**9th Advances Against Aspergillosis and Mucormycosis** | Poster #27

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

Huband MD<sup>1</sup>, Ibrahim AS<sup>2</sup>, Gebremariam T<sup>2</sup>, Andes DR<sup>3</sup>, Bien PA<sup>4</sup>, Shaw KJ<sup>5</sup>, Hodges MR<sup>4</sup>

<sup>1</sup>JMI Laboratories, North Liberty, Iowa; <sup>2</sup>The Lundquist Institute at Harbor-UCLA Medical Center, Torrance, CA; <sup>3</sup>University of Wisconsin, Madison, WI; <sup>4</sup>Amplyx Pharmaceuticals, San Diego, CA; <sup>5</sup>Hearts Consulting Group, San Diego, CA.

### 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
- 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<sup>™</sup> AspergillusEIA
- Pharmacokinetic/Pharmacodynamic (PK/PD) target determinations were evaluated:

#### 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<sub>10</sub> 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:**

Conclusions

- 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<sub>10</sub> 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<sub>10</sub> kill were 48 and 89, respectively

## Acknowledgements

This study was supported by Amplyx Pharmaceuticals and performed by JMI Laboratories, the Lundquist Institute at Harbor-UCLA Medical Center, and the University of Wisconsin.

### References

CLSI. M38. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi: 3<sup>rd</sup> Edition. Wayne, PA, Clinical and Laboratory Standards Institute, 2017.

CLSI. M59. Epidemiological Cutoff Values for Antifungal Susceptibility Testing: 2<sup>nd</sup> Edition. Wayne, PA, Clinical and Laboratory Standards Institute, 2018.

CLSI. M60. Performance Standards for Antifungal Susceptibility Testing of Yeasts: 1<sup>st</sup> Edition. Wayne, PA, Clinical and Laboratory Standards Institute, 2017.

CLSI. M61. Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi: 1<sup>st</sup> Edition. Wayne, PA, Clinical and Laboratory Standards Institute, 2017.

### Contact

- 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)

- 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<sub>max</sub>/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<sub>50</sub> targets were determined

### Results

#### In vitro evaluation:

- Manogepix demonstrated potent *in vitro* activity (MEC<sub>50/90</sub>, ≤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 nonsusceptible to voriconazole
- Manogepix demonstrated notable activity against *Fusarium* spp. (MEC<sub>50</sub>, 0.03 mg/L, n=9), *Gibberella fujikuroi* species complex (MEC<sub>50</sub>,  $\leq$ 0.008 mg/L, n=6) and Scedosporium spp. (MEC<sub>50/90</sub>, 0.03/0.06 mg/L, n=24) where treatment options are limited (Table 1)

complex, Scedosporium spp. and rare mould isolates

Manogepix demonstrated potent in vitro activity against

 Fosmanogepix demonstrated potent in vivo efficacy in a highly immunocompromised mouse model of invasive aspergillosis

Aspergillus spp., Fusarium spp., Gibberella fujikuroi species

- The PK/PD evaluation demonstrated that fosmanogepix has concentration-dependent *in vivo* efficacy against wild-type, azoleresistant, 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

Michael R. Hodges, MD Chief Medical Officer Amplyx Pharmaceuticals 12730 High Bluff Drive, Suite 160 San Diego, CA 92130 Tel: 858.345.1755 Email: mhodges@amplyx.com

#### Figure 1 Fosmanogepix (FMGX) protects mice from IPA

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



- 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<sup>9</sup> mL suspension of conidia with a small particle nebulizer driven by compressed air
- 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				<b>ECV</b> <sup>a</sup>	
(no. tested) antimicrobial agent	MEC <sub>50</sub> / MIC <sub>50</sub>	MEC <sub>90</sub> / MIC <sub>90</sub>	Range	%WT <sup>b</sup>	%NWT°
Aspergillus spp.	( <b>10) (vor</b> i	iconazole	e 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 fumig	atus (35	0)			
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 secti	on <i>Flavi</i> (	55) <sup>d</sup>			
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 secti	on <i>Nigri</i> (				
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	20010	
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 secti	on <i>Terrei</i>	(20) <sup>f</sup>	0120 00 2	10010	
Manogenix	0.015	0.03	0.004 to 0.03		
Anidulafungin	0.015	0.03	0.004  to  0.06		
Casnofungin	0.015	0.06	0.004 to 0.06	100.0	0.0
Micafundin	<0.013	0.015	< 0.009 to 0.000	100.0	0.0
Itraconazolo	_0.5	0.013	0.25 to 1	100.0	0.0
Posaconazolo	0.5	0.25	$0.12 \pm 0.05$	100.0	0.0
Voriconazolo	0.25	0.25	0.12 to 0.5	100.0	0.0
Amphotorioin P	0.5	0.5		100.0	0.0
Amphotencin B	2 0 0 0 0 1 1 1	<u>ک</u>	L (0 4	100.0	0.0
Monogoniy	<b>o o 1</b> 5				
	0.015	0.03			
Caspofungin	0.05	2	$\geq 0.000 \ 10 \ 0.3$		
Mioofuncin	0.015				
Inicalungin	CTO D	0.00	$\geq 0.000 (0.0.12)$		

organisin				ECV <sup>a</sup>	
(no. tested) antimicrobial	MEC <sub>50</sub> / MIC <sub>50</sub>	MEC <sub>90</sub> / MIC <sub>90</sub>	Range	%WT⁵	%NWT
agent					
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	) <sup>h</sup>	·			
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 fuiikur	oi specie	es comple	ex (6)		
Manogenix	<0.008		< 0.008  to  0.03		
Anidulafungin			<u>≤0.000 to 0.05</u>		
Caepofundin	>4		>4		
Micofundin	>4		>4		
Itracopazolo	~4		24 1 to > 9		
	>0		$\frac{1 \text{ to } > 0}{2}$		
Posaconazole	4		2 to > 0		
	8		2 to >8		
Ampnotericin B	2		2 to >2		
Scedosporium sp	<b>p.</b> (24)'	0.00			
Manogepix	0.03	0.06	0.004 to 0.06		
Anidulatungin	4	>4	0.5 to >4		
Caspotungin	>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 Moul	d spp. (1	<b>.3)</b> <sup>j</sup>			
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		
Criteria published by CLSI M %WT = percent wild-type %NWT = percent non-wild-ty Contains A. flavus spices co Contains A. niger (38), A. ni Contains A. terreus (13) and Contains A. terreus (13) and Contains A. lentulus (4), A. H A. ustus species complex (1) Contains Fusarium incarnatic ies complex (4). Contains S. apiospermum (2 S. dehoogii (2) and S. prolification Contains Exophiala attenuation	VI38 (2017). E ope omplex (52), A ger species co d A. terreus spe nidulans specie and A. versico tum-equiseti sp 2), S. apiosperr ans (2). ta (1), E. derma	CV criteria pub nomius (1), A omplex (11) an ecies complex es complex (2) for (3). pecies complex num-boydii spe atitidis (3), Mic	olished in CLSI M59 (2018). . parasiticus (1) and A. tamarii d A. tubingensis (2). (7). ), A. sclerotiorum (1), A. thermo (3), F. oxysporum species cor ecies complex (13), S. aurantia roascus cirrosus (1), Paecilomy	(1). omutatus (1), mplex (2) and acum (2), S. b yces lilacinus	A. ustus (2), I F. solani spe oydii (3), (2), P. variotii

\*\*\*, P < 0.05 versus all other treatments; \*\*, P < 0.0005 versus placebo control;</li>
\*, P < 0.05 versus placebo control by log rank test.</li>

### **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