Novel Echinocandin Rezafungin (CD101) In Vitro Potency Translates to Efficacious Treatment/Prophylaxis in Mouse Models of Disseminated/Pulmonary Aspergillosis

V. Ong1, G. Hough1, S. Flanagan1, K. Bartizal2, A. Sattar2, A. Sharp2, P. Thommes2
1Cidara Therapeutics, San Diego, CA; 2Evotec, Manchester, UK

BACKGROUND
Fungal infections cause significant morbidity and mortality. Disease- and treatment-related immunosuppression in patients with hematological diseases increase the risk of opportunistic infection caused by Candida spp., Aspergillus spp., and Pneumocystis spp. However, safety and tolerability, drug-drug interactions, and variable pharmacokinetics complicate the antifungals currently used for prophylaxis, such as the use of azoles 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 preclinical efficacy and is differentiated from currently available echinocandins by a long-acting pharmacokinetic profile, allowing for once-weekly dosing, and together with its exceptional stability and solubility, allows for subcutaneous dosage form. Whereas currently approved echinocandins are limited to once-daily IV dosing, the potential for intermittent administration may extend the practical utility of rezafungin beyond that of other echinocandins, to include antifungal prophylaxis and treatment in the outpatient setting.

METHODS
The in vitro activity of rezafungin was evaluated against A. fumigatus clinical isolates collected during the 2015 JMI International SENTRY surveillance program. Susceptibility was determined as the minimum effective concentration (MEC) values in accordance with CLSI broth microdilution guidelines (M38-A2)1. Rezafungin (20 µg/mL; human equivalent dose) was measured in the lung epithelial lining fluid (ELF) of CD-1 mice used in efficacy studies. 3 mice/timepoint were euthanized for plasma and bronchoalveolar lavage fluid (BALF) collection with 2x 0.5 mL flushes of saline at 0, 1, 3, 6, 12, 24, 48, and 72 hours post-dose. Urea levels for plasma/BALF normalization for lung ELF volume calculations 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 (6/grp) were challenged with A. fumigatus ATCC 13073 (IV, 106 CFU/mouse) on day 0. Treatment (2x 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 rezafungin (SC; 5, 10, or 20 mg/kg) was dosed on days 1, -3 or -5 prior to infection. Pulmonary (intranasal) aspergillosis: Neutropenic (CPM-induced) ICR mice (10/grp) were challenged with A. fumigatus AF293 (intranasal, 106 CFU/mouse) on day 0. Prophylactic rezafungin 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
Rezafungin exhibited potent in vitro activity against 97 clinical A. fumigatus isolates with MEC50/MEC90 and MEC range values of 0.015, 0.03, and ≤0.0078-0.03 µg/mL respectively (from 2015)1.

<table>
<thead>
<tr>
<th>Drug</th>
<th>MEC50</th>
<th>MEC90</th>
<th>Range</th>
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<tr>
<td>Rezafungin</td>
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<td>0.03</td>
<td>0.008-0.03</td>
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<td>1</td>
<td>0.25-2.2</td>
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<td>Caspofungin</td>
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<td>0.03</td>
<td>0.008-0.03</td>
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<tr>
<td>Micafungin</td>
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<td>0.03</td>
<td>0.008-0.06</td>
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<td>Itraconazole</td>
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<td>1</td>
<td>0.25-1</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>0.5</td>
<td>0.5</td>
<td>0.12-0.5</td>
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Distribution into lung ELF was confirmed by measuring rezafungin concentrations in bronchoalveolar lavage fluid. Rezafungin in ELF reached a maximum by 4 hr and were comparable between plasma and ELF at ≥24 hr post-dose based on total-drug with an ELF/Plasma AUC ratio of 0.80. If plasma concentrations were corrected for protein binding (99.2%), the ELF/Plasma AUC ratio would be 100.

For treatment of disseminated aspergillosis, survival was comparable with either a single 2 mg/kg IP dose or 0.2 mg/kg BID x 5d, confirming that a single, front-loaded dose regimen was equally effective2.

For prophylaxis in disseminated aspergillosis2, survival was monitored for 14 days after challenge. All animals in the 10 and 20 mg/kg groups survived regardless of prophylactic treatment day while the 5 mg/kg group showed increased survival when prophylaxis was given closer to challenge.

For prophylaxis in pulmonary aspergillosis, the human (400 mg) AUC equivalent dose of 20 mg/kg in mice of rezafungin showed an increase in survival relative to vehicle control. Further comparison with micafungin or posaconazole suggests an advantage for rezafungin with higher survival rates compared to micafungin or posaconazole at their respective human AUC equivalent doses. Only posaconazole at 10 mg/kg (5x higher than human AUC) showed a statistically significant increase in survival rate relative to vehicle.

CONCLUSION
Taken together, the in vitro potency as well as excellent lung penetration has translated into robust efficacy in mouse models of aspergillosis suggesting that rezafungin may be a potential new agent for intermittent outpatient echinocandin treatment and prophylaxis of aspergillosis in a clinical setting. As the t1/2 of rezafungin in human (133 hrs) is ~5x longer than in mouse (25 hrs), we anticipate the prophylactic effect from a single dose in mouse given 1 day (~1x mouse t1/2) prior to infection challenge would translate to a comparable prophylactic effect from a single dose given to humans for up to 1 week. Further, rezafungin protein binding results show a higher free fraction (~3x) in human vs mouse plasma suggesting a lower human dose may be equally protective.

REFERENCES
3. 22nd Congress of the European Hematology Association. 2017, Poster P645

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