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EXPERIMENTAL THERAPEUTICS

Determination of Pharmacodynamic Target Exposures for Rezafungin against *Candida tropicalis* and *Candida dubliniensis* in the Neutropenic Mouse Disseminated Candidiasis Model

Alexander J. Lepak,[a] Miao Zhao,[a,b] David R. Andes[a,b]

[a]Department of Medicine, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, USA
[b]Department of Medical Microbiology and Immunology, University of Wisconsin, Madison Wisconsin, USA

ABSTRACT Rezafungin (CD101) is a novel echinocandin under development for once-weekly intravenous (i.v.) dosing. We evaluated the pharmacodynamics (PD) of rezafungin against 4 *Candida tropicalis* and 4 *Candida dubliniensis* strains, using the neutropenic mouse invasive candidiasis model. The area under the concentration-time curve (AUC)/MIC was a robust predictor of efficacy (R^2 = 0.93 and 0.72, respectively). The stasis free-drug 24-h AUC/MIC target exposure for the group ranged from 3 to 25, whereas the 1-log-kill free-drug 24-h AUC/MIC target exposure ranged from 4.3 to 62. These values are similar to those found in previous rezafungin PD studies with other *Candida* spp. Based on recent surveillance susceptibility data, AUC/MIC targets are likely to be exceeded for >99% of *C. tropicalis* and *C. dubliniensis* isolates with the previously studied human dose of 400 mg i.v. once weekly.

KEYWORDS *Candida tropicalis*, *Candida dubliniensis*, pharmacodynamics, rezafungin, echinocandin

Rezafungin is a novel echinocandin with broad activity against fungal pathogens, including all *Candida* and *Aspergillus* species (1–5). Similar to other echinocandin drugs, rezafungin targets 1,3-β-D-glucan synthesis. Unlike other echinocandins, which are administered once daily, rezafungin has the pharmacokinetic (PK) advantage of a prolonged terminal half-life of ~133 h in humans, which allows for extended-interval dosing (6). Once-weekly dosing regimens have been studied in phase 1 and 2 trials demonstrating dose-proportional PK with relatively low interindividual variability and very favorable safety profiles (6).

Invasive candidiasis is the most common invasive fungal infection of hospitalized patients and the fourth most common cause of nosocomial bloodstream infections leading to high morbidity and mortality (7–10). Historically, *Candida albicans* has been the predominant causative species; however, in recent years, epidemiological shifts in prevalence have occurred (11–17). Indeed, in many parts of the world, non-*albicans Candida* species outnumber *C. albicans* strains. For example, a recent large epidemiological study of *Candida* bloodstream isolates from the Asia-Pacific region demonstrated that *C. tropicalis* isolates were almost equal in prevalence to *C. albicans* isolates, and, in sum, non-*albicans Candida* species accounted for almost 65% of all bloodstream isolates over the 2-year study (18). As previous studies have demonstrated species-specific pharmacodynamic (PD) targets for the echinocandin group against different *Candida* species, we sought to examine the PD efficacy of rezafungin against *C. tropicalis* and *C. dubliniensis* in a neutropenic mouse infection model. This included determining the target area under the concentration-time curve (AUC)/MIC exposures to assist in an optimal dosing-regimen design for infections with these species, esti-

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Address correspondence to David R. Andes, dra@medicine.wisc.edu.

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TABLE 1 Susceptibility testing results, fitness, and PD target exposures for net stasis and 1-log kill for rezafungin in the neutropenic mouse disseminated candidiasis model

Species	Strain	MIC (mg/liter)	Growth in untreated animals (CFU/kidneys)	Net stasis			1-Log kill		
				Dose (mg/kg every 72 h)	Free-drug AUC_{0-168}/MIC	Free-drug avg AUC_{0-24}/MIC	Dose (mg/kg every 72 h)	Free-drug AUC_{0-168}/MIC	Free-drug avg AUC_{0-24}/MIC
C. tropicalis	98-234	0.03	4.46	2.28	108.6	15.51	4.06	180.7	25.82
	ATCC 750	0.016	4.74	1.02	107.6	15.37	2.42	214.5	30.64
	CAY2597	0.06	3.30	1.81	44.7	6.39	3.23	73.5	10.50
	1751	0.03	4.46	1.22	65.4	9.35	2.49	117.0	16.71
C. dubliniensis	90	0.06	4.29	1.65	41.6	5.94	2.18	52.2	7.46
	991460	0.06	4.52	1.02	28.6	4.09	23.51	431.5	61.64
	1031899	0.03	3.77	3.92	175.3	25.05	10.34	404.4	57.77
	1032127	0.06	3.86	0.66	21.5	3.07	1.09	30.1	4.30

mation of the probability of clinical target attainment, and proposal of preliminary susceptibility breakpoints.

Four strains each of *C. tropicalis* and *C. dubliniensis* were selected for *in vivo* studies. Unless noted by ATCC number, each of the strains was a clinical isolate and was chosen based on similar growth in untreated animals (Table 1). Antimicrobial susceptibility testing was performed according to CLSI broth microdilution guidelines (19). The MIC values for rezafungin ranged from 0.016 to 0.06 mg/liter against the strain panels selected for both species (Table 1). The neutropenic mouse disseminated candidiasis model was used for all experiments. Neutropenia was induced by subcutaneous cyclophosphamide injection on days −4 (150 mg/kg), −1, +3, and +6 (100 g/kg) to ensure neutropenia throughout the 7-day experiment. Three mice were included in each treatment and control group. Mice were inoculated with one of the eight strains at 5.98 ± 0.06 $\log_{10}$ CFU/ml via the lateral tail vein. Antifungal treatment with rezafungin began 2 h after inoculation. Groups of animals were subjected to five rezafungin dosing regimens that varied from 0.25 to 64 mg/kg. Drug was administered intraperitoneally. We recently characterized the PK in this mouse model across the same dose range (20), and those data were utilized to calculate PK exposures for the present study. Given the prolonged half-life in mice (28 to 41 h), rezafungin doses were administered on days 0, 3, and 6. After 7 days, mice were euthanized for determination of fungal burden using quantitative CFU cultures from kidney homogenates. Organism burden in each treatment group was compared with fungal burden at the start of therapy and

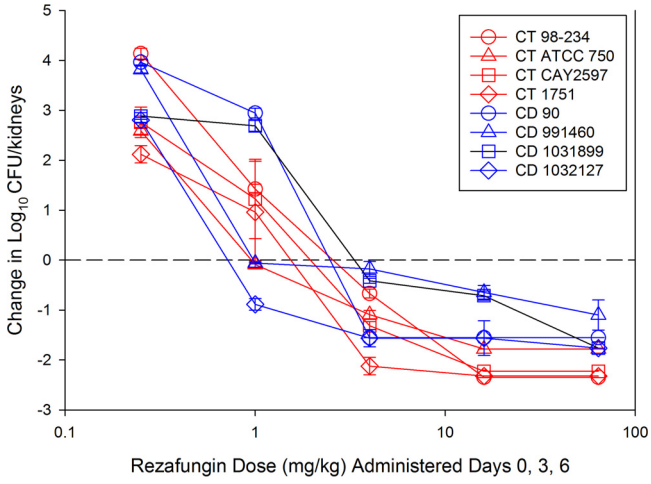

FIG 1 *In vivo* rezafungin dose-response curves for 4 *C. tropicalis* (CT) and 4 *C. dubliniensis* (CD) strains. Each symbol represents the mean and standard deviation (error bars) of the change in fungal burden in kidneys from 3 mice. Dashed horizontal line, burden at the start of therapy (0 h). Points above line, net increase in fungal burden. Points below line, net decrease in fungal burden over the 7-day experiment.

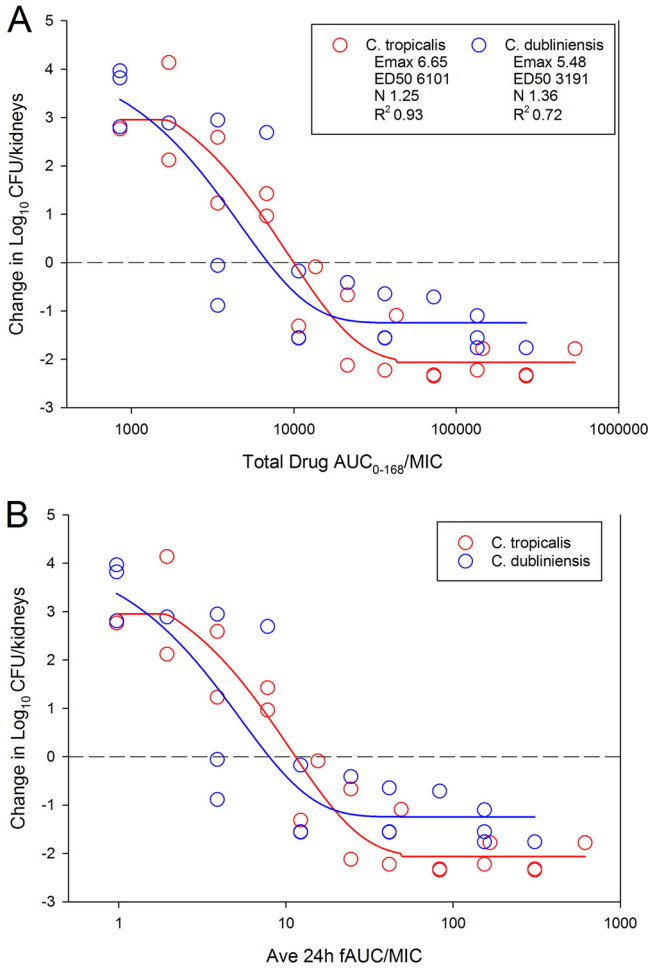

FIG 2 Relationship between rezafungin treatment effect and the PK/PD AUC/MIC index for *C. tropicalis* and *C. dubliniensis* isolates. Each symbol represents the mean change in fungal burden in kidneys from 3 mice. Dashed horizontal line, burden at the start of therapy (0 h). Points above line, net increase in fungal burden. Points below line, net decrease in fungal burden over the 7-day experiment. Rezafungin PK/PD exposure-response relationship using total-drug AUC over the full treatment duration (total-drug AUC_{0-168}/MIC) (A) and using the average free-drug 24-h AUC over the 7-day experiment (average free-drug AUC_{0-24}/MIC) (B).

analyzed using the sigmoid maximum effect (E_{max}) model (Hill equation). Free-drug concentration was determined using a protein binding value of 99.2% in mice (20). The PK/PD parameter AUC/MIC was used for analysis because it is the demonstrated parameter linked to efficacy for echinocandins and rezafungin in previous PK/PD studies (20–22).

The results of the dose-ranging studies against *C. tropicalis* and *C. dubliniensis* are shown in Fig. 1. Dose-dependent activity was noted against all strains, with a $>$1-$\log_{10}$ kill, compared to fungal burden at the start of therapy, achieved for all strains. Compared with vehicle-treated controls, rezafungin-treated animals achieved an almost 6-$\log_{10}$ reduction in fungal burden. When the treatment data were modeled according to the AUC/MIC index, there was a robust relationship, with R^2 values of 0.93 for *C. tropicalis* and 0.72 for *C. dubliniensis* (Fig. 2) strains. The doses necessary to achieve net stasis and a 1-log reduction compared with the start of therapy were calculated for each organism and are shown in Table 1. The respective 7-day (0 to 168 h) AUC/MIC target exposures for each endpoint (stasis and 1-log kill) were calculated, as was the average 24-h AUC/MIC. The latter was included to allow for comparison with other echinocandins in this model, which have traditionally been reported using 24-h AUC/

MIC exposures. Free-drug 24-h average AUC/MIC targets ranged from 3 to 25 and 1-log kill from 4.3 to 62 for all organisms studied (Table 1).

Previous neutropenic mouse invasive candidiasis PK/PD studies with rezafungin against *C. albicans*, *Candida glabrata*, *Candida parapsilosis*, and *Candida auris* species similarly demonstrated potent activity (20, 22). The 24-h free-drug average AUC/MIC target demonstrated in the previous studies was 0.5 to 3 for net stasis, which interestingly was numerically lower than that for other echinocandin (e.g., caspofungin, micafungin, and anidulafungin) PK/PD studies in this model. In the current study, we expanded the understanding of PD activity of rezafungin to *C. tropicalis* and *C. dubliniensis* strains, which are important pathogens in many areas worldwide (23). We demonstrated that the *in vivo* exposure-response activity of rezafungin against *C. tropicalis* and *C. dubliniensis* isolates was comparable to that in previous animal model studies with other *Candida* spp. These data also align with previous *in vitro* evaluations of rezafungin activity, which demonstrated relatively equipotent activity against *C. tropicalis* and *C. dubliniensis* compared with other *Candida* spp. (2, 3). The PK of rezafungin in humans has been well characterized (6), including population PK modeling and target attainment analysis for *C. albicans* and *C. glabrata* isolates (24, 25). According to the highest PK/PD target for each species identified in this study (24-h free-drug AUC/MIC, 15.5 for *C. tropicalis* and 25 for *C. dubliniensis*), the target exposure was met or exceeded for *C. tropicalis* organisms with a rezafungin MIC of ≤0.5 mg/liter and for *C. dubliniensis* organisms with a rezafungin MIC of ≤0.25 mg/liter. To put this in context, previous *in vitro* susceptibility studies for rezafungin have generated MIC_{90} values of 0.06 mg/liter for *C. tropicalis* ($n = 274$) and 0.12 mg/liter for *C. dubliniensis* ($n = 102$) isolates (2, 3, 26–28). Thus, the current rezafungin dosing regimen studied for clinical use would be expected to be efficacious for these two organism groups. Further clinical studies of rezafungin that examine patient outcomes in the context of PK exposure and MIC distribution are necessary to confirm these *in vivo* results.

In summary, rezafungin demonstrated potent *in vivo* efficacy against *C. tropicalis* and *C. dubliniensis* isolates in the neutropenic mouse disseminated candidiasis model. To our knowledge, this is the first *in vivo* PK/PD study in this model to use *C. dubliniensis* isolates. The PK/PD targets identified for net stasis and 1-log kill were similar to those in previous studies of rezafungin against other *Candida* spp. Integrating these PK/PD targets in the context of expected human drug exposure based on PK studies and known MIC distributions suggests that rezafungin is likely to be highly efficacious for these two important pathogen groups.

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REFERENCES

1. Wiederhold NP, Locke JB, Daruwala P, Bartizal K. 2018. Rezafungin (CD101) demonstrates potent in vitro activity against Aspergillus, including azole-resistant Aspergillus fumigatus isolates and cryptic species. J Antimicrob Chemother 73:3063–3067. https://doi.org/10.1093/jac/dky280.
2. Pfaller MA, Messer SA, Rhomberg PR, Castanheira M. 2017. Activity of a long-acting echinocandin (CD101) and seven comparator antifungal agents tested against a global collection of contemporary invasive fungal isolates in the SENTRY 2014 Antifungal Surveillance Program. Antimicrob Agents Chemother 61:e02045-16. https://doi.org/10.1128/AAC.02045-16.
3. Pfaller MA, Messer SA, Rhomberg PR, Castanheira M. 2017. CD101, a long-acting echinocandin, and comparator antifungal agents tested against a global collection of invasive fungal isolates in the SENTRY 2015 Antifungal Surveillance Program. Int J Antimicrob Agents 50:352–358. https://doi.org/10.1016/j.ijantimicag.2017.03.028.
4. Hall D, Bonifas R, Stapert L, Thwaites M, Shinabarger DL, Pillar CM. 2017. In vitro potency and fungicidal activity of CD101, a novel echinocandin, against recent clinical isolates of Candida spp. Diagn Microbiol Infect Dis 89:205–211. https://doi.org/10.1016/j.diagmicrobio.2017.07.007.
5. Berkow EL, Lockhart SR. 2018. Activity of CD101, a long-acting echinocandin, against clinical isolates of Candida auris. Diagn Microbiol Infect Dis 90:196–197. https://doi.org/10.1016/j.diagmicrobio.2017.10.021.
6. Sandison T, Ong V, Lee J, Thye D. 2017. Safety and pharmacokinetics of CD101 IV, a novel echinocandin, in healthy adults. Antimicrob Agents Chemother 61:e01627-16. https://doi.org/10.1128/AAC.01627-16.
7. Pappas PG, Rex JH, Lee J, Hamill RJ, Larsen RA, Powderly W, Kauffman CA, Hyslop N, Mangino JE, Chapman S, Horowitz HW, Edwards JE, Dismukes WE, NIAID Mycosis Study Group. 2003. A prospective observational study of candidemia: epidemiology, therapy, and influences on mortality in hospitalized adult and pediatric patients. Clin Infect Dis 37:634–643. https://doi.org/10.1086/376906.
8. Horn DL, Neofytos D, Anaissie EJ, Fishman JA, Steinbach WJ, Olyaei AJ, Marr KA, Pfaller MA, Chang CH, Webster KM. 2009. Epidemiology and outcomes of candidemia in 2019 patients: data from the prospective antifungal therapy alliance registry. Clin Infect Dis 48:1695–1703. https://doi.org/10.1086/599039.
9. Bassetti M, Merelli M, Ansaldi F, de Florentiis D, Sartor A, Scarparo C, Callegari A, Righi E. 2015. Clinical and therapeutic aspects of candidemia: a five year single centre study. PLoS One 10:e0127534. https://doi.org/10.1371/journal.pone.0127534.

10. Bassetti M, Giacobbe DR, Vena A, Trucchi C, Ansaldi F, Antonelli M, Adamkova V, Alicino C, Almyroudi MP, Atchade E, Azzini AM, Carannante N, Carnelutti A, Corcione S, Cortegiani A, Dimopoulos G, Dubler S, Garcia-Garmendia JL, Girardis M, Cornely OA, Ianniruberto S, Kullberg BJ, Lagrou K, Le Bihan C, Luzzati R, Malbrain M, Merelli M, Marques AJ, Martin-Loeches I, Mesini A, Paiva JA, Peghin M, Raineri SM, Rautemaa-Richardson R, Schouten J, Brugnaro P, Spapen H, Tasioudis P, Timsit JF, Tisa V, Tumbarello M, van den Berg C, Veber B, Venditti M, Voiriot G, Wauters J, Montravers P. 2019. Incidence and outcome of invasive candidiasis in intensive care units (ICUs) in Europe: results of the EUCANDICU project. Crit Care 23:219. https://doi.org/10.1186/s13054-019-2497-3.
11. Pfaller MA, Moet GJ, Messer SA, Jones RN, Castanheira M. 2011. Geographic variations in species distribution and echinocandin and azole antifungal resistance rates among *Candida* bloodstream infection isolates: report from the SENTRY Antimicrobial Surveillance Program (2008 to 2009). J Clin Microbiol 49:396–399. https://doi.org/10.1128/JCM.01398-10.
12. Pfaller MA, Moet GJ, Messer SA, Jones RN, Castanheira M. 2011. *Candida* bloodstream infections: comparison of species distributions and antifungal resistance patterns in community-onset and nosocomial isolates in the SENTRY Antimicrobial Surveillance Program, 2008–2009. Antimicrob Agents Chemother 55:561–566. https://doi.org/10.1128/AAC.01079-10.
13. Pfaller MA, Messer SA, Moet GJ, Jones RN, Castanheira M. 2011. Candida bloodstream infections: comparison of species distribution and resistance to echinocandin and azole antifungal agents in intensive care unit (ICU) and non-ICU settings in the SENTRY Antimicrobial Surveillance Program (2008–2009). Int J Antimicrob Agents 38:65–69. https://doi.org/10.1016/j.ijantimicag.2011.02.016.
14. Pfaller MA, Diekema DJ. 2007. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev 20:133–163. https://doi.org/10.1128/CMR.00029-06.
15. Pfaller M, Neofytos D, Diekema D, Azie N, Meier-Kriesche HU, Quan SP, Horn D. 2012. Epidemiology and outcomes of candidemia in 3648 patients: data from the Prospective Antifungal Therapy (PATH Alliance®) registry, 2004–2008. Diagn Microbiol Infect Dis 74:323–331. https://doi.org/10.1016/j.diagmicrobio.2012.10.003.
16. Diekema D, Arbefeville S, Boyken L, Kroeger J, Pfaller M. 2012. The changing epidemiology of healthcare-associated candidemia over three decades. Diagn Microbiol Infect Dis 73:45–48. https://doi.org/10.1016/j.diagmicrobio.2012.02.001.
17. Guinea J. 2014. Global trends in the distribution of Candida species causing candidemia. Clin Microbiol Infect 20(Suppl 6):5–10. https://doi.org/10.1111/1469-0691.12539.
18. Tan TY, Hsu LY, Alejandria MM, Chaiwarith R, Chinniah T, Chayakulkeeree M, Choudhury S, Chen YH, Shin JH, Kiratisin P, Mendoza M, Prabhu K, Supparatpinyo K, Tan AL, Phan XT, Tran TT, Nguyen GB, Doan MP, Huynh VA, Nguyen SM, Tran TB, Van Pham H. 2016. Antifungal susceptibility of invasive Candida bloodstream isolates from the Asia-Pacific region. Med Mycol 54:471–477. https://doi.org/10.1093/mmy/myv114.
19. Clinical and Laboratory Standards Institute. 2008. Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard—3rd ed. CLSI document M27-A3. Clinical and Laboratory Standards Institute, Wayne, PA.
20. Lepak AJ, Zhao M, VanScoy B, Ambrose PG, Andes DR. 2018. Pharmacodynamics of a long-acting echinocandin, CD101, in a neutropenic invasive-candidiasis murine model using an extended-interval dosing design. Antimicrob Agents Chemother 62:e02154-17. https://doi.org/10.1128/AAC.02154-17.
21. Lepak AJ, Andes DR. 2014. Antifungal pharmacokinetics and pharmacodynamics. Cold Spring Harb Perspect Med 5:a019653. https://doi.org/10.1101/cshperspect.a019653.
22. Lepak AJ, Zhao M, Andes DR. 2018. Pharmacodynamic evaluation of rezafungin (CD101) against *Candida auris* in the neutropenic mouse invasive candidiasis model. Antimicrob Agents Chemother 62:e01572-18. https://doi.org/10.1128/AAC.01572-18.
23. Wang H, Xu YC, Hsueh PR. 2016. Epidemiology of candidemia and antifungal susceptibility in invasive Candida species in the Asia-Pacific region. Future Microbiol 11:1461–1477. https://doi.org/10.2217/fmb-2016-0099.
24. Lakota EA, Ong V, Flanagan S, Rubino CM. 2018. Population pharmacokinetic analyses for rezafungin (CD101) efficacy using phase 1 data. Antimicrob Agents Chemother 62:e02603-17. https://doi.org/10.1128/AAC.02603-17.
25. Bader JC, Lakota EA, Flanagan S, Ong V, Sandison T, Rubino CM, Bhavnani SM, Ambrose PG. 2018. Overcoming the resistance hurdle: pharmacokinetic-pharmacodynamic target attainment analyses for rezafungin (CD101) against *Candida albicans* and *Candida glabrata*. Antimicrob Agents Chemother 62:e02614-17. https://doi.org/10.1128/AAC.02614-17.
26. Castanheira M, Messer SA, Rhomberg PR, Dietrich RR, Pfaller MA. 2018. Activity of a long-acting echinocandin rezafungin (CD101) and comparator antifungal agents tested against contemporary invasive fungal isolates: SENTRY 2016, abstr Friday-485. Abstr ASM Microbe, Atlanta, GA. American Society of Microbiology, Washington, DC.
27. Pfaller MA, Messer SA, Rhomberg PR, Schaefer BA, Castanheira M. 2018. Activity of a long-acting echinocandin rezafungin tested against invasive fungal isolates collected worldwide, abstr 2400. Abstr ID Week 2018, San Francisco, CA. Infectious Diseases Society of America, Arlington, VA.
28. Toth Z, L F, Locke JB, Kardos G, Nagy F, Kovacs R, Szekely A, Borman AM, Majoros L. 2019. Activity of rezafungin against common and rare Candida species in vitro, abstr P2161. Abstr European Congress of Clinical Microbiology and Infectious Diseases, Amsterdam, Netherlands. European Society of Clinical Microbiology and Infectious Diseases, Basel, Switzerland.