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SUSCEPTIBILITY

Manogepix, the Active Moiety of the Investigational Agent Fosmanogepix, Demonstrates *In Vitro* Activity against Members of the *Fusarium oxysporum* and *Fusarium solani* Species Complexes

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ABSTRACT We evaluated the *in vitro* activity of manogepix against *Fusarium oxysporum* and *Fusarium solani* species complex (FOSC and FSSC, respectively) isolates per CLSI document M38 broth microdilution methods. Manogepix demonstrated activity against both FOSC (MEC [minimum effective concentration] range, ≤0.015 to 0.03 μg/ml; MIC_{50} range, ≤0.015 to 0.125 μg/ml) and FSSC (MEC, ≤0.015 μg/ml; MIC_{50}, ≤0.015 to 0.25 μg/ml). Amphotericin B was also active (MIC, 0.25 to 4 μg/ml), whereas the triazoles (MIC, 1 to >16 μg/ml) and micafungin (MEC, ≥8 μg/ml) had limited activity.

KEYWORDS *Fusarium oxysporum*, *Fusarium solani*, manogepix, *in vitro* activity, minimum effective concentration, fosmanogepix, *Fusarium*, susceptibility

Fusarium spp. can cause a wide range of infections in humans, including keratitis and onychomycosis in immunocompetent individuals. *Fusarium* can also cause invasive and disseminated disease in immunocompromised hosts, including patients with neutropenia and hematological malignancies, hematopoietic stem cell transplant recipients, and those with severe T-cell deficiencies, and is associated with marked morbidity and mortality (1, 2). Human infections can be caused by members of eight different *Fusarium* spp. complexes, and those that are commonly seen as causing disease include members of the *Fusarium oxysporum* and *Fusarium solani* species complexes (FOSC and FSSC, respectively) (3). Although response rates have improved over the last 25 years with the use of voriconazole and amphotericin B lipid formulations, clinical outcomes are still suboptimal (4, 5), and both antifungals have unfavorable side-effect profiles. Manogepix (APX001A, Amplyx Pharmaceuticals, San Diego, CA; formerly E1210), the active moiety of the prodrug fosmanogepix (APX001), is a novel antifungal that targets inositol acyltransferase Gwt1, an enzyme in the glycosylphosphatidylinositol (GPI) anchor biosynthesis pathway. Inhibition of this enzyme prevents the maturation of GPI-anchored proteins (6). Both *in vitro* and *in vivo* activity have been demonstrated against *Candida* spp. (with the exception of *Candida krusei*), *Cryptococcus*, and *Coccidioides* spp., as well as molds, such as *Aspergillus* and *Scedosporium* spp. and *Rhizopus arrhizus* (6–18). *In vitro* activity has also been reported against a limited number of *Fusarium* isolates (8, 19), and this has translated to *in vivo* efficacy in murine models of disseminated fusariosis (18, 20). We evaluated the *in vitro* activity of manogepix against a larger number of clinical isolates of FOSC and FSSC.

Clinical isolates of FOSC ($n = 49$) and FSSC ($n = 19$) in the collection of the Fungus Testing Laboratory at the University of Texas Health Science Center at San Antonio were

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TABLE 1 MEC and MIC ranges, MEC/MIC_{50} and MEC/MIC_{90}, and GM MEC/MIC values for manogepix, amphotericin B, posaconazole, isavuconazole, voriconazole, and micafungin against *F. oxysporum* species complex and *F. solani* species complex isolates

	MGX[a] (μg/ml)			MIC (μg/ml) of[b]:				
Antifungal and parameter	MEC	MIC_{50}	MIC	AMB	PSC	ISC	VRC	MFG MEC (μg/ml)
FOSC ($n = 49$)								
Range	≤0.015–0.03	≤0.015–0.125	>8	1–4	1 to >16	>16	4–16	≥8
MEC/MIC_{50}	≤0.015	≤0.015	>8	2	4	16	8	>8
MEC/MIC_{90}	≤0.015	0.125	>8	2	>16	>16	8	>8
GM MEC/MIC	≤0.015	0.021	>8	1.59	6.11	>16	6.94	>8
FSSC ($n = 19$)								
Range	≤0.015	≤0.015–0.25	<0.015 to >8	0.25–2	4 to >16	>16	2 to >16	≥8
MEC/MIC_{50}	≤0.015	≤0.015	>8	1	>16	>16	>16	>8
MEC/MIC_{90}	≤0.015	≤0.015	>8	2	>16	>16	>16	>8
GM MEC/MIC	≤0.015	0.017	>8	1.16	>16	>16	>16	>8

[a]MGX, manogepix.
[b]AMB, amphotericin B; PSC, posaconazole; ISC, isavuconazole; VRC, voriconazole; MFG, micafungin.

used. Each isolate had previously been confirmed to the species complex level by combined phenotypic characteristics and DNA sequence analysis of the translation elongation factor 1-alpha (*TEF1*α) and the RNA polymerase II second largest subunit (*RPB2*), as previously described (21). Antifungal susceptibility testing was performed by broth microdilution methods as described in CLSI document M38 (22), with RPMI 1640 (0.165 M MOPS [morpholinepropanesulfonic acid], pH 7.0, without bicarbonate) as the growth medium. Stock solutions of manogepix (Amplyx); amphotericin B, posaconazole, and voriconazole (Sigma); and isavuconazole and micafungin (Astellas) were prepared in DMSO (dimethyl sulfoxide), with further dilutions prepared in RPMI. For manogepix, activity was measured as the minimal effective concentration (MEC) and MICs at two endpoints: (i) an ~50% reduction in visual growth compared to the growth control, as allowed by the CLSI M38 standard for certain antifungals against filamentous fungi (i.e., fluconazole, ketoconazole, and 5-flucytosine) (22), and (ii) complete inhibition of growth, both of which were measured after 48 h of incubation at 35°C. The MEC is now the standard endpoint used to measure the *in vitro* activity of manogepix against filamentous fungi (6, 8). Similarly, the MEC endpoint was used for micafungin (22, 23). For amphotericin B, posaconazole, isavuconazole, and voriconazole, the MIC after 48 h of incubation was the endpoint used per CLSI recommendations (22). MEC/MIC ranges, MEC/MIC_{50}, MEC/MIC_{90}, and geometric mean (GM) MEC/MIC values were determined.

Manogepix demonstrated *in vitro* activity against FOSC and FSSC isolates when the MEC and MIC_{50} endpoints were used (Table 1 and Tables S1 and S2 in the supplemental material). Against the FOSC isolates, the manogepix MEC range was ≤0.015 to 0.03 μg/ml, which was similar to the range with the MIC_{50} endpoint (≤0.015 to 0.125 μg/ml). The GM MEC and MIC values were ≤0.015 and 0.021 μg/ml, respectively, and only 9 of the 49 FOSC isolates tested had a manogepix MIC_{50} value higher than the lowest concentration tested (0.015 μg/ml) (see Fig. S1 in the supplemental material). Similar results were observed against FSSC isolates. Here, the MEC and MIC_{50} ranges for manogepix were ≤0.015 and ≤0.015 to 0.25 μg/ml, respectively, and the GM MEC and MIC values were ≤0.015 and 0.017 μg/ml, respectively. In contrast, when the MIC endpoint was used, manogepix appeared to have reduced or no *in vitro* activity against *Fusarium* isolates at the highest concentration tested (Table 1; Supplemental Fig. S1).

Of the clinically available antifungals tested, amphotericin B demonstrated activity, with MIC ranges of 1 to 4 and 0.25 to 4 μg/ml against FOSC and FSSC, respectively. In contrast, the azoles demonstrated limited activity overall, with the MIC ranges for posaconazole, isavuconazole, and voriconazole falling between 1 and >16 μg/ml. In addition, the GM MIC values were higher than bloodstream concentrations that can consistently and safely be achieved with these antifungals. No activity was observed with micafungin at the highest concentration tested (MEC, ≥8 μg/ml against all isolates).

The *in vitro* activity of manogepix against *Fusarium* isolates observed in this study is consistent with that previously published by others. Against a limited number of *Fusarium* isolates ($n = 10$) from multiple species complexes, Pfaller et al. (19) reported the manogepix MECs to range between ≤0.008 and 8 μg/ml when determined by CLSI methods. In an earlier study by the same group using both CLSI and EUCAST methods that included a larger number of isolates ($n = 67$), the MECs against various *Fusarium* spp. ranged between 0.008 and 0.5 μg/ml, and essential agreement between the two methods was reported to be 94% to 96.7% (8). In contrast, a recent study reported differences in manogepix activity against *Fusarium* isolates between the EUCAST and CLSI methods, with more potent activity when measured using the CLSI method (24). In the current study and others, the MEC was the endpoint chosen for manogepix *in vitro* activity because, similar to the echinocandins, this agent inhibits hyphal extension but does not completely inhibit growth (6). Here, we also report the MIC_{50} and MIC. The MIC_{50} endpoint demonstrated good agreement with the MEC value, although correlation analysis was not possible because many values for both endpoints were equal to the lowest concentration tested. The MIC is an inappropriate endpoint for manogepix against filamentous fungi, because this would suggest little to no *in vitro* activity against *Fusarium* spp. and other molds, contrary to *in vivo* efficacy model data. Previous studies have reported improved outcomes with manogepix treatment in murine models of invasive fusariosis, and these *in vivo* results are in agreement with the manogepix *in vitro* susceptibility result measured using either the MIC_{50} or the MEC as the susceptibility endpoint (18, 20).

In conclusion, manogepix demonstrated *in vitro* activity against FOSC and FSSC isolates. Clinical studies are ongoing to determine the efficacy and safety of fosmanogepix in patients with invasive fungal infections, and it is important for clinical laboratories to use the correct susceptibility endpoint for determination of *in vitro* activity for manogepix.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

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