Rezafungin is Efficacious Against Invasive Aspergillosis Caused by Azole-Resistant *Aspergillus fumigatus* Harboring the TR_{34}/L98H Mutation

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ABSTRACT

Background: Invasive aspergillosis (IA) caused by azole-resistant *A. fumigatus* is of increasing concern. Isolates harboring the TR34/L98H mutation in the *CYP51A* gene are often pan-azole–resistant, can be found in the environment, and can cause invasive disease in individuals with no previous azole exposure. Rezafungin (formerly CD101) is an investigational echinocandin with a long half-life (130 h in humans) and potent *in vitro* activity against *Aspergillus* species, including azole-resistant *A. fumigatus* (Wiederhold et al. *J Antimicrob Chemother* 2018). Our objective was to evaluate the *in vivo* efficacy of intermittent dosing of rezafungin against IA caused by *A. fumigatus* harboring the TR34/L98H mutation.

Methods: A clinical isolate of *A. fumigatus* with a confirmed TR34/L98H mutation was used (rezafungin MEC 0.06 µg/mL, posaconazole MIC 1 µg/mL). Male ICR mice were rendered neutropenic with cyclophosphamide (200 mg/kg IP) and 5-fluorouracil (5 mg IV) 1 day prior to IV inoculation. Treatment began 1 day after inoculation with vehicle control; subtherapeutic, therapeutic, or supratherapeutic rezafungin (1, 4, or 16 mg/kg IP on days 1, 4, and 7 post-inoculation); or supratherapeutic doses of posaconazole (20 mg/kg PO BID), and therapy was continued through day 7. In the survival arm, mice were monitored off therapy until day 12. In the fungal burden arm, mice were euthanized on day 8 and kidneys were collected for enumeration of colony forming units (CFU/g). Survival was assessed by Kaplan-Meier analysis, and fungal burden by ANOVA.

Results: A survival advantage was observed with intermittent dosing of rezafungin, as median survival was improved in a dose-dependent fashion (10.5 days, 12 days, and >12 days) compared to control (4.5 days; $p \leq 0.001$). Percent survival was also improved with rezafungin (range 50% to 60%) compared to control (0%; $p \leq 0.0325$). Median survival and percent survival were also improved with posaconazole (>12 days and 60%, respectively; $p \leq 0.01$). Fungal burden, as measured by CFU counts, was not significantly reduced in mice treated with rezafungin. However, this is consistent with the mechanism of action of the echinocandins against *Aspergillus* species (Bowman et al. *Antimicrob Agents Chemother* 2001).

Conclusions: Intermittent dosing of rezafungin was associated with *in vivo* efficacy in this mouse model of IA caused by *A. fumigatus* with the TR34/L98H mutation, as survival was improved in a dose-dependent fashion. These results highlight the potential use of rezafungin for the treatment of azole-resistant IA.

BACKGROUND

- Invasive aspergillosis caused by azole-resistant *Aspergillus fumigatus* is of increasing concern.
- Isolates harboring the TR_{34}/L98H mutation in the *CYP51A* gene are of particular concern as they are often pan-azole resistant, can be found in the environment, and can cause invasive disease in patients without previous azole exposure.
- Isolates harboring the TR_{34}/L98H mutation have now been found in numerous countries around the world.
- Rezafungin (formerly CD101) is an investigational echinocandin with a long half-life (130 hours in humans), high plasma exposure and tissue concentrations, and potent *in vitro* activity against *Aspergillus* species, including azole-resistant *A. fumigatus* (Wiederhold et al. *J Antimicrob Chemother* 2018;73:3063-3067).
- This long half-life translates into high exposures, and thus may allow for less frequent dosing compared to other echinocandins (Lepak et al. *Antimicrob Agents Chemother* 2018;24:e01572-18; ; Zhao et al. *Antimicrob Agents Chemother* 2017;61:e01009-17).

OBJECTIVE

- Our objective was to evaluate the *in vivo* efficacy of rezafungin for the treatment of azole-resistant invasive aspergillosis.
- A clinical isolate of *A. fumigatus* harboring a TR_{34}/L98H mutation was used to establish invasive disease in a neutropenic murine model.
- Outcome measures included survival and fungal burden as measured by colony-forming units (CFU/g) and quantitative real-time PCR (qPCR).

MATERIALS AND METHODS

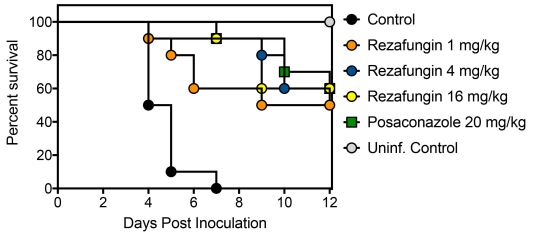
Isolate & Inoculation
- *Aspergillus fumigatus* clinical isolate UTHSCSA DI15-116 (rezafungin MEC 0.06 µg/mL, posaconazole MIC 1 µg/mL) was used
- Male ICR mice were rendered neutropenic with cyclophosphamide (200 mg/kg) and 5-fluoruacil (5 mg/mouse) both administered 1 day prior to inoculation
- Mice were inoculated via the lateral tail vein

Antifungal Treatment
- Antifungal therapy began 1 day post-inoculation and continued through day 7
- Treatment groups consisted of: 1.) Vehicle Control, 2.) rezafungin 1, 4, or 16 mg/kg administered by IP injection on days 1, 4, and 7, and 3.) posaconazole 20 mg/kg PO BID

Outcomes
- In the survival arm mice were monitored off therapy until day 12 post-inoculation
- Fungal burden was measured by CFU/g and quantitative real-time PCR (qPCR) of the 18S rDNA region on day 8 in the fungal burden arm and on day 12 or as mice became moribund in the survival arm
- Survival was assessed by Kaplan Meier analysis and the log-rank test, and fungal burden by ANOVA with Tukey's post-test for multiple comparisons

RESULTS

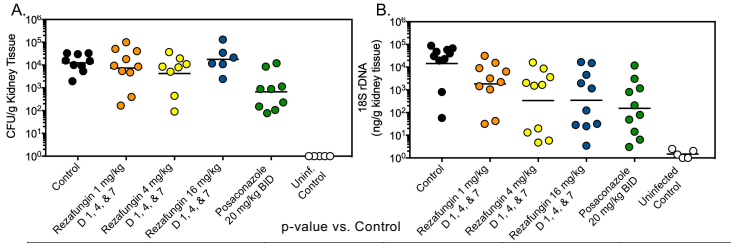
Figure 1. Survival curves in a neutropenic mouse model of invasive aspergillosis caused by an azole-resistant *A. fumigatus* isolate harboring a TR_{34}/L98H mutations.



Group	Control	Rezafungin 1 mg/kg	Rezafungin 4 mg/kg	Rezafungin 16 mg/kg	Posaconazole 20 mg/kg
Median Survival	4.5 days	10.5 days p = 0.0011	12 days p < 0.0001	>12 days p < 0.0001	>12 days p < 0.0001
Percent Survival	0%	50% p = 0.0325	50% p = 0.0325	60% p = 0.0108	60% p = 0.0108

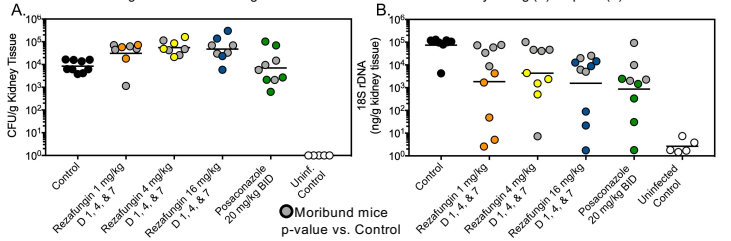
RESULTS (continued)

Figure 2. Fungal burden on day 8 post-inoculation (fungal burden arm) in neutropenic mice with invasive aspergillosis caused by an azole-resistant *A. fumigatus* isolate harboring a TR_{34}/L98H mutation. As measured by CFU/g (A) or qPCR (B).



Group	Control	Rezafungin 1 mg/kg	Rezafungin 4 mg/kg	Rezafungin 16 mg/kg	Posaconazole 20 mg/kg
Mean $\log_{10}$ CFU/g (SD)	4.10 (0.41)	3.87 (0.89)	3.63 (0.88)	4.25 (0.58)	2.83 (0.79) p = 0.0021
Mean $\log_{10}$ ng/g DNA (SD)	4.16 (1.02)	3.26 (1.00)	2.53 (1.39) p = 0.0122	2.54 (1.31) p = 0.0131	2.19 (1.19) p = 0.0017

Figure 3. Fungal burden in the survival arm on day 12 post-inoculation in neutropenic mice with invasive aspergillosis caused by an azole-resistant *A. fumigatus* isolate harboring a TR_{34}/L98H mutations. As measured by CFU/g (A) or qPCR (B).



Group	Control	Rezafungin 1 mg/kg	Rezafungin 4 mg/kg	Rezafungin 16 mg/kg	Posaconazole 20 mg/kg
Mean $\log_{10}$ CFU/g (SD)	3.93 (0.26)	4.49 (0.61)	4.74 (0.31) p = 0.0069	4.68 (0.52) p = 0.0140	3.85 (0.73)
Mean $\log_{10}$ ng/g DNA (SD)	4.87 (0.47)	3.26 (1.72) p = 0.0410	3.64 (1.31)	3.19 (1.46) p = 0.0304	2.94 (1.38) p = 0.0123

CONCLUSIONS

Extended interval dosing of rezafungin was associated with improved outcomes in this mouse model of invasive aspergillosis caused by azole-resistant *A. fumigatus* due to the TR_{34}/L98H mutation. Improvements in survival and reductions in fungal burden as measured by qPCR were observed compared to vehicle control. Further studies are warranted to assess the potential benefits of extended interval dosing of rezafungin for the treatment of aspergillosis caused by azole-resistant *Aspergillus*.

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