Evaluation of the Fungicidal Activity of CD101, a Novel Echinocandin, and Comparators Against Recent Clinical Isolates of *Candida* spp.

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Abstract (Amended)

Background: CD101, a novel, highly stable echinocandin with the potential for once-weekly administration, is currently undergoing development for the treatment of candidemia and invasive candidiasis. To evaluate the fungicidal activity of CD101 and comparators against target pathogens, the MICs, minimum fungicidal concentrations (MFCs), and time-kill (TK) kinetics were determined against recent clinical isolates of *Candida* spp.
Methods: MICs were determined by broth microdilution in accordance with CLSI M27 with the exception that an increased inoculum size (~2.5 x 10^4 CFU/mL) was used. MFCs were determined by enumerating viable yeast from wells at and above the MIC of complete inhibition (MIC_0) at 48 hr. The lowest concentration with >99.9% kill relative to the initial inoculum was reported as the MFC. MFC:MIC_0 ratios ≤4 were considered to be indicative of fungicidal activity. TK was determined based on guidelines provided by CLSI for bacteria (CLSI M26) in which viable yeast (~10^6 CFU/mL) were exposed to 4X, 8X, and 16X the MIC_0 and were quantitated at 2, 4, 6, 24, and 48 hr. Anidulafungin (AFG), micafungin (MFG), and caspofungin (CFG) were included as comparators. For MFC, 10 each of *C. albicans*, *C. parapsilosis*, *C. krusei*, and *C. tropicalis* and 9 *C. glabrata* were tested. For TK, 3 *C. albicans*, 2 *C. glabrata*, 2 *C. parapsilosis*, and 1 each of *C. krusei* and *C. tropicalis* were tested.
Results: For isolates with defined MFCs, MFC:MIC_0 ratios ≤4 for CD101 were observed for 100% of *C. albicans*, *C. parapsilosis*, and *C. krusei*, 89% of *C. glabrata*, and 86% of *C. tropicalis* isolates. Similar results were observed with AFG, MFG, and CFG, though due to the general inactivity by CFG against *C. parapsilosis* by MIC_0, MFCs were largely undetermined. By TK, CD101, AFG, MFG, and CFG generally exhibited a 1 to 2-log kill from 2-6 hr and >3-log kill from 24-48 hr at 4X, 8X, and 16X excluding CFG and *C. parapsilosis* where a >3-log kill was not observed.
Conclusions: Based on MFC and TK, CD101 and the comparator echinocandins were fungicidal across prevalent species of *Candida* with the exception of CFG and *C. parapsilosis* where fungicidal activity was not observed.

Introduction

- CD101 is a novel echinocandin with a long half-life currently under development by Cidara Therapeutics, Inc. for the treatment of candidemia and other forms of invasive candidiasis.
- The potential for an antifungal agent to kill fungi can be evaluated by determining the minimum fungicidal concentration (MFC) and by time-kill kinetic analysis.
- Against *Candida* spp., currently approved echinocandins (anidulafungin, micafungin, and caspofungin) have been shown to be fungicidal by these methods.[1,2]
- In this study, the MFC and time-kill kinetics of CD101 and echinocandin comparators were evaluated against recent clinical isolates of prevalent *Candida* spp.

Objective

To investigate the potential fungicidal activity of CD101 against target *Candida* spp. relative to other currently utilized echinocandins and other antifungal agents.

Methods

- Test isolates for MIC/MFC testing and time-kill kinetic analysis included clinical isolates from the Micromyx Repository (Kalamazoo, MI). A total of 10 each of *C. albicans*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei* and 9 *C. glabrata* were tested for MFC. A subset of these isolates (3 *C. albicans*, 2 *C. glabrata* and *C. parapsilosis*, and 1 *C. tropicalis* and *C. krusei*) were selected for time-kill kinetic analyses.
- For determination of the MFC, the method essentially described by CLSI for bacteria[3] as adapted by Canton et al.[2] for yeast was followed in which the MIC_0 (100% inhibition) was determined by standard CLSI methods[4,5], with the exception that an increased initial inoculum (approximately 2.5 x 10^4 CFU/mL) was used to enable determination of a >99.9% kill.
- For MFC testing, viable yeast were quantitated at the start of the assay to determine the initial inoculum size. Post-incubation and determination of the MIC_0, 0.1 mL aliquots from the wells at the MIC_0 and three wells above the MIC_0 were plated to determine viable counts and the resulting CFU/mL. The MFC was defined as the lowest concentration where a 3-log (99.9%) kill was observed relative to the initial inoculum. An MFC:MIC_0 ratio of ≤4 was considered indicative of fungicidal activity.
- The time-kill kinetic was determined essentially as described by CLSI for bacteria[3] and by Ernst et al.[1] and Canton et al.[2] for yeast. An initial inoculum of 5 x 10^5 – 5 x 10^6 CFU/mL was incubated in the presence of CD101 and comparator echinocandins at 4X, 8X, and 16X the MIC_0 as determined during MFC testing **(Table 1).** A growth control was evaluated in parallel. Viable yeast were quantitated by serial dilution plating at 2, 4, 6, 24 and 48 hr post-inoculation and fungicidal activity was defined as a 3-log (99.9%) kill maintained at 24 – 48 hr.

Table 1. CD101 and Comparator Activity against Time-kill Kinetic Isolates

		MIC_0 (µg/mL)			
Organism	**Isolate**	**CD101**	**Anidulafungin**	**Micafungin**	**Caspofungin**
C. albicans	MMX 7041	0.06	0.12	0.5	0.5
C. albicans	MMX 7409	0.12	0.06	0.25	0.5
C. albicans	MMX 7038	0.03	0.03	0.12	0.25
C. glabrata	MMX 7100	0.06	0.06	0.06	0.25
C. glabrata	MMX 7321	0.12	0.03	1	1
C. parapsilosis	MMX 7204	0.5	1	4	1
C. parapsilosis	MMX 7366	1	2	4	2
C. tropicalis	MMX 7255	0.12	0.03	0.12	0.12
C. krusei	MMX 7277	0.03	0.03	0.25	1

Results

MIC_0/MFC (Table 2)

- Based on MIC_{50} and MIC_{90} as determined by complete inhibition (MIC_0) with an increased inoculum size, the potency of CD101 was similar to anidulafungin across *Candida* spp., and both had lower MIC_{50}s and MIC_{90}s than micafungin and caspofungin.
- For isolates with defined MFCs, an MFC:MIC_0 ratio ≤ 4 indicative of fungicidal activity was observed for nearly all of the *Candida* spp. for CD101 and the comparator echinocandins.
- The MFC:MIC_0 ratio of caspofungin against *C. parapsilosis* was not evaluable for the majority of isolates due to the lack of complete inhibition observed over the tested concentration range.
- Complete inhibition was not commonly observed by MIC over the tested concentrations of fluconazole and voriconazole with the exception of *C. parapsilosis* and *C. krusei* (voriconazole only), preventing the evaluation of MFC for these agents for the majority of isolates.
- In instances where a defined MIC_0 and MFC was observed for fluconazole and voriconazole, MFC:MIC ratios were typically >4 indicating that overall these agents are not fungicidal.
- For amphotericin B, nearly all evaluated isolates had an MFC:MIC_0 ≤4 indicating that amphotericin B is fungicidal.

Table 2. Summary of Activity of CD101 and Comparators by MIC_0 and MFC:MIC_0 Ratio

					MFC:MIC ratio (n[%])		
Organism	**Drug**	**MIC range**	**MIC_{50}**	**MIC_{90}**	**MFC:MIC eval[1] (n)**	**≤4**	**>4**
C. albicans (n=10)	CD101	≤0.015-16	0.06	0.12	8	8 (100%)	
	Anidulafungin	≤0.015->16	0.03	0.12	8	7 (88%)	1 (13%)
	Micafungin	0.12->16	0.25	0.5	9	7 (78%)	2 (22%)
	Caspofungin	0.06->16	0.25	0.5	9	7 (78%)	2 (22%)
	Amphotericin B	0.12-0.5	0.5	0.5	9	9 (100%)	
	Fluconazole	>32	>32	>32	0		
	Voriconazole	>2	>2	>2	0		
C. tropicalis (n=10)	CD101	0.06->16	1	>16	7	6 (86%)	1 (14%)
	Anidulafungin	0.03->16	0.5	>16	6	6 (100%)	
	Micafungin	0.12->16	1	>16	8	8 (100%)	
	Caspofungin	0.06->16	0.25	>16	7	5 (71%)	1 (14%)
	Amphotericin B	0.5-1	0.5	1	10	9 (90%)	1 (10%)
	Fluconazole	2->32	>32	>32	2		2 (100%)
	Voriconazole	0.5->2	>2	>2	1		1 (100%)
C. parapsilosis (n=10)	CD101	0.5-2	1	2	10	10 (100%)	
	Anidulafungin	0.5-2	1	2	10	10 (100%)	
	Micafungin	2-8	4	4	9	8 (89%)	1 (11%)
	Caspofungin	1->16	>16	>16	3	1 (33%)	2 (67%)
	Amphotericin B	0.5-2	1	1	10	10 (100%)	
	Fluconazole	1->32	4	8	10	3 (30%)	7 (70%)
	Voriconazole	0.015->2	0.12	0.12	10	3 (30%)	7 (70%)
C. glabrata (n=9)	CD101	0.06-2	0.06	1	9	8 (89%)	1 (11%)
	Anidulafungin	0.03-1	0.06	1	9	9 (100%)	
	Micafungin	0.06-4	0.12	2	9	9 (100%)	
	Caspofungin	0.12->16	0.25	>16	7	7 (100%)	
	Amphotericin B	0.5-1	1	2	9	9 (100%)	
	Fluconazole	>32	>32	>32	0		
	Voriconazole	>2	>2	>2	0		
C. krusei (n=10)	CD101	≤0.015-0.06	0.03	0.06	8	8 (100%)	
	Anidulafungin	≤0.015-0.12	0.06	0.12	9	9 (100%)	
	Micafungin	0.25-0.5	0.25	0.5	10	10 (100%)	
	Caspofungin	0.5-1	0.5	1	10	10 (100%)	
	Amphotericin B	0.5-2	1	2	10	10 (100%)	
	Fluconazole	>32	>32	>32	0		
	Voriconazole	1-2	1	2	3	3 (100%)	

[1] MFC:MIC ratio only determined where defined MFC and MIC values resulted in the indicated ratios
Analysis based on MIC at which complete inhibition was observed (MIC_0) after 48 hours of incubation at increased inoculum size

Time-kill (Table 3, Figures 1-5)

- Isolates tested by time-kill kinetic analysis and their corresponding CD101 and comparator echinocandin MIC_0s are shown in **Table 1**.
- The occurrences of 2- to 3-log kills and >3-log kills during the course of the study for the evaluated isolates are summarized in **Table 3**.
- Overall, CD101 and comparator echinocandins exhibited fungicidal activity by time-kill kinetic analysis, with ≥3-log kills typically achieved by 24 hr and as early as 6 hr for some strains. 2- to 3-log kills were observed as early as 4 – 6 hr for the majority of strains evaluated.
- In contrast to CD101 and the other evaluated echinocandins, caspofungin did not achieve 3-log kills or even 2- to 3-log kills for the evaluated strains of *C. parapsilosis*.
- The killing observed with CD101 and comparator echinocandins was concentration-independent.
- As shown in **Figures 1-5**, the kill kinetic of CD101 and comparators was very similar at 8X the MIC_0 across evaluated species, with the exception of caspofungin against *C. parapsilosis* where the lack of observed 3-log kill relative to other agents was noted.

Table 3. Summary of CD101 and Comparator Echinocandin Time-kill Kinetic Analysis

			Observed kill[1] by timepoint (hr)															
			CD101					Anidulafungin					Micafungin					Caspofungin				
Organism	**Isolate No.**	**Fold-MIC_0**	**2**	**4**	**6**	**24**	**48**	**2**	**4**	**6**	**24**	**48**	**2**	**4**	**6**	**24**	**48**	**2**	**4**	**6**	**24**	**48**
C. albicans	MMX 7041	4X				++	++				++	++				++	++				++	++
		8X				++	++				++	++				++	++				++	++
		16X				++	++				++	++				++	++				++	++
	MMX 7409	4X				++	++				++	++				++	++				++	++
		8X				++	++				++	++				++	++				+	++
		16X			+	++	++				+	++			+	++	++			+	+	++
	MMX 7038	4X		+	++	++			+	++	++			+	++	++			+	++	++	
		8X		+	++	++	++		++	++	++	++		++	++	++	++		+	++	++	++
		16X		+	++	++	++		+	++	++	++		+	++	++	++			+	++	++
C. glabrata	MMX 7100	4X			+	++	++			+	++	++			+	++	++			+	++	++
		8X			+	++	++			+	++	++			+	++	++			+	++	++
		16X			+	++	++			+	++	++			+	++	++		+	+	++	++
	MMX 7321	4X		+	+	++	++			++	++	++		+	++	++	++			+	++	++
		8X		+	+	++	++		+	+	++	++		+	++	++	++				+	++
		16X		+	+	+	++		+	++	+	++		++	++	++	++				+	++
C. parapsilosis	MMX 7204	4X				+	++				++	++				++	++					
		8X				+	++				++	++				++	++					
		16X				+	++			+	++	++				+	++				+	
	MMX 7366	4X			+	++	++				++	++				++	++				+	+
		8X			+	++	+				++	+				++	+					
		16X		+		++	++				++	++				++	++					
C. tropicalis	MMX 7255	4X				++	++				++	++				++	++				+	++
		8X				++	+				++	+				++	+				+	++
		16X				+	++				+	++				++	++				+	++
C. krusei	MMX 7277	4X		+	+	++	++		+	+	++	++		+	+	++	++	+	++	++	++	++
		8X		+	+	++	++		+	+	++	++		+	++	++	++	++	++	++	++	++
		16X		+	++	++	++		+	++	++	++		+	++	++	++	+	++	++	++	++

[1] "+" denotes a kill of 2 to 3-logs relative to starting inoculum; "++" indicates a kill of >3-logs relative to starting inoculum
Concentrations tested were based on multiples of the MIC_0 (complete inhibition) as observed when testing high concentrations of inocula

Figure 1. Time-kill Kinetic at 8X the MIC_0 – *C. albicans*

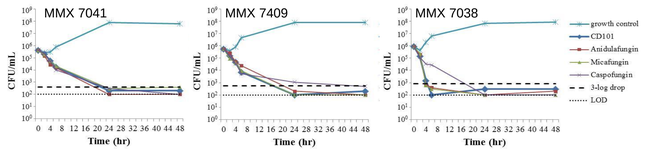


Figure 2. Time-kill Kinetic at 8X the MIC_0 – *C. glabrata*

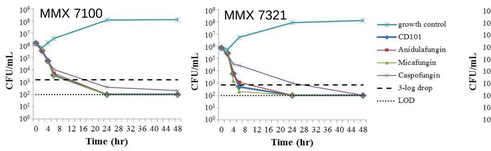


Figure 3. Time-kill Kinetic at 8X the MIC_0 – *C. parapsilosis*

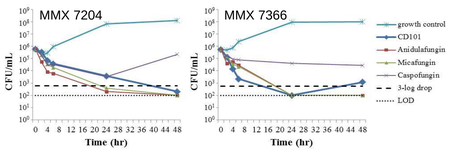


Figure 4. Time-kill Kinetic at 8X the MIC_0 – *C. tropicalis*

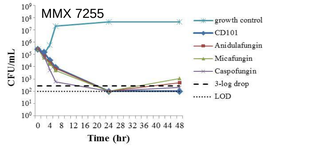
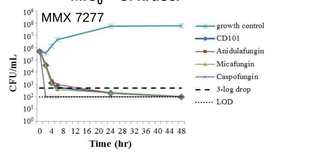


Figure 5. Time-kill Kinetic at 8X the MIC_0 – *C. krusei*



Conclusions

- Results from MFC testing show that both CD101 and comparator echinocandins along with amphotericin B are fungicidal against commonly encountered *Candida* spp.
- The fungicidal activity of CD101 and comparator echinocandins as observed by MFC was confirmed across *Candida* spp. by time-kill kinetic analysis.
- In contrast to CD101, anidulafungin, and micafungin, caspofungin was not fungicidal against *C. parapsilosis* based on time-kill kinetic analysis.
- The kill-kinetic of CD101 is nearly identical to that observed with the comparator echinocandins, with the exception of caspofungin against *C. parapsilosis* as noted above.

Acknowledgements

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