

In Vitro and In Vivo Activity of Manogepix/ Fosmanogepix, a Novel Antifungal with Activity against Aspergillus and Rare Moulds

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Introduction

- The increasing global emergence of resistance to available classes of antifungal therapies has major clinical implications for the treatment of *Aspergillus* spp. and rare moulds
- Despite current antifungal therapy, mortality rates are high and new treatments are needed
- Fosmanogepix (FMGX, APX001), and its active moiety manogepix (MGX, APX001A), is a novel, first-in-class antifungal agent, with broad spectrum of activity, including *Aspergillus* and rare moulds
- Fosmanogepix is an N-phosphonoxyethyl prodrug which is rapidly and completely metabolized by systemic alkaline phosphatases to the active moiety, manogepix
- Manogepix targets the highly conserved fungal enzyme Gwt1, which catalyzes an early step in GPI-anchor biosynthesis
- The *in vitro* activity against a collection of 570 recent (2017–2018), geographically diverse mould isolates, PK/PD studies and *in vivo* efficacy were evaluated to support an ongoing Phase 2 clinical trial in invasive mould infections

Materials and Methods

- The *in vitro* activity of manogepix and comparator agents was determined against 570 recent (2017-2018) mould isolates collected worldwide in the SENTRY Surveillance Program and included *Aspergillus* spp., *Fusarium* spp., *Gibberella fujikuroi* species complex, *Scedosporium* spp. and other rare moulds
 - Isolate identifications were confirmed using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) and molecular methods (as necessary)
 - Broth microdilution antifungal susceptibility testing of manogepix and comparator agents was conducted according to CLSI guidelines (CLSI M38 [2017] and M61 [2017])
 - Minimum effective concentration (MEC) values were evaluated for manogepix and MEC₅₀ and MEC₉₀ values were determined. For species where < 10 isolates were available, MEC₅₀ values were determined
 - Quality control was performed as recommended in CLSI documents M60 (2017) and M61 (2017)
 - Recently published (CLSI M59 [2018]) epidemiologic cutoff values (ECVs) were applied to *Aspergillus* spp. (as available)
- The *in vivo* efficacy of fosmanogepix was evaluated in a highly immunocompromised mouse model of invasive fungal infection (IFI)
 - Male ICR mice were immunosuppressed with cyclophosphamide (200 mg/kg) and cortisone acetate (500 mg/kg) on days -2 and +3, relative to infection
 - Immunosuppressed mice were infected with *Aspergillus fumigatus*, a strain susceptible to MGX, in an inhalation chamber by aerosolizing 12 mL of a 1x10⁸ mL suspension of conidia with a small particle nebulizer driven by compressed air

Table 1 In vitro activity of manogepix and comparator agents against recent (2017–2018) mould isolates collected worldwide

Organism (no. tested) antimicrobial agent	MEC ₅₀ /MIC ₅₀	MEC ₉₀ /MIC ₉₀	Range	ECV ^a	
				%WT ^b	%NWT ^c
Aspergillus spp. (10) (voriconazole NWT)					
Manogepix	0.015	0.015	0.008 to 0.015		
Anidulafungin	0.008	0.015	≤0.002 to 0.03		
Caspofungin	0.015	0.03	0.004 to 0.03	100.0	0.0
Micafungin	≤0.008	0.015	≤0.008 to 0.015		
Itraconazole	4	8	2 to >8	0.0	100.0
Posaconazole	1	1	0.5 to 4		
Voriconazole	2	4	2 to >8	0.0	100.0
Amphotericin B	1	2	1 to 2	100.0	0.0
Aspergillus fumigatus (350)					
Manogepix	0.015	0.03	≤0.008 to 0.06		
Anidulafungin	0.015	0.03	≤0.008 to 0.03		
Caspofungin	0.03	0.03	≤0.008 to 0.06	100.0	0.0
Micafungin	≤0.008	0.015	≤0.008 to 0.03		
Itraconazole	0.5	1	0.25 to >8	94.9	5.1
Posaconazole	0.25	0.5	0.06 to 4		
Voriconazole	0.5	0.5	0.06 to >8	97.4	2.6
Amphotericin B	1	2	0.25 to 2	100.0	0.0
Aspergillus section Flavi (55)^a					
Manogepix	0.015	0.03	≤0.008 to 0.06		
Anidulafungin	≤0.008	0.015	≤0.008 to 0.03		
Caspofungin	0.015	0.03	≤0.008 to 0.03	100.0	0.0
Micafungin	0.015	0.03	≤0.008 to 0.03		
Itraconazole	0.5	1	0.25 to 2	98.1	1.9
Posaconazole	0.5	0.5	0.12 to 1	98.2	1.8
Voriconazole	0.5	1	0.25 to 2	100.0	0.0
Amphotericin B	2	2	1 to 2	94.0	5.8
Aspergillus section Nigri (51)^a					
Manogepix	≤0.008	0.015	≤0.008 to 0.03		
Anidulafungin	≤0.008	0.015	≤0.008 to 0.03		
Caspofungin	0.015	0.03	≤0.008 to 0.06	100.0	0.0
Micafungin	≤0.008	0.015	≤0.008 to 0.015		
Itraconazole	2	4	0.5 to 8	94.0	6.0
Posaconazole	0.5	1	0.25 to 1	100.0	0.0
Voriconazole	1	2	0.12 to 4	98.0	2.0
Amphotericin B	0.5	1	0.25 to 2	100.0	0.0
Aspergillus section Terrei (20)^a					
Manogepix	0.015	0.03	0.004 to 0.03		
Anidulafungin	0.015	0.03	0.004 to 0.06		
Caspofungin	0.015	0.06	0.004 to 0.06	100.0	0.0
Micafungin	≤0.008	0.015	≤0.008 to 0.015		
Itraconazole	0.5	0.5	0.25 to 1	100.0	0.0
Posaconazole	0.25	0.25	0.12 to 0.5	100.0	0.0
Voriconazole	0.5	0.5	0.12 to 1	100.0	0.0
Amphotericin B	2	2	1 to 4	100.0	0.0
Other Aspergillus spp. (14)^a					
Manogepix	0.015	0.03	≤0.008 to 0.25		
Anidulafungin	0.03	0.12	≤0.008 to 0.5		
Caspofungin	0.015	2	0.015 to 2		
Micafungin	0.015	0.06	≤0.008 to 0.12		

- 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. Treatment with placebo (diluent control), fosmanogepix (78 mg/kg or 104 mg/kg, PO, doses which give rise to exposures in mice that are similar to exposures achieved clinically), or posaconazole (POSA, 20 mg/kg, QD or 30 mg/kg, BID [equivalent to 6x the humanized dose]) began 16 h postinfection and continued daily
- Mice were sacrificed 48, 72, or 96 h postinfection 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
- Pharmacokinetic/Pharmacodynamic (PK/PD) target determinations were evaluated:
 - Six *A. fumigatus* isolates were chosen, including three isolates with Cyp51 mutations and one laboratory isolate with an Fks1 mutation
 - Dose-response experiments were performed with the six *A. fumigatus* isolates in the invasive pulmonary aspergillosis model
 - Six dose levels (consisting of 5, 10, 24, 64, 96, and 192 mg/kg/3 h) were administered by the oral route with the duration of treatment of 96 h. ABT was not used in this model since fosmanogepix was administered every 3 h
 - The PK/PD relationships were examined utilizing the plasma free drug concentrations from pharmacokinetic studies
 - Treatment results and associated PK/PD indices AUC/MIC, C_{max}/MIC, and T>MIC were modeled to Hill equation and compared by nonlinear regression PK/PD target studies
 - Correlation between efficacy and AUC/MIC was analyzed by nonlinear regression (Hill equation)
 - Static and ED₅₀ targets were determined

Results

- In vitro evaluation:**
 - Manogepix demonstrated potent *in vitro* activity (MEC_{20/90}^a ≤0.008-0.015/0.015-0.06 mg/L) against all *Aspergillus* spp. isolates tested including azole-nonsusceptible and infrequently encountered *Aspergillus* spp. isolates (Table 1)
 - A total of 2.6% (9/350) of *Aspergillus fumigatus* isolates and 2.0% (1/51) of *Aspergillus* section *Nigri* isolates were non-susceptible to voriconazole
 - Manogepix demonstrated notable activity against *Fusarium* spp. (MEC₅₀, 0.03 mg/L, n=9), *Gibberella fujikuroi* species complex (MEC₅₀, ≤0.008 mg/L, n=6) and *Scedosporium* spp. (MEC_{50/90}^a 0.03/0.06 mg/L, n=24) where treatment options are limited (Table 1)
 - Rare mould isolates including *Exophiala* spp., *Microascus cirrosus*, *Paecilomyces* spp., *Rasamsonia argillacea* species complex, *Scopulariopsis brevicaulis*/S. *brumptii* and *Tricoderma* spp. were inhibited by ≤0.06 mg/L of manogepix (Table 1)

In vivo evaluation:

- In the immunocompromised mouse model of invasive pulmonary aspergillosis, mice treated with fosmanogepix 78 mg/kg once daily (QD), 78 mg/kg twice daily, or 104 mg/kg QD with the addition of ABT significantly enhanced the median survival time and prolonged Day 21 post-infection overall survival compared to the placebo
- In addition, administration of fosmanogepix resulted in a significant reduction in lung fungal burden (4.2 to 7.6 log₁₀ conidial equivalents/g of tissue) versus the untreated control and resolved the infection, as judged by histopathological examination
- The observed survival and tissue clearance were comparable to a clinically relevant posaconazole dose

PK/PD evaluation:

- Evaluation of fosmanogepix PK/PD using 6 strains of *Aspergillus fumigatus* demonstrated that net stasis was achieved against all strains, including those that harbor Cyp51 mutations conferring triazole resistance, and 1-log₁₀ reduction in conidial equivalents was achieved for 5 of 6 strains
- AUC/MEC was the best PK/PD index predictive of efficacy based on dose-fractionation analysis, similar to what was previously observed for *Candida* spp.
- Median 24 h free drug AUC/MEC targets for stasis and 1-log₁₀ kill were 48 and 89, respectively

Conclusions

- Manogepix demonstrated potent *in vitro* activity against *Aspergillus* spp., *Fusarium* spp., *Gibberella fujikuroi* species complex, *Scedosporium* spp. and rare mould isolates
- Fosmanogepix demonstrated potent *in vivo* efficacy in a highly immunocompromised mouse model of invasive aspergillosis
- The PK/PD evaluation demonstrated that fosmanogepix has concentration-dependent *in vivo* efficacy against wild-type, azole-resistant, and echinocandin-resistant *A. fumigatus*
- The AUC/MEC ratios were highly associated with *in vivo* activity
- The PK/PD target exposures derived should be useful for designing optimized drug dosing regimens for continued clinical development of this promising antifungal
- The current data supports the continued clinical evaluation of the novel antifungal agent, fosmanogepix, in the treatment of invasive aspergillosis and rare mould infections

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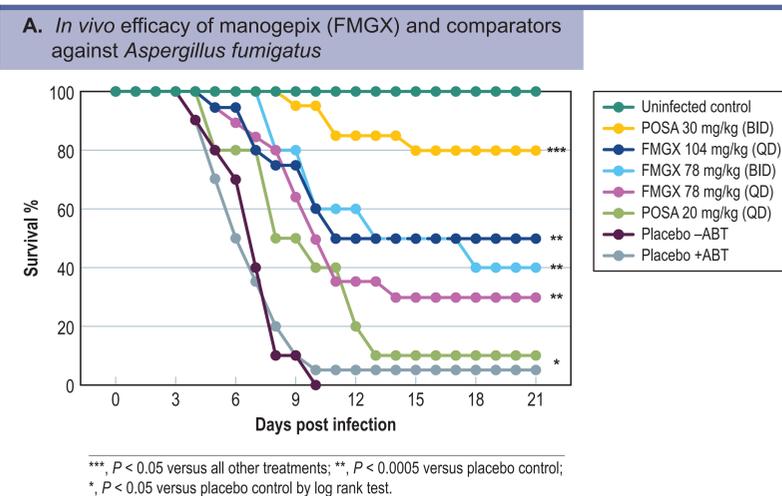
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Figure 1 Fosmanogepix (FMGX) protects mice from IPA



B. Reduction in A. fumigatus lung bioburden by manogepix and comparators

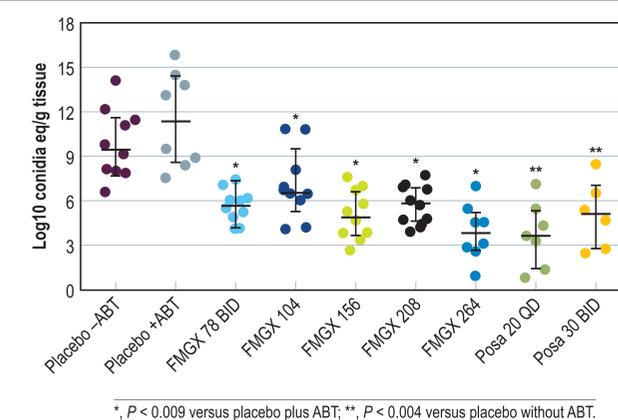


Figure 2 In vivo PK/PD of APX001 against Aspergillus spp.

