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EXPERIMENTAL THERAPEUTICS

Fosmanogepix (APX001) Is Effective in the Treatment of Pulmonary Murine Mucormycosis Due to *Rhizopus arrhizus*

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ABSTRACT Mucormycosis is a life-threatening infection with high mortality that occurs predominantly in immunocompromised patients. Manogepix (MGX) is a novel antifungal that targets Gwt1, a protein involved in an early step in the conserved glycosylphosphotidyl inositol (GPI) posttranslational modification pathway of surface proteins in eukaryotic cells. Inhibition of fungal inositol acylation by MGX results in pleiotropic effects, including inhibition of maturation of GPI-anchored proteins necessary for growth and virulence. MGX has been previously shown to have *in vitro* activity against some strains of Mucorales. Here, we assessed the *in vivo* activity of the prodrug fosmanogepix, currently in clinical development for the treatment of invasive fungal infections, against two *Rhizopus arrhizus* strains with high (4.0 μg/ml) and low (0.25 μg/ml) minimum effective concentration (MEC) values. In both invasive pulmonary infection models, treatment of mice with 78 mg/kg or 104 mg/kg fosmanogepix, along with 1-aminobenzotriazole to enhance the serum half-life of MGX in mice, significantly increased median survival time and prolonged overall survival by day 21 postinfection compared to placebo. In addition, administration of fosmanogepix resulted in a 1 to 2 log reduction in both lung and brain fungal burden. For the 104 mg/kg fosmanogepix dose, tissue clearance and survival were comparable to clinically relevant doses of isavuconazole (ISA), which is FDA approved for the treatment of mucormycosis. These results support continued development of fosmanogepix as a first-in-class treatment for invasive mucormycosis.

KEYWORDS APX001, APX001A, Gwt1, antifungal, *Rhizopus arrhizus*, infection model, mucormycosis, 1-aminobenzotriazole, manogepix, MGX, fosmanogepix, antifungal agents, infectious disease

Mucormycosis is an often-lethal infection that occurs predominantly in immunocompromised patients, including those suffering from diabetic ketoacidosis (DKA) or neutropenia. Due to the rising prevalence of diabetes, cancer, and organ transplantation in the aging population worldwide, the number of patients at risk for this deadly infection is on the rise (1–3). In addition, analysis of invasive mold infections from blast injuries in military personnel in Afghanistan showed that among 31 patients, Mucorales and *Aspergillus* spp. were the predominant organisms isolated (16 patients each), followed by *Fusarium* spp. (9 patients) (4). Unfortunately, despite disfiguring surgical debridement and adjunctive antifungal therapy, the overall mortality of mucormycosis remains >50% (5), and this rate approaches 100% in patients with disseminated disease (6). Thus, new strategies to prevent and treat mucormycosis are urgently needed.

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Glycosylphosphatidylinositol (GPI)-anchored proteins are known to have several functions, including mediating adhesion to and invasion of host tissues by microorganisms (7, 8). Therefore, they play a pivotal role in pathogenesis of infectious diseases. Fosmanogepix (formerly APX001 and E1210) is the prodrug of manogepix (MGX, formerly APX001A and E1210), a broad-spectrum investigational antifungal agent that inhibits inositol acyltransferase, thereby preventing GPI-anchored protein maturation (9). This inhibition results in pleiotropic effects on fungal growth and virulence.

Previous studies have shown that MGX has broad *in vitro* activity against *Candida* spp., *Aspergillus* spp., and some rare molds (9–11). The MGX MEC values against limited numbers of Mucorales strains were more moderate and variable, with reported ranges of 0.12 to 16 μg/ml against *Rhizopus arrhizus*; 2 to 16 μg/ml against *R. microsporus*; 1 to 16 μg/ml against *Rhizomucor pusillus*; and 0.5 to 8 μg/ml against *Mucor circinelloides* using CLSI methodology (9, 12). These MEC values are similar to the ranges of MIC values seen for isavuconazole (ISA) in a study of worldwide isolates from 2015 to 2016 conducted by two reference laboratories (13). In that study, a wide range in MIC values was observed as follows: 0.25 to 4 μg/ml against *R. arrhizus*; 0.5 to 32 μg/ml against *R. microsporus*; 0.5 to 8 μg/ml against *Rhizomucor pusillus*; and 2 to 32 μg/ml against *M. circinelloides* (13).

The correlation between MIC or MEC values and outcome in clinical studies has not been well established, especially for values $>$16 μg/ml (14). However, correlations in MIC (*Candida*) or MEC (*Aspergillus*) values have generally been observed in pharmacokinetic/pharmacodynamic (PK/PD) mouse studies that evaluated isolates with MGX MIC or MEC values below 0.125 μg/ml (15). Thus, we sought to assess the activity of fosmanogepix in neutropenic murine invasive pulmonary mucormycosis (16) models using two strains of *Rhizopus*, the most common cause of mucormycosis, in which the MEC values of MGX were low (0.25 μg/ml for *R. arrhizus* var. *delemar*) and high (4.0 μg/ml for *R. arrhizus* var. *arrhizus*).

We utilized fosmanogepix treatment regimens in mice that resulted in MGX exposures (the area under the plasma drug concentration-time curve [AUC]) similar to what was observed in phase 1 clinical studies (15, 17, 18). This was accomplished by preadministration of 50 mg/kg 1-aminobenzotriazole (ABT), a nonselective suicide inhibitor of cytochrome P450 (CYP) enzymes (19). We have previously shown that ABT administered 2 h prior to fosmanogepix enhanced MGX exposures 16- to 18-fold and enhanced serum half-life from $\sim$1 to 9 h, more closely mimicking human pharmacokinetic values (2 to 2.5 days) observed in phase 1 clinical studies in healthy volunteers (15, 17, 18). As a comparator in the efficacy studies, we orally administered the prodrug isavuconazonium sulfate (ISA) using a dosing regimen that resulted in isavuconazole exposures in mice similar to what has been observed in human clinical studies (20, 21). ISA was utilized as the comparator since it was approved by the FDA in 2015 for the treatment of invasive aspergillosis and invasive mucormycosis (CRESEMBA [isavuconazonium sulfate]). In addition, ISA was approved by the European Medicines Agency (EMA) for treating patients with mucormycosis who cannot be treated with amphotericin B. The efficacy endpoints in the mouse models were survival and reduction in fungal burden in target organs.

RESULTS

Antifungal susceptibility. The microbiological activities of MGX, posaconazole (POSA, another antifungal drug used to treat mucormycosis), and ISA were evaluated against 17 and 19 clinical isolates of *R. arrhizus* var. *arrhizus* and *R. arrhizus* var. *delemar*, respectively. MGX MEC interpretive criteria for molds were as described for the echinocandins and MIC values were determined for the comparators ISA and POSA (22). *R. arrhizus* var. *delemar* strains were more susceptible to MGX than *R. arrhizus* var. *arrhizus*, with MEC geometric means (GM) of 0.75 μg/ml and 3.84 μg/ml, respectively (Table 1). In contrast, *R. arrhizus* var. *arrhizus* isolates were more sensitive to ISA (GM = 0.85 μg/ml) than *R. arrhizus* var. *delemar* strains (GM = 2.5 μg/ml). Both sets of clinical isolates demonstrated similar susceptibility to POSA with GMs of 0.15 and 0.36 μg/ml for *R.*

TABLE 1 Antifungal susceptibility of clinical isolates of *R. arrhizus* var. *delemar* and *R. arrhizus* var. *arrhizus*[a]

Isolate	Assessment type	MEC or MIC in μg/ml		
		MGX	POSA	ISA
R. arrhizus var. *delemar* ($n = 19$)	Range	0.25–8.0	0.125–1.0	1.0–8.0
	MEC/MIC_{50}	0.5	0.25	2.0
	MEC/MIC_{90}	4.0	1.0	4.0
	GM MEC/MIC	0.75	0.36	2.5
R. arrhizus var. *arrhizus* ($n = 17$)	Range	0.25–8.0	0.06–0.5	0.25–2.0
	MEC/MIC_{50}	8.0	0.125	1.0
	MEC/MIC_{90}	8.0	0.25	1.0
	GM MEC/MIC	3.84	0.15	0.85

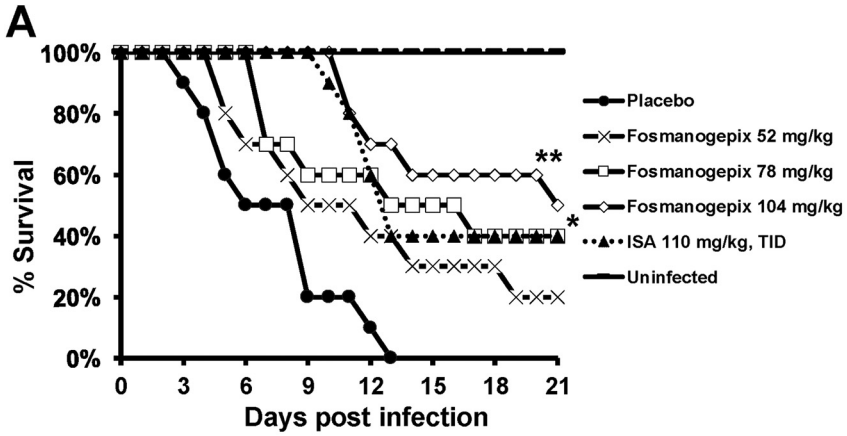
[a]Readings were taken as the minimum effective concentration (MEC) for MGX and MIC_{50} or MIC_{90} for POSA and ISA. MGX, manogepix; ISA, isavuconazole; POSA, posaconazole; GM, geometric mean.

arrhizus var. *arrhizus* and *R. arrhizus* var. *delemar*, respectively (Table 1). The MGX MEC values for the two clinical isolates used in the animal models were 0.25 μg/ml and 4 μg/ml for *R. arrhizus* var. *delemar* strain 99-880 and *R. arrhizus* var. *arrhizus* strain 99-892, respectively, representing a 16-fold difference between the two strains. For both isolates, the MIC values for POSA was 0.125 μg/ml, while the ISA MICs were 2.0 μg/ml for *R. arrhizus* var. *arrhizus* 99-892 and 1.0 μg/ml for *R. arrhizus* var. *delemar* 99-880. The selection of these two strains allowed examination of the efficacy of fosmanogepix against *Rhizopus* infections where the strains demonstrated high and low MEC values, compared to a control drug (ISA) where the two strains demonstrated similar MIC values (1 and 2 μg/ml, for isolates 99-880 and 99-892, respectively).

Fosmanogepix demonstrates efficacy in a highly immunosuppressed mouse model of pulmonary mucormycosis caused by *R. arrhizus* var. *delemar*. In order to assess fosmanogepix in our established model of neutropenic murine mucormycosis, ICR mice were immunosuppressed with cyclophosphamide (200 mg/kg) and cortisone acetate (500 mg/kg) on days −2, +3, and +8, relative to intratracheal infection with 2.5×10^5 cells of *R. arrhizus* var. *delemar* 99-880 on day 0 (17). Treatment with placebo (diluent control), fosmanogepix (52, 78, or 104 mg/kg, once daily orally [p.o.]), or ISA (110 mg/kg three times a day [TID], p.o.) began 16 h postinfection and continued for 7 days. To extend the half-life of MGX in mice, 50 mg/kg of the cytochrome P450 inhibitor 1-aminobenzotriazole (ABT) was administered 2 h prior to fosmanogepix administration, as previously described (23). Control mice did not receive ABT because ABT has no *in vitro* activity against *Rhizopus* strains and we previously demonstrated no difference in survival of mice infected with pulmonary invasive aspergillosis and treated with or without ABT (24). ISA was dosed at 110 mg/kg p.o. TID to achieve an exposure in mice that is equivalent to clinical exposures achieved in humans (20, 21). Similarly, ABT plus fosmanogepix (78 mg/kg and 104 mg/kg once daily) achieved exposures in mice that approximate exposures achieved in clinical doses in phase 1 trials (17, 18), whereas the 52 mg/kg dose achieved a lower exposure.

(i) Survival. Mice survival was assessed over 21 days ($n = 10$ mice/cohort). Doses of fosmanogepix in mice that achieved clinically relevant exposures (78 mg/kg and 104 mg/kg) demonstrated significantly improved survival compared to the placebo control ($P < 0.01$ by log rank test), whereas the low dose of 52 mg/kg fosmanogepix, although numerically better than placebo control, did not achieve significance ($P = 0.07$) (Fig. 1A). As we previously reported (20), a clinically relevant exposure of ISA in mice also improved survival compared to placebo ($P = 0.001$), but was not significantly different from any of the three fosmanogepix dosing groups ($P > 0.25$) (Fig. 1A). The two highest dosing groups of fosmanogepix resulted in 40% and 50% survival at day 21 postinoculation, respectively, similar to ISA (40% survival at this time point) (Fig. 1A). Furthermore, fosmanogepix demonstrated a dose-dependent prolongation in median survival time of mice (9, 13, and 21 days) versus placebo (6 days). The median survival time for ISA was 13 days (Fig. 1B).

A



B

	Placebo	Fosmanogepix + ABT QD			ISA TID
		52 mg/kg	78 mg/kg	104 mg/kg	110 mg/kg
Median Survival	6	9	13	21	13
Percent Survival	0%	20%	40%	50%	40%

FIG 1 Survival of immunosuppressed mice infected with *R. arrhizus* var. delemar 99-880 (low MEC). Mice ($n = 10$/group) were infected intratracheally with *R. arrhizus* var. *delemar* (inhaled inoculum of 7×10^3 spores/mouse) and 16 h later treated with ISA 110 mg/kg TID p.o., or with fosmanogepix QD p.o. for 7 days. ABT was administered 2 h prior to each fosmanogepix treatment to enhance the half-life of MGX in mice. *, $P = 0.001$ for 78 mg/kg fosmanogepix and 110 ISA; **, $P < 0.0001$ for 104 mg/kg fosmanogepix versus placebo mice by log rank test. (A) Kaplan-Meier survival curve. (B) Median and percent survival at day 21.

(ii) Tissue fungal burden. Mice were immunosuppressed and infected as described above; however, mice ($n = 10$/group) were sacrificed at day +4 after infection to determine conidial equivalents (CE)/g of lung and brain tissue by quantitative PCR (qPCR) using 18S primers. In the placebo group, fungal burdens (CE) at day +4 were $\log_{10}$ 4.74 $\pm$ 0.96 (lung) and $\log_{10}$ 3.48 $\pm$ 0.67 (brain) (Fig. 2). For the 78 mg/kg and 104 mg/kg fosmanogepix dosing groups, a 1.3 and 1.97 $\log_{10}$ reduction in CE/g of lung tissue was observed, respectively, which was similar to what was observed for ISA (1.79 $\log_{10}$ reduction in CE). Reductions in $\log_{10}$ CE/g of brain tissue were 0.93, 1.78, and 1.65 for 78 mg/kg fosmanogepix, 104 mg/kg fosmanogepix, and 110 mg/kg TID ISA, respectively. In all three treatment groups, the CE counts observed for tissue fungal burden in lung and brains were significantly lower than the placebo control group (Fig. 2). However, the 104 mg/kg fosmanogepix dosing regimen reduced tissue fungal burden to a greater degree than the 78 mg/kg fosmanogepix treatment ($P = 0.001$), and was equivalent to tissue burden reductions observed for the ISA treatment ($P > 0.13$).

Fosmanogepix demonstrates efficacy in a highly immunosuppressed mouse model of pulmonary mucormycosis caused by *R. arrhizus* var. *arrhizus*. The efficacy of fosmanogepix was assessed in the immunosuppressed murine pulmonary mucormycosis model using a strain of *R. arrhizus* var. *arrhizus* that had a 16-folder higher MEC value than the *R. arrhizus* var. *delemar* 99-880 strain. ICR mice were immunosuppressed and infected intratracheally as above with 2.5×10^5 spores of *R. arrhizus* var. *arrhizus* 99-892/mouse on day 0 (with confirmed lung-delivered inoculum of 1.1×10^4 spores). Due to the better performance of 104 mg/kg fosmanogepix versus the 78 mg/kg treatment group in the *R. arrhizus* var. *delemar* mucormycosis model, only the higher dosing regimen was evaluated versus ISA in this efficacy model.

(i) Survival. In the survival model, mice ($n = 10$ mice/cohort), were assessed for 21 days. Both treatments of 104 mg/kg fosmanogepix or ISA (110 mg/kg TID, PO) demonstrated

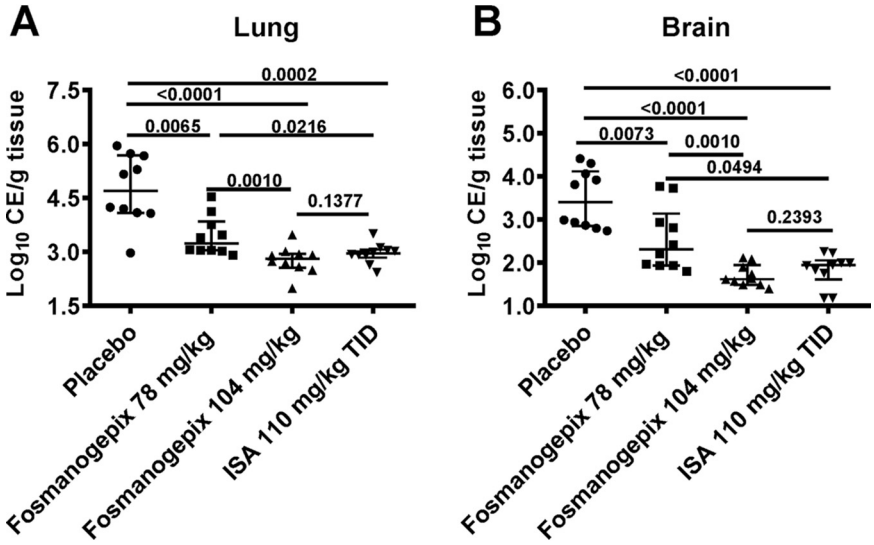


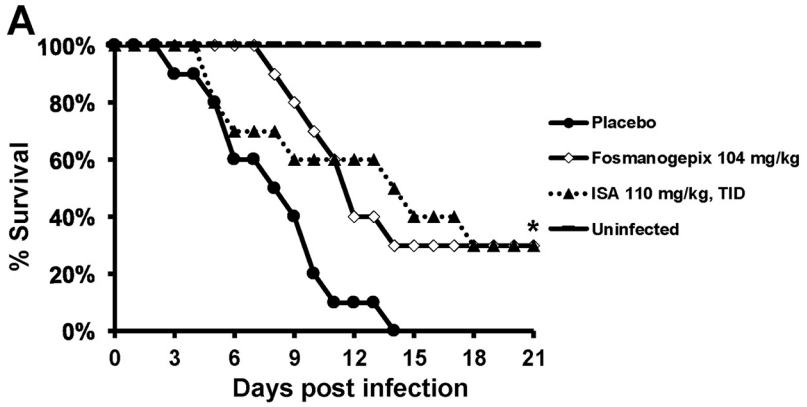
FIG 2 Reduction in tissue fungal burden of immunosuppressed mice infected with *R. arrhizus* var. *delemar* 99-880. Mice ($n = 10$/group) infected intratracheally with *R. arrhizus* var. *delemar* (inhaled inoculum of 1.1×10^4 spores/mouse) and 16 h later treated with ISA 110 mg/kg TID p.o., or with fosmanogepix 104 mg/kg QD p.o. On day +4, organs were collected and processed for tissue fungal burden by qPCR. Data are presented as the median ± interquartile range and the *y* axis represents the lower limit of detection. Intergroup *P* values are shown as a dark line. All dosing groups resulted in a statistically significant reduction in brain and lung fungal burden versus placebo control using the Wilcoxon rank sum test.

efficacy in this mucormycosis model with 30% overall survival compared to 0% survival for placebo-treated mice ($P < 0.05$ versus placebo control [Fig. 3]). Despite the difference in MEC/MIC values against this strain, fosmanogepix (4 μg/ml) and ISA (2 μg/ml) survival curves were not significantly different from each other ($P = 0.80$) (Fig. 3).

(ii) Tissue fungal burden. Mice were infected and treated as above but sacrificed on day +4 postinfection. Lung and brain tissue were harvested to determine conidial equivalents (CE)/g of lung and brain tissue by qPCR using 18S primers. Fungal burdens at day +4 for the placebo group were $\log_{10}$ 4.21 ± 1.0 (lung) and 3.18 ± 0.4 $\log_{10}$ (brain) (Fig. 4). Compared to placebo, both fosmanogepix and ISA treatment groups demonstrated significant reductions in lung ($P \leq 0.001$) and brain ($P < 0.0001$) CE. Treatment with 104 mg/kg fosmanogepix reduced lung and brain CE by $\log_{10}$ 1.15 (lung) and $\log_{10}$ 1.14 (brain), whereas ISA reduced CE by $\log_{10}$ 1.69 (lung) and $\log_{10}$ 1.14 (brain). ISA and fosmanogepix CE reductions were not significantly different ($P > 0.28$) for either brain or lung.

DISCUSSION

Although relatively rare, mucormycosis is difficult to treat and is frequently life-threatening, with all-cause mortality estimated to be 54% (25). These infections are most often seen in solid organ and stem cell transplant recipients, diabetics, neutropenic patients, or patients treated with corticosteroids. Guidelines for the treatment of mucormycoses recommend high dose liposomal amphotericin B, posaconazole, or isavuconazole as first line therapy, and these infections often require surgical debridement and immune recovery to improve the chances of a successful outcome (26). Isavuconazole (administered as the prodrug isavuconazonium sulfate, CRESEMBA) is approved for the treatment of adults with invasive mucormycosis. In a phase 3, open-label, noncomparative trial (VITAL) for isavuconazole, the most common organisms identified were *Rhizopus arrhizus* (*oryzae*) and Mucormycetes (27). Of 22 Mucorales isolated and evaluated during the VITAL trial, ISA MIC values ranged from 0.25 μg/ml for a single strain of *Actinomucor elegans* to 32 μg/ml for some strains of *M. circinelloides* and *Rhizopus* species (27). These values are similar to the range of ISA MIC values (0.25 to ≥16 μg/ml) observed for 292 Mucorales obtained from 2015 and 2016 at two



B

	Placebo	Fosmanogepix + ABT QD	ISA TID
		104 mg/kg	110 mg/kg
Median Survival	8	12	14
Percent Survival	0%	30%	30%

FIG 3 Survival of immunosuppressed mice infected with *R. arrhizus* var. *arrhizus* 99-892 (high MEC). Mice ($n = 10$/group) infected intratracheally with *R. arrhizus* var. *arrhizus* (inhaled inoculum of 1.1×10^4 spores/mouse) and 16 h later treated with ISA 110 mg/kg TID p.o., or with fosmanogepix 104 mg/kg QD p.o. for 7 days. ABT was administered 2 h prior to fosmanogepix treatment to enhance the half-life of manogepix in mice. *, $P = 0.01$ for 104 mg/kg fosmanogepix; $P = 0.02$ for ISA 110 mg/kg, TID versus placebo mice by log rank test. (A) Kaplan-Meier survival curve. (B) Median and percent survival at day 21.

reference laboratories (13). Although no correlations were observed between trough ISA plasma concentrations, MIC values, and key outcomes, the authors indicate that this is possibly due to the relatively small number of patients in the study. Similarly, Andes et al. evaluated the efficacy of ISA and voriconazole against *Aspergillus* spp. and found that although the drugs were clearly ineffective against strains where MICs were

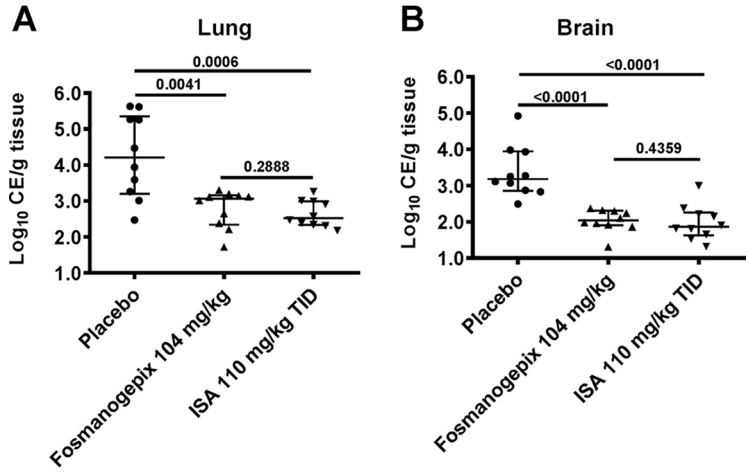


FIG 4 Reduction in tissue fungal burden of immunosuppressed mice infected with *R. arrhizus* var. *arrhizus* 99-892. Mice ($n = 10$/group) infected intratracheally with *R. arrhizus* var. *arrhizus* (inhaled inoculum of 6.4×10^3 spores/mouse) and 16 h later treated with ISA 110 mg/kg TID p.o., or with fosmanogepix 104 mg/kg QD p.o. On day +4, organs were collected and processed for tissue fungal burden by qPCR. Data are presented as the median ± interquartile range and the *y* axis represents the lower limit of detection. Intergroup *P* values are shown as a dark line. Both fosmanogepix and ISA resulted in a statistically significant reduction in brain and lung fungal burden versus placebo control using the Wilcoxon rank sum test.

≥16 μg/ml, there was no clear relationship with clinical outcomes in cases where the MIC was <16 μg/ml for either drug (14).

Fosmanogepix is an intravenously (i.v.) and orally (p.o.) available antifungal prodrug that is currently in clinical development for the treatment of life-threatening invasive fungal infections. The broad-spectrum activity of this first-in-class agent has been demonstrated *in vivo* using multiple mouse models of invasive pulmonary and disseminated fungal infections, including those of *Candida* spp., *Coccidioides* spp., *Cryptococcus* spp., *Aspergillus* spp., *Fusarium* spp., and *Scedosporium* spp. (15, 24, 28–31). These studies demonstrated increased survival, reduced fungal burden in lung, kidney, and brain tissues of infected mice, as well as histological improvement (15, 23, 30, 32, 33). Here, we extend these findings to *Rhizopus*, where we evaluated the efficacy against two different strains with MGX MEC values of 0.25 μg/ml and 4.0 μg/ml.

We previously evaluated the efficacy of ISA in murine models of pulmonary mucormycosis due to *R. arrhizus* var. *delemar* 99-880 or *M. circinelloides* and demonstrated improved mouse survival and clearance of fungal burden versus placebo control (20, 34). In this study, as a comparator in both experiments, we also utilized 110 mg/kg ISA (TID), a dose which gives rise to exposures in mice that are similar to exposures achieved clinically by ISA. MIC values of ISA against *R. arrhizus* var. *delemar* 99-880 and *R. arrhizus* var. *arrhizus* 99-892 were similar (1.0 and 2.0 μg/ml, respectively). Similar to our historical data (20, 34), in this study ISA significantly improved survival and reduced fungal burden in brain and lung by 1 to 2 log reductions versus the placebo control. Importantly, in the current study, similar results were seen for the 104 mg/kg fosmanogepix cohorts in both infection models. Total exposures for the 78 mg/kg and 104 mg/kg doses in mice (14) are ~200 and ~280 μg · h/ml, respectively (K. J. Shaw, unpublished data) and were previously shown to be associated with *A. fumigatus* stasis and 1 log kill in PK/PD studies (28). These exposures in mice are consistent with exposures achieved in fosmanogepix phase 1 single and multiple ascending-dose studies (17, 18) and anticipated in future fosmanogepix clinical trials. Thus, although the MGX MEC values of the two strains differed by 16-fold (0.25 versus 4.0 μg/ml), the efficacy of fosmanogepix at clinically relevant total exposures was equivalent to ISA, which is approved for the treatment of mucormycosis. Fosmanogepix has now been shown to be efficacious against both varieties of *Rhizopus arrhizus*, which together are the main cause of the disease and responsible for ~50% of cases of lethal mucormycosis (25).

In this study, we used a highly immunocompromised mouse model to show that fosmanogepix has equivalent efficacy to ISA, a current standard of care antifungal drug, in treating pulmonary mucormycosis. Findings from both oral and i.v. fosmanogepix phase 1 clinical studies have shown favorable PK, allowing once-daily dosing, with high bioavailability (~90%), and no food effect (17, 18). These data support further investigations into the development of this first-in-class agent for a broad range of difficult-to-treat invasive fungal infections.

MATERIALS AND METHODS

Antifungal agents. For *in vitro* studies, the active moiety manogepix (Amplyx Pharmaceuticals), ISA active compound, and POSA (both from Sigma-Aldrich Corp. St. Louis, MO, USA) were used. For efficacy studies, the water-soluble *N*-phosphonooxymethyl prodrug fosmanogepix (Amplyx Pharmaceuticals) was dissolved in a final prodrug solution of 5% dextrose. The water soluble isavuconazonium sulfate (Astellas Pharam US) was purchased from Bellavida Pharmacy, Torrance, CA. POSA (Merck & Co., Inc., Rahway, NJ) was purchased as an oral suspension (200 mg/5 ml) and kept at room temperature. All drugs were prepared fresh and orally (p.o.) dosed per gram mouse body weight on a daily basis. A 5 mg/ml solution of ABT (Fisher Scientific, Hampton, NH) in water was administered orally 2 h prior to administration of fosmanogepix at 10 μl per gram mouse body weight resulting in a dose of 50 mg/kg.

Microorganisms. *R. arrhizus* var. *delemar* 99-880 and *R. arrhizus* var. *arrhizus* 99-892 are brain and lung isolates obtained from the Fungus Testing Laboratory at the University of Texas Health Sciences Center at San Antonio (UTHSCSA). Other strains from *R. arrhizus* var. *delemar* and *R. arrhizus* var. *arrhizus* were also obtained from the culture collection of the Fungus Testing Laboratory, and species identification was confirmed as previously described (13). Strains were routinely grown on potato dextrose agar plates for 4 days until confluent at 37°C. Spores were collected by flooding the plates with sterile

phosphate-buffered saline (PBS) containing 0.01% (vol/vol) Tween 80. The spores were concentrated by centrifugation, washed in the same buffer, diluted, and counted using a hemocytometer.

***In vitro* testing.** The *in vitro* susceptibility to manogepix, ISA, and POSA by fungal agents of mucormycosis was evaluated using the Clinical Laboratory and Standards Institute (CLSI) M38 method using minimum effective concentration (MEC) endpoints, as per the echinocandins, and MIC endpoints for POSA and ISA (22).

Efficacy models. The pulmonary mucormycosis model has been previously described (16). Briefly, ICR mice (Envigo) were immunosuppressed with cyclophosphamide (200 mg/kg) and cortisone acetate (500 mg/kg) on days −2, +3, and +8 relative to infection. This regimen was shown to result in ~16 days of leucopenia (16). To prevent bacterial infection, 50 μg/ml enrofloxacin (Baytril; Bayer, Leverkusen, Germany) was added to the drinking water from day −3 to day 0. Ceftazidine (5 μg/dose/0.2 ml) replaced enrofloxacin treatment on day 0 and was administered daily by subcutaneous injection from day 0 until day +4 (tissue fungal burden) or day +13 (survival). Mice were challenged with *R. arrhizus* var. *delemar* or *R. arrhizus* var. *arrhizus* (2.5×10^5/mouse) through intratracheal instillation of 25 μl after sedation with isoflurane gas (16). Immediately after infection, a subset of mice was sacrificed and lungs were removed to determine conidial equivalent (CE) by qPCR. All drug treatments (by oral gavage) were initiated at 16 h postinfection and continued for 7 consecutive days (survival) or 4 days (tissue burden assessment). To extend the half-life of MGX after fosmanogepix administration, 50 mg/kg of ABT was administered 2 h prior to each daily fosmanogepix dose. The prodrug fosmanogepix was dosed at 78 mg/kg and 104 mg/kg once daily by oral gavage. Using a conversion factor of 1.3 to account for the methyl phosphate group in the prodrug, the doses were equivalent to MGX at 60 mg/kg and 80 mg/kg, respectively. ISA was dosed at 110 mg/kg TID, the dose that gives rise to exposures equivalent to the human clinical dose (21). For the survival experiments, mice were monitored for 21 days. To assess tissue fungal burden, mice were sacrificed on day +4 and organs processed for conidial equivalent (CE) by real-time qPCR using 18S primers (sense amplification primer, 5′-GCGGATCGCATGGCC-3′; antisense amplification primer, 5′-CCATGATAGGGCAGAAAATCG-3′.)

Ethics statement. All animal-related study procedures were compliant with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Laboratory Animal Welfare and were conducted under an IACUC-approved protocol by The Lundquist Institute for Biomedical Innovations at Harbor-UCLA Medical Center.

Statistical analyses. 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. All analyses were corrected for multiple comparisons with the Bonferroni correction. A P value of <0.05 was considered significant.

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Conflict of interest statement. A.S.I. has received research support to The Lundquist Institute at Harbor-UCLA Medical Center from Amplyx, Astellas, and Cidara and has served on advisory boards of Amplyx, Astellas, Cidara, and Navigen. N.P.W. has received research support to UT Health San Antonio from Astellas, bioMérieux, Cepheid, Cidara, Covance, F2G, and Viamet and has served on advisory boards for Astellas and Mayne Pharma and as a speaker for Gilead. T.F.P. has received a research grant to UT Health San Antonio from Cidara and has served as a consultant for Basilea, Gilead, Mayne, Merck, Pfizer, Scynexis, and Toyama. S.G.F. has received a research grant to The Lundquist Institute at Harbor-UCLA Medical Center from Merck. K.J.S. was an employee of Amplyx and is now an independent consultant at Hearts Consulting Group. All other authors have no conflicts.

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