Fosmanogepix (APX001) is Effective in an Immunosuppressed Mouse Model of *Rhizopus oryzae* Infection

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ABSTRACT

Background: Mucormycosis is a life-threatening infection that predominantly occurs in immunocompromised hosts. The antifungal fosmanogepix (APX001) is a novel antifungal prodrug currently in Phase 2 clinical trials. The active moiety manogepix (MGX, APX001A) inhibits Gwt1, an enzyme required for the conserved glycosylphosphatidyl inositol (GPI) post-translational modification in eukaryotes. We previously reported the efficacy of fosmanogepix against *Rhizopus delemar* (MGX minimum effective concentration [MEC] = 0.25 µg/ml). Here we assessed the efficacy against *R. oryzae,* which has an elevated MEC value.

Materials/methods: The minimum effective concentration (MEC) of MGX, the active moiety of fosmanogepix, was determined against 10 clinical isolates of *R. oryzae* and *R. delemar* using CLSI M38 methodology. ICR mice were immunosuppressed with cyclophosphamide and cortisone acetate on days -2, +3 and +8, relative to intratracheal infection with 2.5×10^5 cells of *R. oryzae* 99-892. For survival studies, treatment with fosmanogepix (104, mg/kg, PO, a dose which achieves MGX exposures anticipated to provide efficacy in clinical trials)), was compared to isavuconazole (ISA, 110 mg/kg, TID, PO, a dose which achieves exposures in mice equivalent to exposures in human]). Oral treatment started 24 h post infection and continued through Day +7, relative to infection for survival studies and through Day +4 for tissue fungal burden studies (assessed by conidial equivalent [CE] using qPCR). Placebo mice received vehicle control. To extend the half-life of fosmanogepix, mice were administered 50 mg/kg of the cytochrome P450 inhibitor 1-aminobenzotriazole (ABT) 2 h prior to fosmanogepix administration.

Results: *R. oryzae* 99-892 MIC and MEC values were 0.125 µg/mL and 4.0 µg/mL for ISA and manogepix, respectively. Treatment with fosmanogepix (104 mg/kg QD) and ISA (110 mg/kg TID) for 7 days equally prolonged median survival time of mice (n=10) versus placebo (12 and 14 days for fosmanogepix and ISA, respectively vs. 8 days for placebo, *P*<0.05). Further, fosmanogepix and ISA treatment both resulted in 30% survival by day 21 when the experiment was terminated vs. 0% survival for placebo mice (*P*<0.05). Both drug treatments resulted in ~1.5 $\log_{10}$ reduction in lung, and brain CE vs. placebo-treated mice (n=10 mice/group, *P*<0.005 by Wilcoxon Rank Sum).

Conclusions: Despite a higher MEC value, fosmanogepix showed significant efficacy against *R. oryzae* that was as protective as ISA in immunosuppressed mice. Given the previously reported activity of fosmanogepix against a strain of *R. delemar* with a lower MEC value, fosmanogepix has now been shown to be efficacious against both species of *Rhizopus*, which together are responsible for ~60-70% of isolates causing lethal mucormycosis. Thus, continued investigation of fosmanogepix against mucormycosis is warranted.

INTRODUCTION/AIMS

- Mucormycosis is a life-threatening infection commonly caused by *Rhizopus* species (1).
- It occurs mostly in immunocompromised hosts such as neutropenic patients with mortality rates >50% and can approach 100% in prolonged neutropenia, brain involvement or disseminated disease (2).
- Fosmanogepix is a first-in-class small molecule antifungal that is currently in clinical development for the treatment of invasive fungal infections (3).
- Fosmanogepix is an N-phosphonooxymethyl prodrug which is rapidly and completely metabolized by systemic phosphatases to the active moiety, manogepix (4).
- Manogepix targets the highly conserved fungal enzyme Gwt1, which catalyzes an early step in GPI-anchor biosynthesis (5).
- Here, we assessed the *in vitro* and *in vivo* activity of manogepix and fosmanogepix, respectively against agents of mucormycosis.

REFERENCES

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METHODS

- ***In vitro* testing**. The *in vitro* susceptibility of manogepix against agents of mucormyosis was evaluated using the Clinical Laboratory and Standards Institute (CLSI) M38-A2 method. MEC values were determined as per the echinocandins.
- **Immunosuppression.** Male ICR mice were immuno-suppressed with cyclophosphamide (200 mg/kg) and cortisone acetate (500 mg/kg) on days -2, +3 and +8, relative to infection.
- **Infection.** Immunosuppressed mice were intratracheally infected with 2.5 x 10^5 spores of *R. oryzae 99-892*, a strain with an elevated MEC value to manogepix (MEC = 4 µg/ml).
- **Treatment.** Treatment with placebo (diluent control), fosmanogepix (104 mg/kg, PO), or ISA (110 mg/kg TID, PO), began 24 h post infection and continued for 7 days for survival and through Day +4 for tissue fungal burden studies. To extend the half-life of fosmanogepix, mice were administered 50 mg/kg of the cytochrome P450 inhibitor 1-aminobenzotriazole (ABT) 2 h prior to fosmanogepix administration (6).
- **Efficacy endpoints.** Survival of mice for 21 days and tissue fungal burden (assessed by qPCR) on Day +4 post infection.
- **Statistical Analysis.** The nonparametric log-rank test was used to determine differences in survival times. Differences in lung, and brain CE were compared by the nonparametric Wilcoxon rank-sum test. A *P* value < 0.05 was considered significant.

SUMMARY/CONCLUSIONS

- Manogepix has a potent *in vitro* activity against all tested *Rhizopus* clinical isolates.
- Fosmanogepix showed significant efficacy against a strain of *R. oryzae* with a high MEC that was as protective as ISA in prolonging survival of immunosuppressed mice at a dose in mice that is similar to the projected dose in man.
- Fosmanogepix also significantly reduced tissue fungal burden in target organs vs. placebo-treated mice to levels comparable to ISA treatment.
- Given the previously reported efficacy of fosmanogepix against a strain of *R. delemar* with a lower MEC value, fosmanogepix has now been shown to be efficacious against both species of *Rhizopus*, which together are responsible for ~60-70% of isolates causing lethal mucormycosis.
- Continued investigation of fosmanogepix as a novel antifungal drug against mucormycosis is warranted.

ACKNOWLEDGEMENTS

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RESULTS

Figure 1. Chemical structures of fosmanogepix and manogepix

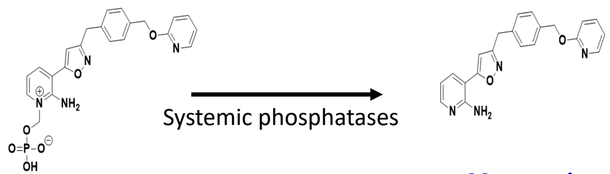
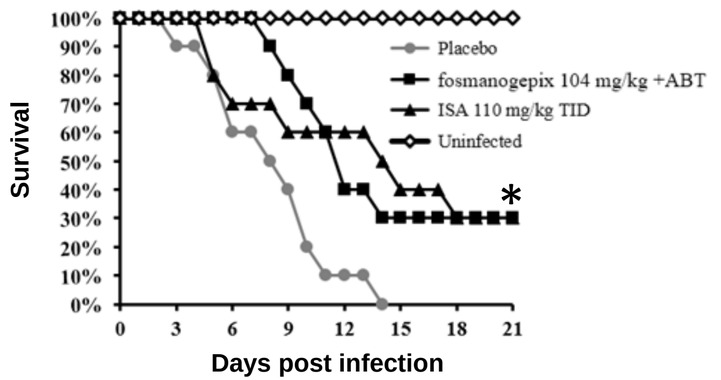


Table 1: Minimum effective concentration (MEC) of manogepix against the two *Rhizopus* species in µg/ml.

Strain	Manogepix	ISA
R. oryzae 99-892	4.0	0.125
R. oryzae 99-892	0.25	0.25

Figure 2. Fosmanogepix prolonged survival of immunosuppressed mice (n=10/group) infected with *R. oryzae* 99-892. * *P* <0.03.



Dose	*t*AUC
Fosmanogepix 104 mg/kg (ABT)	282

Treatment	Median survival time (days)	*P* values (vs. Placebo)
Placebo	8	
Fosmanogepix 104 mg/kg QD	12	*P* <0.05
ISA 110 mg/kg TID	14	*P* <0.05

Figure 3. Fosmanogepix treatment demonstrated statistically significant reduction in tissue CE burden vs. placebo-treated mice. The reduction in CE burden was and equally efficacious to ISA treatment.