Delayed Initiation of the Novel Oral Glucan Synthase Inhibitor, Ibrexafungerp, is Effective in a Murine Model of Invasive Candidiasis caused by *Candida auris*

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ABSTRACT

Background: *Candida auris* is an emerging pathogen that is associated with significant morbidity and mortality as well as antifungal resistance. Ibrexafungerp (IBX) is the first representative of a novel antifungal family, the triterpenoids ('fungerps'), that, inhibits glucan synthase (similar to echinocandins) but can be administered orally. Previous studies have demonstrated *in vitro* activity of this agent against *C. auris* and *in vivo* efficacy when treatment is started early after inoculation (e.g., 2 hours post-inoculation). We assessed the *in vivo* efficacy of IBX in a neutropenic murine model of *C. auris* invasive candidiasis following delayed initiation of therapy (24 hours post-inoculation).

Methods: Mice were rendered neutropenic with 5-fluorouracil (5 mg/mouse IV) and infected IV with *C. auris* (MICs 1, >64, 0.25 μg/mL for IBX, fluconazole, and caspofungin). Treatment was initiated after a 24-hour delay post-inoculation and consisted of placebo, IBX (20, 30, or 40 mg/kg PO BID), fluconazole (20 mg/kg PO BID), or supratherapeutic caspofungin (10 mg/kg IP QD) and continued through day 7. In the survival arm, mice were monitored off therapy until day 21. 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 IBX. Median survival was significantly improved in the IBX 30 and 40 mg/kg groups vs. placebo (>21 days vs. 6.5 days; $p \leq 0.05$). Caspofungin also improved survival compared to placebo (>21 days). Kidney fungal burden on day 8 was also dose-dependently reduced in the IBX groups (mean $\log_{10}$ CFU/g 3.85, 2.70, and 1.83 in the 20, 30, and 40 mg/kg dose groups, respectively) vs. placebo (5.36 $\log_{10}$ CFY/g), and these differences were significant for the IBX 30 and 40 mg/kg groups vs. placebo ($p \leq 0.01$). Similar fungal burden results were also observed in the survival arm on day 21 or as the mice became moribund with the IBX 30 and 40 mg/kg groups (5.10 and 4.14 $\log_{10}$ CFU/g) vs. placebo (7.42 $\log_{10}$ CFU/g). In contrast, no improvements in survival or reductions in fungal burden were observed with the lower IBX dose or with fluconazole.

Conclusions: IBX demonstrated *in vivo* efficacy following delayed initiation of therapy against *C. auris*. Significant improvements in survival and reductions in fungal burden were observed in mice treated with the higher doses of IBX. These data further support the potential utility of IBX as therapy against invasive infections caused by *Candida auris*.

BACKGROUND

- *Candida auris* is an emerging pathogen that has now been detected in institutions on multiple continents.
- Invasive infections caused by this species are associated with high mortality rates, up to 59% in one retrospective study (Lockhart et al. *Clin Infect Dis* 2017;64:134-40).
- Treatment options are limited as *C. auris* isolates are often resistant to multiple antifungals, including fluconazole and other azoles, and up to one third of isolates may be resistant to amphotericin B, with echinocandin resistance also being reported.
- Ibrexafungerp (IBX) is the first representative of a novel antifungal family, the triterpenoids ('fungerps'), that, inhibits glucan synthase (similar to the echinocandins) but can be administered orally.
- This agent has been shown to be effective in murine models of invasive candidiasis caused by echinocandin-resistant *C. glabrata* (Wiederhold et al. *J Antimicrob Chemother* 2018).
- Previous studies have demonstrated *in vitro* activity of this agent against *C. auris* and *in vivo* efficacy when treatment is started early after inoculation (e.g., 2 hours post-inoculation).

OBJECTIVE

- Our objective was to evaluate the *in vivo* efficacy of ibrexafungerp following delayed initiation of therapy (24 hours post-inoculation) for the treatment of invasive candidiasis caused by *C. auris*. *In vivo* outcome measures included survival and fungal burden as measured by colony-forming units (CFU/g).

MATERIALS AND METHODS

Isolate & *In vitro* Susceptibility
- *In vitro* susceptibility was determined for *Candida auris* clinical isolate UTHSCSA DI 17-46 by broth microdilution according to the CLSI M27 standard
- This clinical isolate was used to establish infection in the murine model

Murine Model
- Male ICR mice were rendered neutropenic with 5-fluoruacil (5 mg/mouse IV) administered 1 day prior to inoculation
- Mice were inoculated via the lateral tail vein
- Antifungal therapy began 1 day post-inoculation and continued through day 7
- Treatment groups consisted of: Vehicle Control (methyl cellulose) PO BID, Ibrexafungerp 20, 30, or 40 mg/kg PO BID, fluconazole 20 mg/kg PO BID, and caspofungin 10 mg/kg IP QD

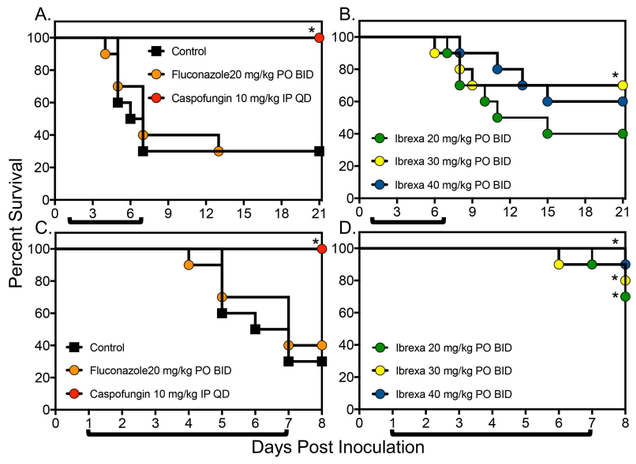
Outcomes
- In the survival arm mice were monitored off therapy until day 21 post-inoculation
- Fungal burden was measured by colony-forming units (CFU/g) on day 8 in the fungal burden arm (one day after treatment stopped) and on day 21 or as mice became moribund in the survival arm
- Survival was assessed by Kaplan Meier analysis and the log-rank test, and fungal burden by ANOVA with Tukey's post-test for multiple comparisons

RESULTS

Table 1. MIC of ibrexafungerp, fluconazole, and caspofungin against *C. auris* DI 17-46.

Parameter	Ibrexafungerp	Fluconazole	Caspofungin
MIC (μg/ml)	1	>64	0.25

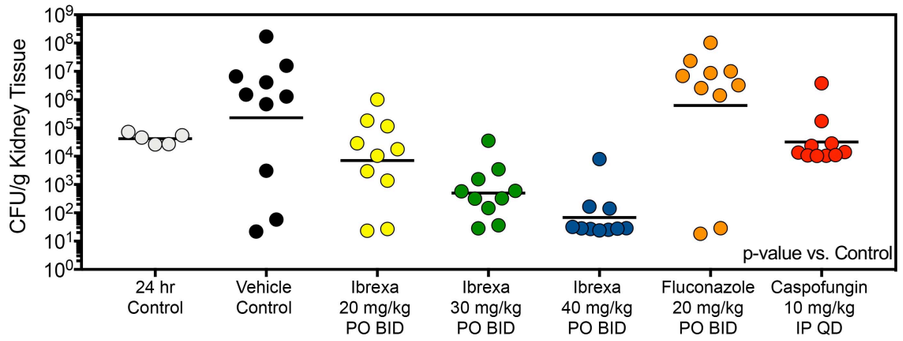
Figure 1. Survival curves to day 21 (A & B) or on therapy to day 8 (C & D) in a neutropenic murine model of invasive candidiasis caused by *C. auris*. Mice were treated with vehicle control PO BID, fluconazole 20 mg/kg PO BID, caspofungin 10 mg/kg IP QD (A & C), or ibrexafungerp (Ibrexa) 20, 30, or 40 mg/kg PO BID (B & D).



Brackets show treatment period (days 1-7) * p-value <0.05 vs. Control

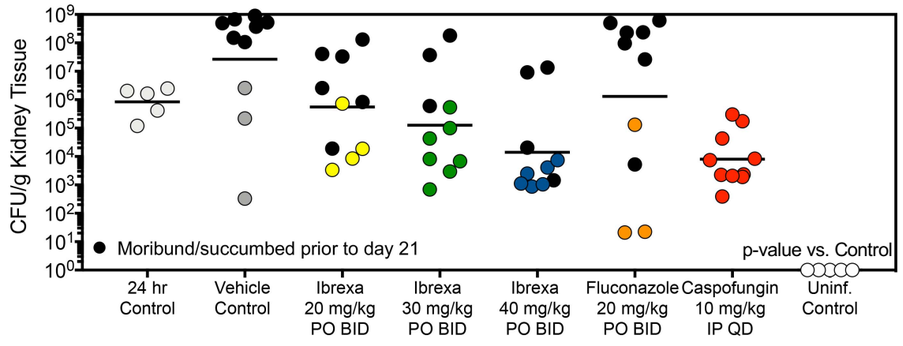
RESULTS (continued)

Figure 2. Kidney (CFU/g) on day 8 post-inoculation (fungal burden arm) in neutropenic mice with invasive candidiasis caused by *C. auris*.



Group	24 hr Control	Control	Ibrexa 20 mg/kg	Ibrexa 30 mg/kg	Ibrexa 40 mg/kg	Fluconazole 20 mg/kg	Caspofungin 10 mg/kg
Mean $\log_{10}$ CFU/g (SD)	4.62 (0.19)	5.36 (2.34)	3.85 (1.54) p = 0.1527	2.70 (0.92) p = 0.0026	1.83 (0.79) p < 0.0001	5.79 (2.39) p = NS	4.50 (0.82) p = NS

Figure 3. Kidney (CFU/g) on day 21 or as mice became moribund (survival arm) in neutropenic mice with invasive candidiasis caused by *C. auris*.



Group	24 hr Control	Control	Ibrexa 20 mg/kg	Ibrexa 30 mg/kg	Ibrexa 40 mg/kg	Fluconazole 20 mg/kg	Caspofungin 10 mg/kg
Mean $\log_{10}$ CFU/g (SD)	5.92 (0.56)	7.42 (2.09)	5.74 (1.68) p = NS	5.10 (1.76) p = 0.0415	4.14 (1.59) p = 0.0019	6.11 (3.01) p = NS	3.90 (0.93) p = 0.0008

CONCLUSIONS

Ibrexafungerp demonstrated *in vivo* efficacy against *C. auris* following delayed initiation of therapy, as improvements in survival and reductions in kidney fungal burden were also observed in mice treated with the two highest doses evaluated in this study. These data demonstrate the potential utility of ibrexafungerp as therapy against invasive *C. auris* infections.

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