Objective: To evaluate the in vivo efficacy of HPLC-purified antifungal lipopeptides (AF4 and AF5) in a murine model of disseminated candidiasis.

Methods: C. albicans AMR16294 isolate was used for all the in vivo experiments. A total of 6-week-old pathogen-free, female BALB/c mice, weighing 20-25 g were used for all animal experiments. For Kaplan-Meier analysis, mice were rendered neutropenic by a loading dose of 200 mg/kg cyclophosphamide three days prior (D-3) to infection and 150 mg/kg (D = 0) maintenance dose on day 1 post-infection (D + 1). A total of 40 mice were randomized into 8 different groups with 5 or 6 animals in each group. Animals were infected with 1000 cfu of \(1 \times 10^8\) blastospores (corresponding to LD₉₀) via the lateral tail vein. AF4 and AF5 were formulated in sterile PBS and administered intraperitoneally at doses of 5 mg/kg and 10 mg/kg body weight and compared with a clinically-relevant human equivalent dose of caspofungin. AF4, AF5, caspofungin, or vehicle were administered at 1 h and 24 h post-infection. The survival of the mice was monitored for 14 days post-infection. For organ fungal-burden assessment, mice from each group were euthanized by CO₂ inhalation, and the organs were aseptically removed, homogenized, and cultured on SDA.

Results: Both the doses of AF4 significantly reduced the mortality of mice compared to vehicle-treated mice. The survival over 2 weeks in 5 mg/kg, 10 mg/kg, and caspofungin arms were similar and no death was reported in the three groups \(P < .001\). In contrast, the mortality in vehicle-administered group was 80% with a median survival of 6 days. A similar survival benefit was observed in AF5-treated mice. While the median survival in the vehicle-treated arm was 8 days, the 2-week survival in 1 mg/kg and 10 mg/kg arms was 80%-100%, comparable to that in the caspofungin arm \(P < .01\) (Fig. 1).

The median CFU/kg kidney tissue in 5 mg/kg arm of AF4 was \(3.3 \times 10^4\) equivalent to a 4-log reduction compared to the vehicle arm \((3.8 \times 10^6\) CFU/kg kidney, \(P < .0001\)). The in vivo efficacy was higher at a higher dose with the kidney homogenates of 10 mg/kg yielding sterile cultures comparable to that of CAS arm (Fig. 2). Similar organ fungal-burden reduction was noted in heart and splenic tissues with a median cfu/kg tissue of \(3.5 \times 10^6\) to \(10^9\) mg/kg, while CAS arms yielded sterile cultures.

In AF5-treated groups, the median cfu/kg kidney tissue in 1 mg/kg arm was \(1.5 \times 10^4\), however, the heart and splenic tissue homogenates yielded less fungal burden with median (cfu/kg) cfu/kg as 6, while cf of \(6.7 \times 10^2\) and \(1.3 \times 10^3\), respectively (Fig. 2).

Conclusions: Both the antifungal compounds demonstrated a remarkable in vivo efficacy against C. albicans with a significant improvement in survival and a reduction in the organ-fungal burden.

Figure 1. Kaplan-Meier survival curves demonstrating the in vivo efficacy of AF4 and AF5 in a neutropenic murine model of disseminated C. albicans infection.

Figure 2. Assessment of tissue fungal-burden determined as \(\log_{10}\) CFU/gram of kidney, spleen, and heart after administration of AF4 and AF5 at two doses each of 5 mg/kg and 10 mg/kg body weight.