Poster 554

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

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INTRODUCTION

Fungal infections cause significant morbidity and mortality. Disease- and treatment-related immunosuppression in patients with hematological diseases increases the risk of opportunistic infections caused by *Candida* spp., *Aspergillus* spp., and *Pneumocystis* spp. However, safety and tolerability, drug-drug interactions, and variable pharmacokinetics complicate antifungal options 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 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 terms of in vitro minimum inhibitory/effective concentration (MIC/MEC) against a variety of fungi[1]. 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) as well as non-neutropenic (immunecompetent) mice infected with *Candida*, *Aspergillus*, or *Pneumocystis* species have previously been successfully treated by rezafungin[2], the focus of this presentation will be on prophylaxis/treatment of aspergillosis, where there is greatest unmet need.

METHODS

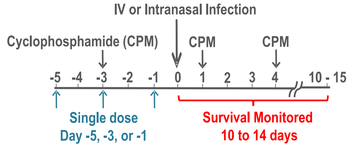
The in vitro activity of rezafungin was evaluated against *A. fumigatus* clinical isolates collected during the 2015 JMI international SENTRY surveillance program. Susceptibility was determined as the minimum effective concentration (MEC) values in accordance with CLSI broth microdilution guidelines (M38-A2)[1].

The plasma and tissue exposures of rezafungin were initially evaluated in Sprague-Dawley rats (3 rats/timepoint) after a 5 mg/kg IV dose. At 0.08 (5 minutes), 1, 4, 8, 24, 72, and 120 hours post-dose, rats were euthanized and plasma as well as various tissues (liver, lungs, kidneys, heart, spleen and brain) were collected for rezafungin concentration measurement by LC-MS/MS.

METHODS (con't)

Rezafungin (20 mg/kg IP; human equivalent dose) was also measured in the lung epithelial lining fluid of CD-1 mice used in efficacy studies. Three mice/timepoint were euthanized for plasma and bronchoalveolar lavage fluid (BALF) collection with 2 x 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 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 (6/grp) were challenged with *A. fumigatus* ATCC 13073 (IV, 10^4 CFU/mouse) on day 0. Treatment (2 hr 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, 10^5 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.

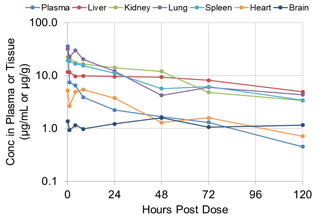
RESULTS

Rezafungin exhibited potent in vitro activity against 97 clinical *A. fumigatus* isolates with MEC_{50}, MEC_{90} and MEC range values of 0.015, 0.03, and ≤0.0078-0.03 µg/mL, respectively (from 2015)[1].

Drug	MEC_{50}	MEC_{90}	Range
Rezafungin	0.015	0.03	≤0.008 - 0.03
Anidulafungin	≤0.008	0.03	≤0.008 - 0.03
Caspofungin	0.015	0.03	≤0.008 - 0.06
Micafungin	≤0.008	0.015	≤0.008 - 0.03
Itraconazole	0.5	1	0.25 - 1
Posaconazole	0.25	0.5	0.12 - 0.5
Voriconazole	0.5	0.5	0.12 - 1
Amphotericin B	1	2	0.25 - 2

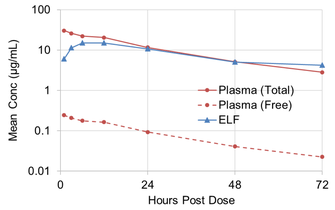
RESULTS (con't)

In the rat, homogenized tissue/plasma exposure ratios (~4) were comparable among different organs (liver, kidney, lung, spleen) suggesting good tissue penetration, with the exception of the heart and brain. Longer tissue residence times were observed as $t_{1/2}$ of rezafungin in all tissues (40 – 77 hr) exceeded plasma $t_{1/2}$ (39 hr).

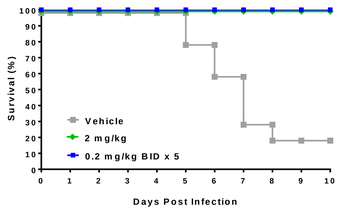


Matrix	Tissue/Plasma AUC Ratio
Liver	4.14
Kidney	4.62
Lung	4.33
Spleen	3.87
Heart	1.09
Brain	0.609

Distribution into lung ELF was confirmed by measuring rezafungin concentrations in bronchoalveolar lavage fluid. Rezafungin in ELF reached a maximum by 4 hr and was 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.

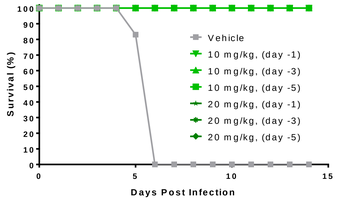


For treatment of disseminated aspergillosis, survival was comparable with either a single 2 mg/kg dose or 0.2 mg/kg BID x 5d, confirming that a single, front-loaded dose regimen was equally effective[3].

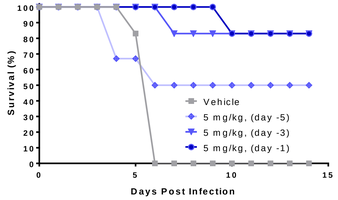


RESULTS (con't)

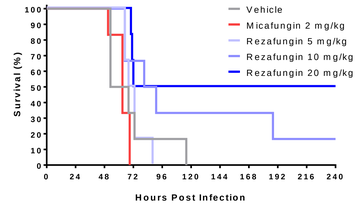
For prophylaxis in disseminated aspergillosis[2], survival was monitored for 14 days after challenge. All animals in the 10 and 20 mg/kg groups survived regardless of prophylactic treatment day.



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

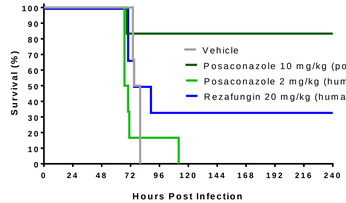


For prophylaxis in pulmonary aspergillosis, dose-dependent survival was observed from a single dose of rezafungin. The human dose (400 mg) AUC equivalent to 20 mg/kg in mice showed better survival compared with the micafungin human dose (50 mg) AUC equivalent to 2 mg/kg in mice (Fig 3). Further, rezafungin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



RESULTS (con't)

In another prophylaxis pulmonary aspergillosis study, the human dose (400 mg) equivalent of 20 mg/kg in mice showed 30% survival whereas posaconazole at its human equivalent dose of 2 mg/kg was not protective and only showed a significant increase in survival at 10 mg/kg (5x higher than human equivalent dose).



CONCLUSION

Taken together, the in vitro potency and good tissue 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. Further, as the $t_{1/2}$ of rezafungin in humans ($t_{1/2}$ 133 hr) is ~5x longer than in mouse ($t_{1/2}$ 25 hrs), it is anticipated that the prophylactic effect from a single dose in mouse given 1 day (~1x mouse $t_{1/2}$) prior to fungal challenge would translate to a comparable prophylactic effect from a single dose given to humans for up to 1 week.

REFERENCES

1. Pfaller et al., International Journal of Antimicrobial Agents 50 (2017) 352–358
2. 22nd Congress of the European Hematology Association, 2017, Poster P645
3. Lakota et al. 2017. Pharmacological basis of CD101 efficacy: exposure shape matters. Antimicrob Agents Chemother 61:e00758-17.

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DISCLOSURES

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