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-phosphonooxymethyl 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 MEC_{50} and MEC_{90} values were determined. For species where < 10 isolates were available, MEC_{50} 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 1×10^9 mL suspension of conidia with a small particle nebulizer driven by compressed air
  - 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 ED_{50} targets were determined

Results

***In vitro* evaluation:**

- Manogepix demonstrated potent *in vitro* activity ($MEC_{50/90}$, ≤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. (MEC_{50}, 0.03 mg/L, n=9), *Gibberella fujikuroi* species complex (MEC_{50}, ≤0.008 mg/L, n=6) and *Scedosporium* spp. ($MEC_{50/90}$, 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)

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]	ECV[a] %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)[d]**					
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)[e]**					
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)[f]**					
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)[g]					
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		
Itraconazole	1	8	0.25 to >8		
Posaconazole	0.5	4	0.12 to >8		
Voriconazole	1	8	0.12 to 8		
Amphotericin B	1	2	0.25 to 2		
***Fusarium* spp. (9)[h]**					
Manogepix	0.03	—	0.015 to 8		
Anidulafungin	>4	—	>4		
Caspofungin	>4	—	>4		
Micafungin	>4	—	>4		
Itraconazole	>8	—	2 to >8		
Posaconazole	>8	—	1 to >8		
Voriconazole	4	—	2 to >8		
Amphotericin B	2	—	1 to 2		
***Gibberella fujikuroi* species complex (6)**					
Manogepix	≤0.008	—	≤0.008 to 0.03		
Anidulafungin	>4	—	>4		
Caspofungin	>4	—	>4		
Micafungin	>4	—	>4		
Itraconazole	>8	—	1 to >8		
Posaconazole	4	—	1 to >8		
Voriconazole	8	—	2 to >8		
Amphotericin B	2	—	2 to >2		
***Scedosporium* spp. (24)[i]**					
Manogepix	0.03	0.06	0.004 to 0.06		
Anidulafungin	4	>4	0.5 to >4		
Caspofungin	>4	>4	0.06 to >4		
Micafungin	0.5	>4	0.12 to >4		
Itraconazole	8	>8	2 to >8		
Posaconazole	2	>8	1 to >8		
Voriconazole	1	8	0.25 to >8		
Amphotericin B	>2	>2	0.5 to >2		
Uncommon Mould spp. (13)[j]					
Manogepix	≤0.008	0.06	≤0.008 to 0.06		
Anidulafungin	0.25	>4	≤0.008 to >4		
Caspofungin	0.25	>4	0.008 to >4		
Micafungin	0.12	>4	0.008 to >4		
Itraconazole	0.5	>8	0.25 to >8		
Posaconazole	0.25	>8	0.12 to >8		
Voriconazole	2	>8	0.03 to >8		
Amphotericin B	1	>2	0.25 to >2		

[a] Criteria published by CLSI M38 (2017). ECV criteria published in CLSI M59 (2018).
[b] %WT = percent wild-type
[c] %NWT = percent non-wild-type
[d] Contains *A. flavus* spices complex (52), *A. nomius* (1), *A. parasiticus* (1) and *A. tamarii* (1).
[e] Contains *A. niger* (38), *A. niger* species complex (11) and *A. tubingensis* (2).
[f] Contains *A. terreus* (13) and *A. terreus* species complex (7).
[g] Contains *A. lentulus* (4), *A. nidulans* species complex (2), *A. sclerotiorum* (1), *A. thermomutatus* (1), *A. ustus* (2), *A. ustus* species complex (1) and *A. versicolor* (3).
[h] Contains *Fusarium incarnatum-equiseti* species complex (3), *F. oxysporum* species complex (2) and *F. solani* species complex (4).
[i] Contains *S. apiospermum* (2), *S. apiospermum-boydii* species complex (13), *S. aurantiacum* (2), *S. boydii* (3), *S. dehoogii* (2) and *S. prolificans* (2).
[j] Contains *Exophiala attenuata* (1), *E. dermatitidis* (3), *Microascus cirrosus* (1), *Paecilomyces lilacinus* (2), *P. variotii* (1), *Rasamsonia argillacea* species complex (1), *Scopulariopsis brevicaulis*/*S. brumptii* (1), unspeciated *Paecilomyces* (2) and unspeciated *Tricoderma* (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 log_{10} 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-log_{10} 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-log_{10} 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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References

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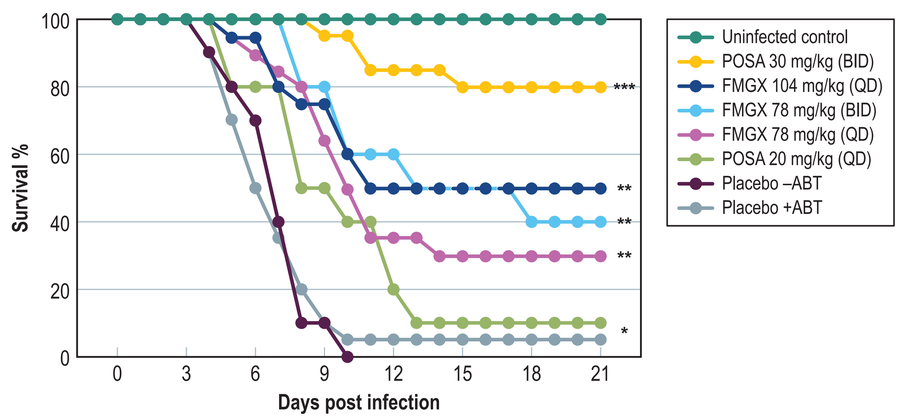
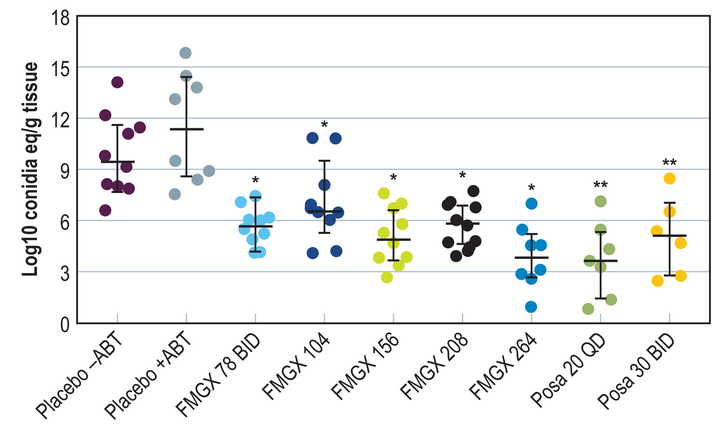
Figure 1 Fosmanogepix (FMGX) protects mice from IPA

A. *In vivo* efficacy of manogepix (FMGX) and comparators against *Aspergillus fumigatus*



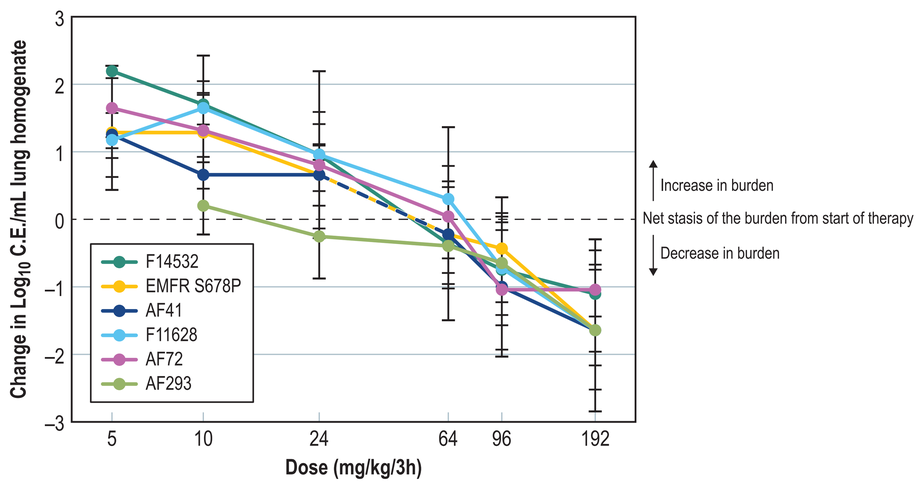
***, $P < 0.05$ versus all other treatments; **, $P < 0.0005$ versus placebo control; *, $P < 0.05$ versus placebo control by log rank test.

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



*, $P < 0.009$ versus placebo plus ABT; **, $P < 0.004$ versus placebo without ABT.

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



Dose-response curves for each isolate. Each symbol represents the mean and standard deviation for four mice. Six total drug dose levels of APX001 were given orally.
• Stasis exposure for these *Aspergillus* strains ~tAUC 200 ug.hr/mL (mean+SD)
• 1–2 log killing observed at higher doses/exposures