**CD101, ‘A Perfect Storm’ Against Aspergillus: In Vitro Microbiology, In Vivo Tissue Distribution, and Front-Loaded Treatment and Prophylaxis Efficacy in Mouse Disseminated and Pulmonary Aspergillosis Infection Models**

V. Ong, G. Hough, S. Flanagan, K. Bartizal, A. Sattar, A. Sharp, P. Thommes

1Cidara Therapeutics, San Diego, CA; 2Evotec, Manchester, UK

**INTRODUCTION**

Fungal infections cause significant morbidity and mortality. Disease and treatment-related immunosuppression in patients with cancer, hematopoietic stem cell transplantation, and patients on immunosuppressive medications account for a large number of infections caused by Candida spp., Aspergillus spp., and Pneumocystis spp., respectively. The use of drug interactions, and variable pharmacokinetics complicate antifungal options currently used for prophylaxis, such as the use of access for Candida spp. and Aspergillus spp. Unmet needs in antifungal prophylaxis remain.

Rezafungin (previously known as CD101) is a novel echinocandin in phase 2 clinical development that has demonstrated robust predicted efficacy and is differentiated by its currently available echinocandins by its long-acting pharmacokinetic profile that allows for once-weekly dosing and, together with its exceptional stability and solubility potential for subcutaneous administration. Whereas currently approved echinocandins are limited to once-daily IV dosing, the potential for intermittent administration may extend the practical utility of rezafungin to include antifungal prophylaxis and treatment in the outpatient setting.

Rezafungin shows potent activity in vitro in minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) against a variety of fungi. Additional evidence or support for the translatability from in vitro potency to in vivo efficacy was sought by measuring tissue exposures, particularly in the lungs, where most cases of invasive pulmonary aspergillosis originate. Both neutropenic (cyclophosphamide-induced) and non-neutropenic (uncompromised) mice infected with Candida, Aspergillus, or Pneumocystis species have previously been successfully treated by rezafungin, the focus of this presentation will be on tissue/plasma exposure ratios, where there is greatest unmet need.

**METHODS (cont’)

Rezafungin (20 mg/kg; human equivalent dose) was also measured in the lung epithelial lining fluid of CD1-1 mice used in efficacy studies. The tissue/plasma ratio was determined using bronchoalveolar lavage fluid (BALF) collection with 2.5 mL flushes of saline at 0, 1, 3, 6, 12, 24, 48, and 72 hours post-dose. Ultra levels for plasma/BALF normalization for lung ELF volume calculation were quantified using a spectrophotometry-based assay. Rezafungin concentrations in plasma/BALF samples were measured by LC-MS/MS.

Disseminated aspergillosis: Neutropenic (cyclophosphamide-induced) ICR mice (50g) were challenged with A. fumigatus ATCC 13873 [10⁵ IU/mL] (0.02% gum tragacanth) 24 hours after infection with rezafungin was given as a single IP or SC dose. Survival was monitored for 10 days. The same model was used for prophylaxis except CD1-1 mice (5, 10, or 20 mg/kg) was dosed on days -1, -3 or -5 prior to infection.

Pulmonary (intranasal) aspergillosis: Neutropenic (CPCR-induced) ICR mice (100g) were challenged with A. fumigatus ATCC 29233 [10⁷ IU/mL] on day 0. Prophylactic rezagafungin was given as a single dose, IP, 5, 10, or 20 mg/kg, 1 day prior to infection. Survival was monitored for 10 days.

**RESULTS (cont’)

In the rat, homogenized tissue/plasma exposure ratios (EPR=) were determined through different organs (liver, kidney, lung, spleen) using good tissue penetration, with the exception of the heart and brain. Longer residence times were observed as t1/2 for tissues (40 – 77) exceeded t1/2 for tissues (39 hr).

In the same study, the 5 mg/kg group showed increased survival when prophylaxis was given closer to challenge.

**RESULTS (cont’)

For prophylaxis in disseminated aspergillosis, survival was monitored for 14 days after challenge. All animals in the 10 and 20 mg/kg groups survived to the end of prophylactic treatment.

For prophylaxis in pulmonary aspergillosis, dosed-dependent survival was observed from a single dose of rezagafungin. The human dose (400 mg) AUC equivalent to 20 mg/kg in mice showed better survival compared with the rezagafungin human dose (50 mg) AUC equivalent to 2 mg/kg in mice (Fig 3). Further, rezagafungin protein binding studies show a higher free fraction (~3%) in human plasma compared to human plasma suggesting a lower human dose may be equally protective.

**CONCLUSION**

Taken together, the in vitro potency and good tissue penetration has translated into robust efficacy in mouse models of aspergillosis suggesting that rezagafungin may be a potential new agent to intermittent outpatient echinocandin treatment and prophylaxis of invasive pulmonary aspergillosis in a clinical setting. Further, the t1/2 for rezagafungin in humans (133 hr) is ≤50% longer than in mouse (25 hr). It is anticipated that the prophylactic effect from a single dose (400 mg) given 1 day-1 (≤4x mouse t1/2) prior to fungal challenge would translate to a comparable effective dose from a single dose given to humans for up to 1 week.

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**DISCLOSURES**

V.O., G.H., S.F., K.B.: employees and shareholders of Cidara Therapeutics, Inc.

A.S., A.S.: PT. None.

**REFERENCES**


2. 20th Congress of the European Hematology Association, 2017, Poster #H195


**METHODS**

Rezafungin exhibited potent in vitro activity against 97 clinical A. fumigatus isolates collected during the 2015 JMIA international Sentry surveillance program. Susceptibility was determined as the minimum effective concentration (MEC) values in accordance with CLSI broth microdilution guidelines (2017).

The plasma and tissue exposures of rezagafungin were initially evaluated in Sprague-Dawley rats (3 rats/group) after a 5 mg/kg IV dose. At 0.05, 0.1, 0.5, 1, 2, 4, 8, and 24 hours post-dose, rats were euthanized and plasma as well as various tissues (liver, kidney, heart, spleen, brain) were collected for rezagafungin concentration measurement by LC/MS/MS.

**RESULTS**

Rezagafungin (20 mg/kg; human equivalent dose) was also measured in the lung epithelial lining fluid of CD1-1 mice used in efficacy studies. The tissue/plasma ratio was determined using bronchoalveolar lavage fluid (BALF) collection with 2.5 mL flushes of saline at 0, 1, 3, 6, 12, 24, 48, and 72 hours post-dose. Ultra levels for plasma/BALF normalization for lung ELF volume calculation were quantified using a spectrophotometry-based assay. Rezagafungin concentrations in plasma/BALF samples were measured by LC-MS/MS.