Galactomannan is a Biomarker of Fosmanogepix (APX001) Efficacy in Treating Experimental Invasive Pulmonary Aspergillosis

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ABSTRACT

Background:. Invasive pulmonary aspergillosis (IPA) is a serious fungal infection afflicting immunocompromised patients. Galactomannan (GM) detection in biological samples using the Platelia ELISA has been shown to predict therapy response by azoles, and polyenes. We previously reported on the activity of fosmanogepix (APX001) in treating murine IPA. Here, we investigated the potential use of GM as a biomarker of fosmanogepix efficacy in an immunosuppressed murine model of IPA.

Materials/methods: ICR mice (n=8-16/group) were immunosuppressed with cyclophosphamide and cortisone acetate on days -2, and +3, relative to infection with *Aspergillus fumigatus* via inhalation. Treatment with placebo (diluent control), fosmanogepix (78 or 104 mg/kg, PO, doses which achieve exposures anticipated to provide efficacy in clinical trials), or posaconazole (POSA, 20 mg/kg, QD or 30 mg/kg, BID [a dose which achieves exposures in mice equivalent to 6x the human exposure achieved by POSA]) began 16 h post-infection and continued daily. 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. Mice were sacrificed 48, 72, or 96 h post-infection and their lungs, bronchoalveolar lavage (BAL) and sera were collected. Lung fungal burden was determined by conidial equivalent (CE) using qPCR, while GM was determined using the Platelia ELISA .

Results Compared to placebo, fosmanogepix or POSA treatment resulted in gradual decrease in tissue fungal burden over time with fosmanogepix or POSA showing significant reduction as early as 96 h and 48 h, respectively (P <0.005). Although the *supra* therapeutic dose of POSA resulted in faster reduction in lung fungal burden after 48 h, both drugs resulted in similar reduction (~6-7 log) in lung CE vs. placebo after 96 h. Changes in GM levels in BAL or serum samples mirrored reductions in lung CE with significant decrease seen after 96 h or 72 h for fosmanogepix or POSA, respectively vs. placebo (P <0.02).

Conclusions: Exposures of manogepix anticipated to achieve efficacy in humans and *supra* humanized exposures of POSA both resulted in time-dependent reduction of lung fungal burden and GM levels when compared to placebo. These results show that GM can be used as a biomarker of fosmanogepix efficacy in immunosuppressed mice.

INTRODUCTION/AIMS

- IPA due to *A. fumigatus* is a serious fungal infection in immunosuppressed patients.
- Despite current antifungal therapy, mortality rates remain high, thus new treatments are needed (1).
- Fosmanogepix (APX001) is a first-in-class small molecule antifungal that is currently in clinical development for the treatment of invasive fungal infections (2).
- Fosmanogepix is an N-phosphonooxymethyl prodrug which is rapidly and completely metabolized by systemic alkaline phosphatases to the active moiety, manogepix (MGX, APX001A) (3).
- Manogepix targets the highly conserved fungal enzyme Gwt1, which catalyzes an early step in GPI-anchor biosynthesis (4).
- Galactomannan (GM) detection in biological samples using the Platelia ELISA has been shown to predict therapy response by azoles, and polyenes (5).
- Here, we investigated the potential use of GM as a biomarker of fosmanogepix efficacy in an immunosuppressed murine model of IPA.

METHODS

- **Immunosuppression.** Male ICR mice were immuno-suppressed with cyclophosphamide (200 mg/kg) and cortisone acetate (500 mg/kg) on days -2, and +3, relative to infection.
- **Infection.** Immunosuppressed mice were infected with *A. fumigatus* a strain susceptible to MGX in an inhalation chamber by aerosolizing 12 ml of a 1×10^9 ml suspension of conidia with a small particle nebulizer driven by compressed air (6).
- **Treatment.** Treatment with placebo (diluent control), fosmanogepix (78 mg/kg or 104 mg/kg, PO, clinically relevant doses), or posaconazole (POSA, 20 mg/kg, QD or 30 mg/kg, BID [equivalent to 6x the humanized dose]) began 16 h post-infection and continued daily. To extend the half-life of MGX, mice were administered 50 mg/kg of the cytochrome P450 inhibitor 1-aminobenzotriazole (ABT) 2 h prior to fosmanogepix administration (7).
- **Efficacy endpoints.** Mice were sacrificed 48, 72, or 96 h post-infection and their lungs, BAL and sera were collected. Lung fungal burden was determined by conidial equivalent (CE) using qPCR, while GM was determined using the Platelia™ AspergillusEIA.
- **GM index.** The GM index was calculated as the OD value of the specimen divided by the mean OD of the wells containing cut-off control provided in the kit. Values of an index < 0.50 and >0.50 are considered negative and positive for GM, respectively.
- **Statistical Analysis.** Differences in fungal CE of lung and GM index were compared by the nonparametric Wilcoxon rank-sum test. A P value < 0.05 was considered significant.

SUMMARY/CONCLUSIONS

- Fosmanogepix or POSA treatment resulted in gradual decrease in fungal CE/g of lungs over time with fosmanogepix or POSA showing significant reduction as early as 96 h and 48 h, respectively compared to placebo.
- Although the *supra* therapeutic dose of POSA resulted in faster reduction in lung CE after 48 h, both drugs resulted in similar reduction (~6-7 log) vs. placebo after 96 h.
- Changes in GM levels in BAL or serum samples mirrored reductions in lung CE with significant decrease seen after 96 h or 72 h for fosmanogepix or POSA, respectively vs. placebo.
- 104 mg/kg fosmanogepix and a *supra* humanized dose of POSA resulted in a time-dependent reduction of lung CE and GM levels vs. placebo.
- These results show that GM can be used as a biomarker of fosmanogepix efficacy in immunosuppressed mice.

RESULTS

Figure 1. Chemical structures of fosmanogepix and manogepix

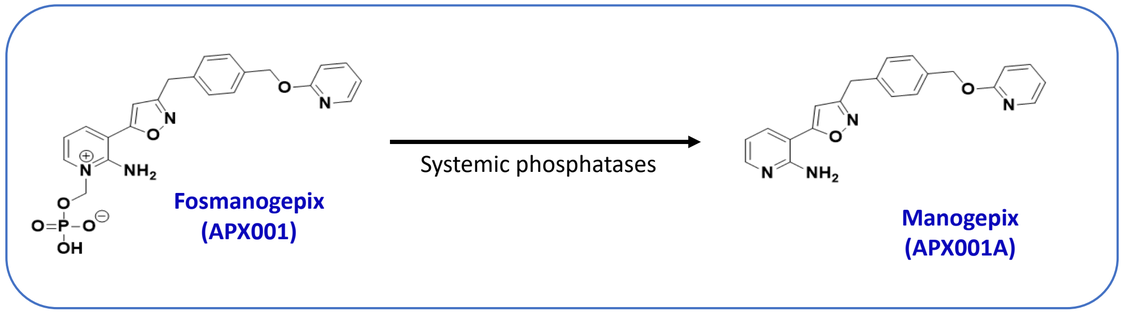
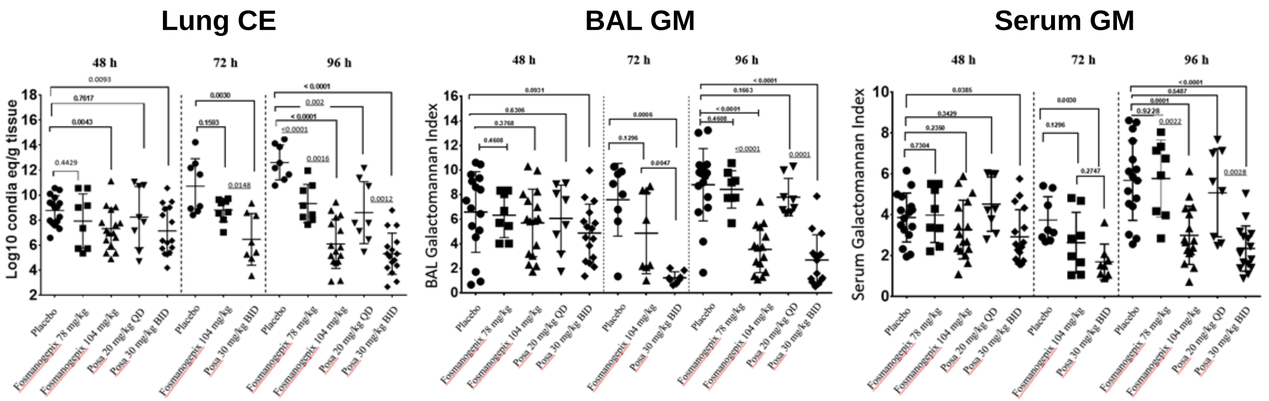


Figure 2. Effect of antifungal treatment on $\log_{10}$ CE/g lung tissue (A), BAL GM (B), and serum GM (C) levels. Mice (n= 8-16 mice/group) were infected with *A. fumigatus* (average inhaled inoculum of 5.1 x 10^3 conidia from 2 experiments). Data were presented as medians ± interquartile ranges) and evaluated using the nonparametric Wilcoxon rank sum test (Prism 5; GraphPad Software, Inc., San Diego, CA). The *y* axis 2.0 value in (A) represents the lower limit of detection of the assay.



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