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 [MIC90 = 0.06, 0.12 and 0.03 mg/L, respectively (CLSI method values)] was comparable to that of anidulafungin [MIC90 = 0.03, 0.12 and 0.03 mg/L, respectively] and caspofungin [MIC90 = 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 (MIC50/90 = 0.1/2 mg/L) was comparable to that of anidulafungin (MIC50/90 = 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 (MEC90 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.1,7–12

Although the currently available echinocandins are highly efficacious and relatively easy to use in the treatment of invasive fungal infections,5,1,6 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,\(^\text{13}\) a long half-life\(^\text{14,15}\) and \textit{in vivo} efficacy against \textit{Candida} and \textit{Aspergillus} spp.\(^\text{16–18}\) The pharmacokinetic profile of CD101 enables once-weekly iv administration and front-loaded antimicrobial plasma exposure.\(^\text{17}\) 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. \textit{In vitro} susceptibilities of 173 \textit{Candida} and \textit{Aspergillus} spp. to CD101, anidulafungin and caspofungin as determined by the CLSI BMD method\(^a\)

| Organism (no. tested)/antifungal agent | \(<0.008\) | 0.015 | 0.03 | 0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | \(\geq 8\) |
|--------------------------------------|---------|-------|-------|-------|-------|-------|-----|---|---|---|-------|
| \textit{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) | | | | |
| \textit{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) | 1 (1) | 1 (1) | 1 (1) |
| caspofungin                           | 1       | 11 (1) | 7 (3) | 3 (3) | 1 (1) | 1 (1) | 1 (1) | 1 (1) | | | |
| \textit{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) | 1 (1) | 2 (2) | | | | |
| \textit{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) | 1 (1) | | | | | | |
| \textit{C. parapsilosis} (15)        |         |       |       |       |       |       |     |   |   |   |       |
| CD101                                | 1       | 3     | 10    | 1     | | | | | | | |
| anidulafungin                         | 2       | 5     | 8     | | | | | | | | |
| caspofungin                           | 1       | 3     | 10    | 1     | | | | | | | |
| \textit{A. fumigatus} (20)           |         |       |       |       |       |       |     |   |   |   |       |
| CD101                                | 15      | 4     | 1     | | | | | | | | |
| anidulafungin                         | 15      | 4     | 1     | | | | | | | | |
| caspofungin                           | 1       | 11    | 7     | 1     | | | | | | | |
| \textit{A. terreus} (19)             |         |       |       |       |       |       |     |   |   |   |       |
| CD101                                | 8       | 10    | 1     | | | | | | | | |
| anidulafungin                         | 11      | 7     | 1     | | | | | | | | |
| caspofungin                           | 11      | 8     | | | | | | | | | |
| \textit{A. flavus} (12)              |         |       |       |       |       |       |     |   |   |   |       |
| CD101                                | 11      | 1     | | | | | | | | | |
| anidulafungin                         | 11      | 1     | | | | | | | | | |
| caspofungin                           | 3       | 8     | 1     | | | | | | | | |
| \textit{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 \((\geq 50\%)\) inhibition (MIC) or the lowest concentration that results in growth of \textit{Aspergillus} spp. producing conspicuously aberrant growth: small, round, compact microcolonies (MEC).

\(^{b}\)No. of mutants = number of \textit{fks} mutant strains of each \textit{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,\(^{2,23}\) 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; \(\pm 2 \log_2\) 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 \(\text{fks}\) (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.\(^{2,27}\)

#### Antifungal susceptibility testing

Candida spp. \(\text{(n = 106)}\) and Aspergillus spp. \(\text{(n = 67)}\) isolates were tested for susceptibility to CD101, anidulafungin and caspofungin, determined by the CLSI and EUCAST BMD methods.\(^{26-28,29}\) 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\(^{25}\) and M27-S4\(^{25}\) by using round-bottomed 96-well plates containing RPMI 1640 medium with 0.2% glucose, inocula of 0.5–2.5 \(\times 10^6\) 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 \((\geq 50\%)\) 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 \(\pm 2 \log_2\) 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. fumigatus 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. fumigatus 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 1. In vitro susceptibilities of Candida and Aspergillus spp. to CD101 as determined by 24 h CLSI and EUCAST BMD methods using prominent \((\geq 50\%)\) 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      | \(\leq0.008\)           | \(\leq0.008\) |
| C. glabrata (25)     | CLSI        | 0.015–2                 | 0.06          | 100.0         |
|                      | EUCAST      | \(<0.008\)–2            | \(\leq0.008\) |
| 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         | \(\leq0.008\) | 95.0          |
|                      | EUCAST      | \(<0.008\)–0.03         | \(\leq0.008\) |
| A. terreus (19)      | CLSI        | \(<0.008\)–0.03         | \(\leq0.008\) | 100.0         |
|                      | EUCAST      | \(<0.008\)–0.03         | \(\leq0.008\) |               |
| A. flavus (12)       | CLSI        | \(<0.008\)–0.015        | \(\leq0.008\) | 100.0         |
|                      | EUCAST      | \(<0.008\)–0.03         | \(\leq0.008\) |               |
| A. niger (16)        | CLSI        | \(<0.008\)–0.03         | \(\leq0.008\) | 100.0         |
|                      | EUCAST      | \(<0.008\)–0.03         | \(\leq0.008\) |               |

\(\text{Note:} \) \(\text{MIC} = \text{minimum} \text{inhibitory} \text{concentration}\), \(\text{MEC} = \text{minimum} \text{effective} \text{concentration}\), \(\text{CLSI} = \text{Clinical} \text{and} \text{Laboratory} \text{Standards} \text{Institute}\), \(\text{EUCAST} = \text{European} \text{Committee} \text{on} \text{Antifungal} \text{Treatment} \text{and} \text{Quality} \text{Control}\).
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 \( \geq 0.12 \) mg/L for \( C. albicans \) and \( C. glabrata \) and \( \geq 0.25 \) 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 \( \leq 0.06 \) mg/L for all isolates tested (Table1). 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. Overall EA between the methods was 92.0%–100.0% for \( C. albicans \) and \( C. glabrata \) and 95.0%–100.0% 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).

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

| Organism      | FKS1 | FKS2 | CLSI MIC (mg/L) | EUCAST MIC (mg/L) |
|---------------|------|------|-----------------|--------------------|
|               |      |      | 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    | \( \geq 8 \) | 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  | \( \leq 0.008 \) | 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   | \( \geq 8 \) |
| WT            | WT   | P667T| 0.25  | 0.25 | 0.5 | 0.25  | 0.12| 1   |
| \( C. krusei \) |      |      |       |     |     |       |     |     |
| WT            | WT   | R1361G| 1     | 2    | 8   | 0.5   | 1   | \( \geq 8 \) |
| 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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