Pharmacological Basis of CD101 Efficacy: Exposure Shape Matters

Elizabeth A. Lakota\textsuperscript{1}, Justin C. Bader\textsuperscript{1}, Voon Ong\textsuperscript{2}, Ken Bartizal\textsuperscript{2}, Lynn Miesel\textsuperscript{3}, David R. Andes\textsuperscript{4}, Sujata M. Bhavnani\textsuperscript{1}, Christopher M. Rubino\textsuperscript{1}, Paul G. Ambrose\textsuperscript{1}, Alexander J. Lepak\textsuperscript{4}

\textsuperscript{1} Institute for Clinical Pharmacodynamics (ICPD), Schenectady, NY
\textsuperscript{2} Cidara Therapeutics, San Diego, CA
\textsuperscript{3} Eurofins Panlabs, Ltd., St. Charles, MO
\textsuperscript{4} University of Wisconsin, Madison, WI

Corresponding Author:
Elizabeth A. Lakota, Pharm.D., M.S.
242 Broadway
Schenectady, NY 12305
Email: elakota@icpd.com
Telephone: 518-631-8125
Fax: 518-631-8199

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ABSTRACT

CD101 is a novel echinocandin with concentration-dependent fungicidal activity in vitro and a long half-life (~133 hours in humans, ~70-80 hours in mice). Given these characteristics, it is likely that the shape of the CD101 exposure (i.e. the time course of CD101 concentrations) influences efficacy. To test this hypothesis, the same total area under the concentration-time curve (AUC) was administered to groups of neutropenic ICR mice infected with *Candida albicans* R303 using three different schedules. A total CD101 dose of 2 mg/kg was administered as a single IV dose or in equal divided doses of either 1 mg/kg twice weekly or 0.29 mg/kg/day over seven days. The studies were performed using a murine disseminated candidiasis model. Animals were euthanized at 168 hours following the start of treatment. Fungi grew well in the no-treatment control group with variable activity in treatment groups. When the CD101 AUC\(_{0-168}\) was administered as a single dose, a >2-log\(_{10}\) CFU reduction from baseline at 168 hours was observed. When twice weekly and daily regimens with similar AUC values were administered, net fungal stasis and a >1-log\(_{10}\) CFU increase from baseline were observed, respectively. These data support the hypothesis that the shape of the CD101 AUC influences efficacy. Thus, CD101 demonstrated a greater degree of fungal killing when administered once per week relative to when the same dose was divided into twice weekly or daily regimens.
INTRODUCTION

CD101 is a novel echinocandin antifungal agent with activity against *Aspergillus* and *Candida* species, including azole- and echinocandin-resistant isolates [1,2]. This compound is a structural analog of anidulafungin but differs in certain beneficial ways with regard to toxicologic and pharmacokinetic properties. Regarding the former, no changes were observed in the hepatocytes of Sprague-Dawley rats exposed to supratherapeutic doses of CD101 in a two-week repeated-dose study [3]. In contrast, rats in this study administered comparable doses of anidulafungin displayed hepatocellular necrosis ranging from mild to moderate. Compared to anidulafungin and other echinocandins, CD101 has a considerably longer half-life [4-8]. This finding was observed pre-clinically across multiple animal species [4] and in healthy volunteers [5].

Results from the latter study demonstrated that the terminal half-life of CD101 is approximately 133 hours [5], a value far greater than those reported for anidulafungin, caspofungin, and micafungin (9 to 52 hours) [6-8].

CD101 demonstrates a concentration-dependent pattern of fungal killing [9], as previously observed with anidulafungin, caspofungin, and micafungin [9]. This property, in conjunction with CD101’s long half-life in humans, led to the hypothesis that a front-loaded CD101 dosing regimen would provide superior fungal killing over multiple-dose regimens. In order to test this hypothesis, front-loaded dose studies were conducted using a neutropenic murine disseminated candidiasis model. Our objective was to evaluate a single-dose regimen fractionated to yield multiple dosing regimens with equivalent areas under the CD101 concentration-time curve (AUC) and to assess their...
corresponding changes in log$_{10}$ colony forming units (CFU) at 168 hours to determine if exposure shape impacts antifungal activity.

**METHODS**

**Study Drug, Challenge isolate and In Vitro Susceptibility Testing**

CD101 was supplied by Cidara Therapeutics, Inc. (San Diego, CA). Stock solutions of CD101 were prepared in dimethyl sulfoxide for the susceptibility testing. The vehicle used for dosing in the in vivo studies was 10% DMSO/ 1% Tween 20 in saline. The challenge isolate utilized in these studies was *Candida albicans* R303. Susceptibility testing was done in accordance with the Clinical and Laboratory Standards Institute (CLSI) M27-A3 methods, using RPMI 1640 medium, an initial inoculum of 0.5 to 2.5 x 10$^3$ CFU/mL, and incubation at 35 °C [10]. MIC plates were read following a 24 hour incubation and MIC values are reported as concentrations resulting in prominent growth inhibition (~50%). Susceptibility testing was performed on three separate occasions. Final results were expressed as modal values. Quality control was performed on each day of testing using CLSI-recommended reference strains (*C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019).

**Pharmacokinetic Study**

Healthy female ICR mice weighing 20-24 g were administered a single dose of CD101 via intraperitoneal (IP) injection. Doses of 1, 4, and 16 mg/kg were studied, utilizing 3 animals per dose level. Whole blood samples (one sample per animal) were collected at 1, 3, 6, 12, 24, 48, 72, and 96 hours post-dose. Plasma was collected by centrifugation and samples were stored at -20 °C or below. Samples were analyzed...
using liquid chromatography with tandem mass spectrometry detection using an AB-SCIEX API 4000 mass spectrometry system. The method used in the analysis was qualified as fit-for-purpose: calibration standards were matrix-matched and, for each analytical batch, triplicate calibration standards were included at the beginning, approximately in the middle, and at the end of the batch. Following analysis, quantitation is carried out by a calibration curve comprising analyte/internal standard area ratio versus concentration. Calibration standards ranged from 0.5 (lower limit of quantitation, LLOQ) to 100 mg/L. Analytical batch acceptance followed the general guidance that standards/quality control be within ±20% for accuracy (% of nominal concentration) and ≤20% for precision (% coefficient of variation). For analysis, plasma samples were quenched with acetonitrile (4:1, acetonitrile:plasma ratio) containing diclofenac as internal standard. Animals were maintained in accordance with the American Association for Assessment and Accreditation of Laboratory Animal Care criteria (AAALAC). The pharmacokinetic study was approved by the Animal Research Committee of the William S. Middleton Memorial Veterans Affairs Hospital and the University of Wisconsin.

Protein Binding

The extent of protein binding of CD101 to murine K:EDTA plasma proteins was determined using ultracentrifugation (500,000 x g for 2.5 hours at 37°C). CD101 was tested at concentrations of 7, 10, 20, 30 and 60 mg/L. An experimental control was included in order to assess compound recovery (matrix stability) under the assay conditions. The level of protein binding was calculated using the following equation: %
protein binding: 100% - % Unbound, where % Unbound = Mean concentration of supernatant / Mean concentration of total x 100.

**Front-Loaded Dose Studies**

A neutropenic, murine, disseminated candidiasis model was used for the evaluation of CD101 dosing regimens on efficacy. Male or female ICR mice (n=5 per regimen and observation time) weighing 22 ± 2 g were rendered neutropenic by the injection of cyclophosphamide treatment four days (-Day 4) and one day (-Day 1) prior to infection with 150 and 100 mg/kg IP, respectively. Neutropenia was sustained for the duration of the study with cyclophosphamide doses (100 mg/kg IP) every 48 hours on Days 1, 3, 5 and 7 after infection. Each animal was inoculated, using a lateral tail vein injection, with 1 x 10^3 CFU of *Candida albicans* (Isolate R303). CD101 (or vehicle) was administered 24 hours post-infection via IP injection. The dosing regimens studied are shown in Table 1. Mice were euthanized with CO₂ 168 hours (7 days) following the start of treatment. Mice in the control group were euthanized 0, 24, and 48 hours post-administration of vehicle. Paired kidneys were aseptically harvested, homogenized, and serial dilutions were plated for colony counts to determine the fungal burden (CFU/g).

Kidney counts were not measured in animals that expired prior to the scheduled sacrifice time. The front-loaded dose studies was performed under Animal Biosafety Level 2 conditions in an Association for Assessment and Accreditation of Laboratory Animal Care accredited vivarium, at Eurofins Panlabs Taiwan, Ltd, with the oversight of veterinarians to assure compliance with the Eurofins Panlabs Institutional Animal Care and Use Committee regulations and the humane treatment of laboratory animals.

**Data Analysis**
Data collected from the above-described pharmacokinetic study were used to develop a pharmacokinetic model describing the disposition of CD101 in mice in S-ADAPT [11]. The developed pharmacokinetic model, along with the CD101 fraction unbound, was used to simulate free-drug concentration-time profiles for each dosing regimen administered in the front-loaded dose studies. Free-drug AUC<sub>0-168</sub> values were calculated through numeric integration of CD101 free-drug concentration-time profiles. Relationships between change in log<sub>10</sub> CFU at 168 hours and free-drug AUC<sub>0-168</sub>:MIC ratio were explored.

**RESULTS**

**In Vitro Susceptibility Testing**

The modal CD101 MIC value against C. albicans R303, determined using broth microdilution, was 0.125 mg/L.

**Pharmacokinetic Study**

Following IP administration of CD101, maximum plasma concentrations were observed at the first sampling occasion (1 hour) with values of 3.97, 13.6, and 52.0 mg/L for the doses of 1, 4, and 16 mg/kg, respectively. CD101 exhibited linear pharmacokinetics over the dose range studied (1 to 16 mg/kg IP). A four-compartment model best described the disposition of this agent in murine plasma. Observed and model predicted pharmacokinetic profiles are displayed in Figure 1. The coefficient of determination (r<sup>2</sup>) for the model predicted versus observed concentration was 0.97.

Final model parameter estimates are displayed in Table 2. All model parameters were estimated with excellent precision, as indicated by a %SEM of less than 20% for 7 out of
8 of the model parameters. Results of the protein binding evaluation demonstrated that the magnitude of CD101 protein binding in mouse serum was 99.2% across concentrations ranging from 7 to 60 mg/L.

**Front-Loaded Dose Studies**

The results of the front-loaded dose studies are presented in Figure 2. Fungi in the no-treatment control group grew well and reached a bacterial density of greater than $1 \times 10^6$ CFU/g by 48 hours. The magnitude of net change in $\log_{10}$ CFU from baseline at 168 hours was similar regardless of fractionation schedule within the CD101 0.7 and 7 mg/kg dosing groups. In the 0.7 mg/kg dose group, the magnitude of net change was similar to the no-treatment control group, regardless of fractionation schedule. In the 7 mg/kg dose group, the magnitude of net change was greater than $2 - \log_{10}$ CFU reduction from baseline at 168 hours, regardless of fractionation schedule. Conversely, results within the CD101 2 mg/kg group varied across the fractionation schedules. These data are displayed in Figure 3. When a total dose of 2 mg/kg was delivered daily (0.29 mg/kg/day), the magnitude of net change in $\log_{10}$ CFU from baseline at 168 hours was similar to the no-treatment control group. However, when this dose was front-loaded (i.e., delivered entirely on Day 1), there was a greater than $2 - \log_{10}$ CFU reduction from baseline at 168 hours. While these regimens, 0.29 mg/kg daily x 7 and 2 mg/kg x 1, had comparable cumulative CD101 exposures at 168 hours (Figure 2), the treatment effects differed greatly.

Simulated free-drug plasma concentration-time profiles of the three fractionated CD101 2 mg/kg dosing regimens are shown in Figure 4. The regimens displayed dissimilar exposure profiles. In particular, the single-dose regimen resulted in substantially larger
CD101 exposures early in therapy. The free-drug plasma AUC during the first 24 hours of therapy (AUC0-24) was 0.520, 0.260, and 0.0754 mg∙h/L following administration of CD101 2 mg/kg as a front-loaded, twice-weekly, and daily regimen, respectively. Further, as shown in Figure 4, administration of a front-loaded regimen resulted in free-drug plasma concentrations that remained above those for the twice-weekly and daily regimens for 84 and 48 hours, respectively. No overt signs of toxicities were observed in any of the mice evaluated.

DISCUSSION

The goal of these studies was to examine the impact of the shape of drug exposure, as measured by AUC, on the fungicidal activity of CD101. Using a front-loading experiment design, in which a similar AUC was achieved through single, twice weekly, or daily dosing, we successfully demonstrated that the shape of CD101 exposure greatly influenced fungicidal activity.

More specifically, a 2-log reduction in log10 CFU from baseline was achieved for all five mice administered CD101 2 mg/kg (free-drug AUC0-168 = 1.84 mg∙h/L) as a single dose. However, when a twice weekly regimen with a comparable AUC was administered, net stasis was observed. Further, when a comparable AUC was administered daily over seven days, a 1-log increase in log10 CFU from baseline was observed (similar to the no treatment control group). Given that echinocandins exhibit a concentration-dependent pattern of in vitro fungal killing [9] and that the front-loaded and daily CD101 regimens resulted in similar CD101 free-drug AUC0-168 values, one would expect to see similar magnitudes of fungal killing regardless of fractionation schedule. However,
the results of the front-loaded dose studies suggest that the shape of the CD101 AUC is a determinant of efficacy, with front-loaded regimens demonstrating greater benefit. When CD101 was administered as a single dose, free-drug concentrations remained higher than those for the fractionated regimens over the first several days of therapy. CD101 free-drug plasma trough concentrations were comparable on Day 5 between the front-loaded and daily dosing regimens. Additionally, the single-dose regimen yielded a free-drug CD101 AUC$_{0-24}$ seven-fold greater than that of the daily CD101 regimen. These pharmacokinetic characteristics provide support for front-loaded single-dose regimen, which is highly beneficial given the importance of achieving efficacious drug exposures early in therapy.

As demonstrated in Figure 2, a daily 1 mg/kg dosing regimen was required to achieve a comparable reduction in log$_{10}$ CFU from baseline over 168 hours as that obtained after administration of a front-loaded 2 mg/kg regimen. Administration of the daily 1 mg/kg regimen resulted in an approximate three-fold greater CD101 AUC$_{0-168}$ relative to the 2 mg/kg front-loaded regimen. Thus, front-loaded CD101 doses provided comparable efficacy with reduced total drug exposures, a characteristic which may reduce the risk of AUC-driven drug-related toxicities.

Similar findings were seen during the development of oritavancin, a lipoglycopeptide antibacterial indicated for the treatment of patients with acute bacterial skin and skin structure infections due to Gram-negative organisms. Oritavancin is another agent which exhibits a remarkably long half-life (terminal half-life ~245 hours) [12]. This agent is unique in that a full course of therapy consists of a single 1200 mg intravenous dose. Two characteristics of oritavancin’s pharmacokinetics and pharmacodynamics enable
this front-loaded dosing regimen to provide prolonged efficacious drug exposures and
produce superior outcomes to multiple-dose regimens [13,14]. The first of these, a
pharmacodynamic characteristic, is that oritavancin displays a concentration-
dependent pattern of \textit{in vitro} bacterial killing [15]. Given this characteristic, as drug
concentration increases, so too do the rate and extent of bacterial killing. The second
characteristic is oritavancin’s distinct pharmacokinetic profile, which provides consistent
exposure over a prolonged period. This is a product of the protracted terminal half-life
of oritavancin. As stated previously, CD101 exhibits a concentration-dependent
pattern of \textit{in vitro} fungal killing and possesses a long half-life.

Several authors have demonstrated similar findings as those described herein for other
echinocandins with shorter plasma half-lives with respect to similar or greater reductions
in CFU associated with the administration of front-loaded compared to more
fractionated dosing regimens in mice [17, 18, 19, 20]. These investigators postulated that
their results were due to therapeutic drug concentrations being maintained within
peripheral tissues over a prolonged period. However, in order to translate these findings
to humans, two assumptions must be made. First, the magnitude of echinocandin
distribution in peripheral tissue is similar or greater in humans relative to mice. Second,
the time-course of echinocandin disposition into and out of peripheral tissues is similar in
humans and mice. Using the example of ceftobiprole and oritavancin for the former
and later assumptions, respectively, these characteristics cannot always be assumed to
be concordant across species [21]. Additional studies characterizing the disposition of
CD101 in tissue in both mice and humans may be useful to further interpret these
data. In summary, the studies described herein demonstrated that front-loaded CD101
dosing regimens exhibited greater fungicidal activity than more fractionated regimens.
This apparent relationship between exposure shape and fungal killing greatly differentiates CD101 relative to approved antifungal therapies and poses several beneficial clinical implications. Front-loaded dosing provides the opportunity to deliver drug exposures in a pharmacokinetic-pharmacodynamic optimized manner, improve patient compliance, and reduce resources required for therapeutic drug monitoring.

These data for CD101 provide dose selection support for future clinical studies.
REFERENCES


Table 1. Summary of CD101 dosing regimens evaluated in front-loaded dose studies

<table>
<thead>
<tr>
<th>Total Dose</th>
<th>Dosing Interval</th>
<th>Fractionated Doses</th>
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<tbody>
<tr>
<td>0.7 mg/kg IP</td>
<td>Single Dose</td>
<td>0.7 mg/kg x 1</td>
</tr>
<tr>
<td>0.7 mg/kg IP</td>
<td>Twice Weekly&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.35 mg/kg x 2</td>
</tr>
<tr>
<td>0.7 mg/kg IP</td>
<td>Daily</td>
<td>0.1 mg/kg x 7</td>
</tr>
<tr>
<td>2 mg/kg IP</td>
<td>Single Dose</td>
<td>2 mg/kg x 1</td>
</tr>
<tr>
<td>2 mg/kg IP</td>
<td>Twice Weekly&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1 mg/kg x 2</td>
</tr>
<tr>
<td>2 mg/kg IP</td>
<td>Daily</td>
<td>0.29 mg/kg x 7</td>
</tr>
<tr>
<td>7 mg/kg IP</td>
<td>Single Dose</td>
<td>7 mg/kg x 1</td>
</tr>
<tr>
<td>7 mg/kg IP</td>
<td>Twice Weekly&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.5 mg/kg x 2</td>
</tr>
<tr>
<td>7 mg/kg IP</td>
<td>Daily</td>
<td>1 mg/kg x 7</td>
</tr>
</tbody>
</table>

<sup>a</sup> Second dose administered 84 hours (3.5 days) after first dose
### Table 2. Final CD101 Pharmacokinetic Model Parameter Estimates

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Final Estimate</th>
<th>%SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLt (L/h/kg)</td>
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<td>3.55</td>
</tr>
<tr>
<td>V1 (L/kg)</td>
<td>0.201</td>
<td>0.585</td>
</tr>
<tr>
<td>Q2 (L/h/kg)</td>
<td>0.0153</td>
<td>1.24</td>
</tr>
<tr>
<td>V2 (L/kg)</td>
<td>0.871</td>
<td>1.62</td>
</tr>
<tr>
<td>Q3 (L/h/kg)</td>
<td>0.312</td>
<td>19.7</td>
</tr>
<tr>
<td>V3 (L/kg)</td>
<td>0.0341</td>
<td>19.4</td>
</tr>
<tr>
<td>Q4 (L/h/kg)</td>
<td>0.0723</td>
<td>43.8</td>
</tr>
<tr>
<td>V4 (L/kg)</td>
<td>0.165</td>
<td>18.5</td>
</tr>
</tbody>
</table>
Figure 1. Observed (points) and model predicted (lines) CD101 total-drug concentration versus time following IP administration of CD101.
Figure 2. Mean (solid circles) and range (error bars) change in log_{10} CFU from baseline at 168 hours versus CD101 free-drug AUC_{0-168}/MIC ratio by fractionation schedule.
Figure 3. Mean (bar) and range (error bars) change in log_{10} CFU from baseline at 168 hours after administration of CD101 2 mg/kg by fractionation schedule.
Figure 4. Simulated free-drug concentration time profiles for the fractionated CD101 2 mg/kg regimen.