7	1	5 (5)	4 (4)	1 (1)		
C. glabrata (25)											
CD101		1	4	10 (2)	4 (2)	2 (2)	1 (1)	2 (2)	1 (1)		
anidulafungin	1		2	8 (1)	6 (1)	4 (4)		2 (2)	1 (1)	1 (1)	
caspofungin			1		11 (1)	7 (3)	3 (3)	1 (1)	1 (1)		1 (1)
C. tropicalis (21)											
CD101	1	6	4	4		4 (3)	1 (1)	1 (1)			
anidulafungin	2	5	7	1		3 (2)	1 (1)	2 (2)			
caspofungin				4	9	4 (1)	1 (1)	1 (1)	2 (2)		
C. krusei (20)											
CD101		1	8	9		1 (1)		1 (1)			
anidulafungin		1		14	3		1 (1)		1 (1)		
caspofungin					2	16		1 (1)			1 (1)
C. parapsilosis (15)											
CD101							5	7	3		
anidulafungin							2	5	8		
caspofungin					1	3	10	1			
A. fumigatus (20)											
CD101	15	4		1							
anidulafungin	15	4		1							
caspofungin			1	11	7		1				
A. terreus (19)											
CD101	8	10	1								
anidulafungin	11	7	1								
caspofungin				11	8						
A. flavus (12)											
CD101	11	1									
anidulafungin	11	1									
caspofungin			3	8	1						
A. niger (16)											
CD101	14		2								
anidulafungin	15	1									
caspofungin				14	2						

[a]CLSI BMD method[24,25,29] using 24 h incubation and prominent (≥50%) inhibition (MIC) or the lowest concentration that results in growth of *Aspergillus* spp. producing conspicuously aberrant growth: small, round, compact microcolonies (MEC).
[b]No. of mutants = number of *fks* mutant strains of each *Candida* species.

convenient outpatient prophylaxis or maintenance treatment regimens.

The similarities and differences between the CLSI and EUCAST international standards for broth microdilution (BMD) testing of *Candida* spp. have been discussed in several publications.[19–21] Whereas we have shown previously that the two reference methods provide concordant results when testing both azoles and echinocandins against *Candida* and *Aspergillus* spp., similar data are not available for CD101.[21,22] Given the important role that both methods play in antifungal development and resistance surveillance,[3,23] it is important to demonstrate the comparability of the susceptibility testing results in preclinical studies of new antifungal agents.

In the present study, we performed a methods comparison where the activity and potency of CD101, anidulafungin and caspofungin were determined against a panel of 106 *Candida* spp. (five species) and 67 *Aspergillus* spp. (four species) isolates selected to represent phenotypically and genotypically antifungal-resistant strains. All isolates were tested using both CLSI[24,25,29] and EUCAST[26] BMD methods and the species-specific and overall essential agreement (EA; ± 2 $\log_2$ dilutions) were determined.

Materials and methods

Organisms

A total of 106 clinical isolates of *Candida* spp. and 67 isolates of *Aspergillus* spp. were tested, including 25 *Candida albicans*, 25 *Candida glabrata*, 21 *Candida tropicalis*, 20 *Candida krusei*, 15 *Candida parapsilosis*, 20 *Aspergillus fumigatus*, 19 *Aspergillus terreus*, 12 *Aspergillus flavus* and 16 *Aspergillus niger* isolates. Isolates were selected to represent both WT and antifungal-resistant strains. The collection contained 46 fluconazole-resistant strains of *Candida* spp. (11 *C. albicans*, 5 *C. glabrata*, 4 *C. parapsilosis*, 6 *C. tropicalis* and 20 *C. krusei*), 27 strains of *Candida* spp. with documented mutations in *fks* (10 *C. albicans*, 10 *C. glabrata*, 5 *C. tropicalis* and 2 *C. krusei*) and 12 itraconazole non-WT (MIC >4 mg/L) strains of *Aspergillus* spp. (10 *A. fumigatus* and 2 *A. flavus*). All isolates were identified to the species level using a combination of conventional, molecular and proteomic methods as described previously.[3,27]

Antifungal susceptibility testing

Candida spp. ($n=106$) and *Aspergillus* spp. ($n=67$) isolates were tested for susceptibility to CD101, anidulafungin and caspofungin using CLSI and EUCAST BMD methods.[24–26,28,29] The reference powder of CD101 was obtained from Seachaid Pharmaceuticals (Durham, NC, USA). Stock solutions of all three echinocandins were prepared in DMSO and the final range of concentrations tested was 0.008–16 mg/L.

CLSI BMD testing of *Candida* spp. was performed and interpreted as outlined in documents M27-A3[24] and M27-S4[29] by using round-bottomed 96-well plates containing RPMI 1640 medium with 0.2% glucose, inocula of $0.5–2.5\times10^3$ cells/mL and incubation at 35°C. MIC values were determined visually after 24 h of incubation. The MIC endpoint criterion was the lowest concentration of drug that caused significant diminution (≥50% inhibition) of growth relative to that of the growth control. EUCAST BMD testing of *Candida* spp. was performed as outlined in document EDef 7.2[26] by using flat-bottomed 96-well plates containing RPMI 1640 broth with 2.0% glucose, inocula of $0.5–2.5\times10^5$ cells/mL and incubation at 35°C. MIC values were determined spectrophotometrically (at 530 nm) after 24 h of incubation, as the lowest concentration of drug that resulted in ≥50% inhibition of growth relative to that of the growth control.

In vitro testing of *Aspergillus* susceptibility to CD101, anidulafungin and caspofungin was performed using BMD methods of the CLSI[25] and EUCAST.[28] Incubation for both methods was at 35°C for 24 h and minimum effective concentrations (MECs) were defined as the lowest concentration of drug in which abnormal, short and branched hyphal clusters were observed in contrast to the long, unbranched, hyphal elements (confluent hyphal growth) seen in the growth control.

MIC and MEC results of CD101 obtained with the CLSI method were compared with those obtained with the EUCAST method in order to determine the EA between MIC values. High off-scale MIC or MEC results were converted into the next highest concentration and low off-scale MIC results were left unchanged. Discrepancies of at least ± 2 $\log_2$ dilutions among MIC or MEC results were used to calculate the EA.

Quality control (QC)

QC was ensured by concurrent testing with the following strains recommended by CLSI and EUCAST: *C. krusei* ATCC 6258, *C. parapsilosis* ATCC 22019, *A. flavus* ATCC 204304 and *A. fumigatus* ATCC MYA-3626. QC strains were tested a total of five times each against CD101, anidulafungin and caspofungin over the course of the study. The results for both anidulafungin and caspofungin were within control limits for all tested QC strains. MIC or MEC values of CD101 were 0.03 mg/L for *C. krusei* ATCC 6258, 0.5 mg/L for *C. parapsilosis* ATCC 22019 and ≤0.008 mg/L for both *A. flavus* ATCC 204304 and *A. fumigatus* ATCC MYA-3626.

Results and discussion

Table 1 summarizes the *in vitro* susceptibilities of 173 isolates of *Candida* spp. (106 isolates) and *Aspergillus* spp. (67 isolates) to CD101, anidulafungin and caspofungin, determined by the CLSI

Table 2. *In vitro* susceptibilities of *Candida* and *Aspergillus* spp. to CD101 as determined by 24 h CLSI and EUCAST BMD methods using prominent (≥50%) MIC endpoint criteria

Species (no. tested)	Test method	CD101 MIC or MEC (mg/L)		Percentage EA
		range	mode	
C. albicans (25)	CLSI	≤0.008–1	0.015	92.0
	EUCAST	≤0.008–1	≤0.008	
C. glabrata (25)	CLSI	0.015–2	0.06	100.0
	EUCAST	≤0.008–2	0.06	
C. tropicalis (21)	CLSI	≤0.008–1	0.015	100.0
	EUCAST	≤0.008–0.5	0.03	
C. krusei (20)	CLSI	0.015–1	0.06	100.0
	EUCAST	0.03–0.5	0.06	
C. parapsilosis (15)	CLSI	0.5–2	1	100.0
	EUCAST	0.5–1	1	
A. fumigatus (20)	CLSI	≤0.008–0.06	≤0.008	95.0
	EUCAST	≤0.008–0.03	0.015	
A. terreus (19)	CLSI	≤0.008–0.03	≤0.008	100.0
	EUCAST	≤0.008–0.03	≤0.008	
A. flavus (12)	CLSI	≤0.008–0.015	≤0.008	100.0
	EUCAST	≤0.008–0.03	≤0.008	
A. niger (16)	CLSI	≤0.008–0.03	≤0.008	100.0
	EUCAST	≤0.008–0.03	≤0.008	

BMD method. The number of *fks* mutant strains for each species of *Candida* is listed in parentheses. The activity of CD101 against both WT and *fks* mutant strains of *Candida* spp. was comparable to that of anidulafungin against all species tested. The MIC values of both CD101 and anidulafungin that were associated with the *fks* mutant strains were generally ≥0.12 mg/L for *C. albicans* and *C. glabrata* and ≥0.25 mg/L for *C. tropicalis* and *C. krusei*. Caspofungin was 2- to 4-fold less active than either CD101 or anidulafungin against both WT and *fks* mutant strains of each species with the exception of *C. parapsilosis* (Table 1).

Both CD101 and anidulafungin were most active against all four species groups of *Aspergillus* spp. with MEC values ≤0.06 mg/L for all isolates tested (Table 1). By comparison, caspofungin was generally 4-fold less active than either CD101 or anidulafungin. CD101 and anidulafungin were comparably active against both WT and itraconazole non-WT isolates.

The challenging nature of the selected *Candida* spp. tested in this study is evident from the high levels of resistance to anidulafungin (0.0%–16.0%) and caspofungin (0.0%–24.0%) (data not shown). In addition to echinocandin resistance, 43.3% of these isolates were also resistant to fluconazole when categorized using CLSI interpretive criteria.[29] Table 2 displays the *in vitro* activity of CD101 tested against the five species of *Candida* and four species groups of *Aspergillus* using CLSI and EUCAST BMD methods. Whereas CD101 showed comparable activity against all isolates tested with both methods, the MIC or MEC values obtained with the EUCAST method were generally one dilution lower than those obtained with the CLSI method. The EA between the methods was 92.0%–100.0% for *Candida* spp. (overall EA = 98.1%) and 95.0%–100.0% for *Aspergillus* spp. (overall EA = 98.5%).

The activity of CD101, anidulafungin and caspofungin against the *fks* mutant strains as determined by both CLSI and EUCAST methods is shown in Table 3. The vast majority of *fks* mutants exhibited intermediate or resistant echinocandin MIC values as per current CLSI and/or EUCAST interpretive criteria. Although the MIC results of CD101 were comparable for both reference methods, the MIC values obtained for anidulafungin were generally 2- to 4-fold higher and for caspofungin were 2-fold lower with the CLSI method versus the EUCAST method.

These data demonstrate the comparable *in vitro* activity of CD101 versus anidulafungin and caspofungin when tested against a select group of highly resistant strains of *Candida* and *Aspergillus* spp. as determined by both CLSI and EUCAST BMD methods. CD101 and other echinocandins showed potent activity against azole-resistant strains of *Candida* and *Aspergillus* spp. (data not shown). In general, CD101 MIC values of ≤0.06 and ≤0.12 mg/L provided separation between WT strains of *Candida* spp. and those harbouring mutations in *fks1/fks2*. These findings are in agreement with those of Perez *et al.*,[30] who found that WT GS purified from *C. albicans* and *C. glabrata* was as sensitive

Table 3. MICs of CD101, caspofungin and anidulafungin for *Candida* spp. strains possessing *fks* hotspot mutations leading to amino-acid alterations

	FKS1		FKS2	CLSI MIC (mg/L)			EUCAST MIC (mg/L)		
Organism	HS1	HS2	HS1	CD101	ANF	CAS	CD101	ANF	CAS
C. albicans	F641I	WT	NT	0.12	0.12	0.5	0.12	0.03	1
	F641S	WT	NT	0.25	0.12	0.5	0.12	0.03	1
	S645P	WT	NT	0.5	0.5	2	0.5	0.25	4
	F641S	WT	NT	0.25	0.25	1	0.25	0.12	2
	S645Y	WT	NT	1	2	1	0.5	0.25	2
	S645F	WT	NT	0.5	1	1	0.5	0.25	1
	D698Y	WT	NT	0.25	0.25	0.5	0.12	0.03	1
	P649H	WT	NT	0.25	0.25	0.5	0.12	0.06	1
	S645P	WT	NT	1	1	1	1	0.25	4
C. glabrata	WT	WT	F659V	1	1	0.5	0.5	0.12	2
	S629P	WT	WT	2	4	≥8	1	1	8
	D632Y	WT	WT	0.12	0.25	0.25	0.06	0.03	0.5
	L630I	WT	WT	0.06	0.06	0.25	0.06	≤0.008	0.5
	WT	WT	D648E	0.25	0.25	0.25	0.12	0.06	0.5
	F625Y	WT	WT	0.06	0.12	0.12	0.06	0.03	0.25
	F625S	WT	WT	0.5	1	2	0.25	0.25	2
	WT	WT	S663P	1	2	1	2	2	≥8
	WT	WT	P667T	0.25	0.25	0.5	0.25	0.12	1
C. krusei	WT	R1361G	WT	1	2	8	0.5	1	≥8
	F655C	WT	WT	0.25	0.5	1	0.12	0.12	1
C. tropicalis	S645P	WT	WT	0.5	1	2	0.25	0.25	2
	F641S	WT	WT	0.25	0.25	0.25	0.12	0.06	1

HS1, hotspot 1; HS2, hotspot 2; ANF, anidulafungin; CAS, caspofungin; NT, not tested.

to inhibition by CD101 as to inhibition by micafungin. Likewise, GS from echinocandin-resistant strains of *C. albicans* and *C. glabrata* with well-defined mutations in either *fks1* or *fks2* showed a decrease in sensitivity to CD101 similar to micafungin. CD101 has been shown to be fungicidal against *Candida* spp.[31] and to have *in vivo* activity against both *C. albicans* and *A. fumigatus* over a range of exposures and dosing schedules in murine models of disseminated infection.[16–18] CD101 has also been demonstrated to be efficacious in treating IC caused by both *fks* WT and heterozygous *fks* mutant *C. albicans* in mice.[18] Most notably, Ong *et al*.[17] demonstrated prolonged efficacy following one dose of CD101 in a neutropenic mouse model of disseminated candidiasis. CD101 displayed a concentration-dependent pattern of activity *in vivo* at doses that are projected to be achievable in the clinic. The prolonged efficacy and favourable pharmacokinetics suggest that a front-loaded CD101 dosing regimen is the optimal approach to maximize drug effect early in the course of infection.

In summary, the *in vitro* activity of CD101 against common WT and antifungal-resistant species of *Candida* and *Aspergillus* isolated from invasive infections was assessed using reference CLSI and EUCAST methods. The level of concordance between the two methods for testing CD101 was excellent and comparable to other studies evaluating systemically active antifungal agents.[20–22] The potency and spectrum of CD101 against *Candida* and *Aspergillus* spp. was excellent and comparable to that of anidulafungin and the other echinocandins. The activity of CD101 against *Candida* and *Aspergillus* spp., together with its exceptional stability, long half-life and front-loaded CD101 plasma exposure, warrant the continued clinical development of CD101.

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