Efficacy of a Non-Peptide, Small Molecule Mimic of Host Defense Proteins in Mouse Models of Disseminated Candidiasis and Aspergillosis

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1. BACKGROUND

- Invasive fungal infections (IFIs) are a growing problem in numerous medical settings, especially in immuno-compromised patients.
- The most common fungal pathogens are *Candida, Aspergillus*, *Cryptococcus*, *Mucorales* and *Fusarium* spp. and infections are associated with a significant incidence of treatment failure and high mortality.
- The incidence of IFIs continues to rise and several important medical needs persist for their prevention and treatment:
  - mortality rates remain high
  - long treatment times increase the potential for resistance development
  - emerging drug-resistant pathogens such as *Fusarium* and *Scedosporium* spp. and *Candida auris* are a serious medical threat
  - resistance is building to the current antifungals
  - safety issues related to multiple therapeutic regimens in patients raise the risk of drug – drug interactions.
- There is an urgent need for new broadly active and rapidly cidal antifungal agents with distinct mechanisms of action to more effectively control these life-threatening infections.

2. APPROACH

- We have developed a series of small nonpeptide mimics of the host defense proteins (smHDP) as antimicrobial agents.
- The overarching goal was to recapitulate the biological and structural properties of HDPs into oligomeric backbones creating small structurally constrained compounds. Expected advantages include:
  - better pharmacokinetic and tissue distribution properties
  - improved stability
  - economical production
  - considerably more phase space expanded beyond amino acids to fine-tune structures for desired activities
- Current lead series show potent activity against multiple fungal pathogens including *Candida, Cryptococcus*, *Aspergillus* and *Fusarium* spp. (see Poster Submission ID 896957).
- Lead compound FL-1 and back-up analog FL-6 have been tested *in vivo* in mouse models of disseminated candidiasis and aspergillosis

3. METHODS

MIC

- Minimal Inhibitory Concentrations (MICs) were determined vs. ATCC strains in standardized assays according to CLSI-M27-A3 (yeast) and CLSI-M38-A2 (molds) guidelines

Disseminated Candidiasis

- Neutropenic CD-1 mice
  - Cyclophosphamide administered intraperitoneally (IP) 4 days (150 mg/kg) and 1 day (150 mg/kg) prior to infection.
- T=0 hrs: infected intravenously (IV) with *C. albicans* R303 @ 2 – 3 x10^4 CFU/inoculum
- T=2 hrs: treated with test agents daily by subcutaneous (SC) administration for 1 day (burden) or 5 days (survival) at indicated dosages (n = 8/grp)
- Kidney burdens were determined by plating serial dilutions of lysates prepared 24 hours post-infection
- Survival was monitored over 2 weeks post-treatment initiation

Disseminated Aspergillosis

- Neutropenic CD-1 mice
  - Cyclophosphamide IP 4 days (150 mg/kg) and 1 day (100 mg/kg) prior to infection
- T = 0: infect IV with app. 4.46 $\log_{10}$ CFU *A. fumigatus* spores (clinical isolate)
- T = 24 hrs: Treat with test agents daily by SC administration for 5 days at indicated dosages (n = 10/grp)
- Survival was monitored daily over the treatment period and tissue burdens were determined by plating serial dilutions of lysates prepared 24 hrs after the last dose

4A. RESULTS

Table 1: MIC Results for Lead FL-1 and Back-up Analog FL-6

Cmpd	MIC (µg/ml)						
	Candida albicans GDH2346*	*Aspergillus fumigatus* ATCC 3626	*Aspergillus flavus* ATCC 3631	*Fusarium falciforme* ATCC *3636*	*Fusarium solani* ATCC *58877*	*Mucor circinelloides* ATCC *26759**	*Mucor ramosissimus* ATCC *90286**
FL-1	**0.1**	**0.05**	**0.05**	**0.006**	**0.05**	**0.2**	**0.78**
FL-6	0.2	0.05	0.2	0.05	0.05	0.39	3.13
AmpB	0.2	1.6	1.6	1.6	1.6	0.8	0.8
PCZ	0.05	0.8	0.4	0.8	0.8	0.2	0.2

*MIC = 50% growth inhibition @ 48 hrs.; All MICs read at 48 hours of treatment; AmpB: Amphotericin B; PCZ: Posaconazole; NT: Not Teseted

4B. RESULTS

Figure 1: Disseminated Candidiasis
(*C. albicans* R303)

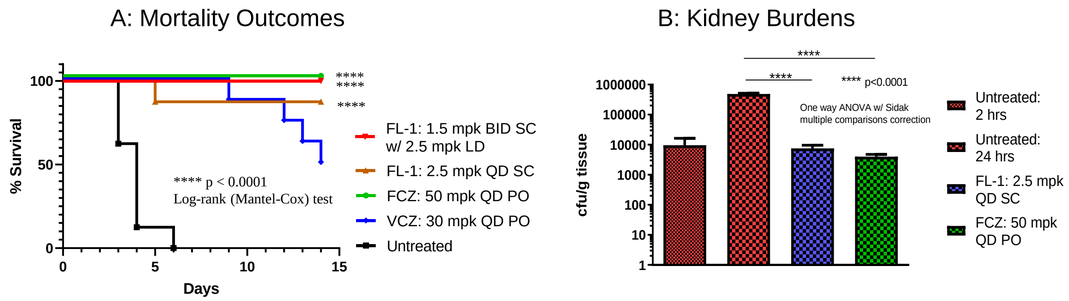
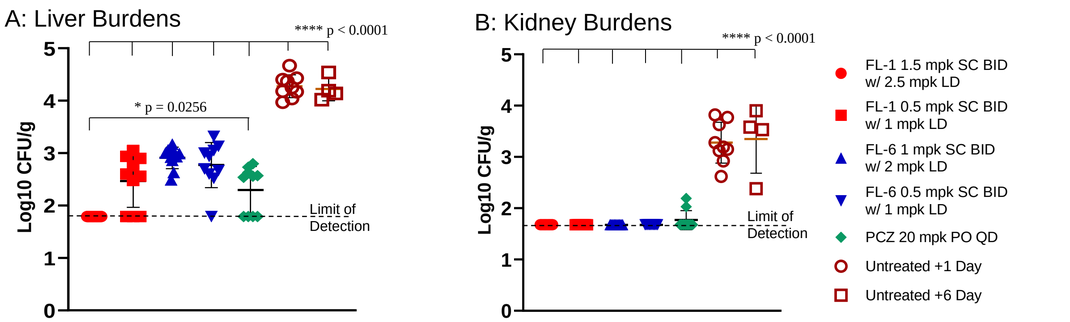


Figure 2: Disseminated Aspergillosis
(*A. fumigatus* clinical isolate)



C. Survival at Day 6

Untreated	FL-1 (2.5/1.5 mpk)	FL-1 (1/0.5 mpk)	FL-6 (2/1 mpk)	FL-6 (1/0.5 mpk)	PCZ (20 mpk)
4/10	10/10	10/10	10/10	10/10	10/10

5. RESULTS AND CONCLUSIONS

- **Table 1.** FL-1 and back-up FL-6 were potently active against *C. albicans* and mold strains. MICs were superior to amphotericin B (AmpB) and posaconazole (PCZ) versus *Aspergillus* and *Fusarium* spp.
  - Clinical isolate susceptibilities reported in poster presentation by Rhomberg et al. (Submission ID: 896957)
- **Figure 1.** FL-1 was fully protective in a mouse model of disseminated candidiasis caused by *C. albicans* when administered twice daily for 5 days. Treatment for 1 day reduced kidney burden to levels found at treatment onset. Activity was comparable to fluconazole (FCZ).
- **Figure 2.** Significant reductions in *A. fumigatus* burdens in liver and kidney were found with FL-1 after twice daily treatments for 5 days. A back-up compound, FL-6, showed similar efficacy to FL-1 at the same doses. FL-1 efficacies measuring survival and tissue burden reductions were comparable to posaconazole (PCZ).

Animal efficacy results support further development of FL-1 as a therapy for disseminated fungal infections

6. ACKNOWLEDGEMENTS

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