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# Noncystic fibrosis bronchiectasis A singlecentre retrospective study in Johannesburg South Africa

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# *In vitro* manogepix susceptibility testing of South African *Emergomyces africanus, Emergomyces pasteurianus*, and *Blastomyces emzantsi* clinical isolates

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**ABSTRACT** We performed *in vitro* antifungal susceptibility testing of manogepix against the yeast phase of 78 *Emergomyces africanus*, 2 *Emergomyces pasteurianus,* and 5 *Blastomyces emzantsi* isolates using a reference broth microdilution method following Clinical and Laboratory Standards Institute recommendations. All three pathogens had low minimum inhibitory concentrations ranging from <0.0005 to 0.008 mg/L. Manogepix should be investigated in animal models and potentially in future human clinical trials for endemic mycoses.

**KEYWORDS** manogepix, APX001A, antifungal susceptibility testing, thermally dimorphic fungi, emergomycosis, blastomycosis

The fungal genera *Emergomyces, Blastomyces, Histoplasma*, and *Sporothrix* are endemic to the Southern African region. The first three are phylogenetically related thermally dimorphic fungal genera and belong to the family Ajellomycetaceae (1). *Emergomyces africanus* is the main cause of emergomycosis in Southern Africa among people living with HIV (2). Although *E. pasteurianus* is of worldwide distribution, a few cases have been detected in South Africa. Blastomycosis, caused in South Africa by two species namely *Blastomyces emzantsi* and *Blastomyces percursus,* is less frequently diagnosed compared to emergomycosis (2). For both emergomycosis and blastomycosis, there are no comparative treatment trials. Non-randomized studies, case series, and expert opinions have been used to justify antifungal treatment recommendations. The recommended initial treatment for severe forms of emergomycosis and blastomycosis is amphotericin B, either liposomal or deoxycholate formulations, followed by a step-down to itraconazole (3). Manogepix is a first-in-class small-molecule antifungal agent that targets the highly conserved Gwt1 enzyme and blocks glycosylphosphatidylinositol post-translational modification, which facilitates anchoring of surface proteins to the fungal cell membrane (4, 5). While manogepix has been found to be highly active *in vitro* against clinical isolates of *Coccidioides* and *Histoplasma* (6, 7), *in vitro* testing has not been performed for other thermally dimorphic fungi. We performed *in vitro* manogepix susceptibility testing for three thermally dimorphic fungi, two of which are uniquely associated with human disease in Southern Africa.

## Isolates

We included 78 *Emergomyces africanus* and two *Emergomyces pasteurianus* isolates collected through passive laboratory surveillance performed by the National Institute for Communicable Diseases (NICD) from 2008 to 2021 in South Africa. Of the eight *B. emzantsi* isolates in the NICD's culture collection of pathogenic fungi, only five were included; these had been isolated from clinical cases between 1967 and 1999. *Blastomyces percursus* was excluded from the study because the revival of isolates stored at −70°C

**Editor** Andreas H. Groll, University Children's Hospital Münster, Münster, Germany

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The authors declare no conflict of interest.

See the funding table on p. 3.

**Received** 29 August 2023
**Accepted** 6 October 2023
**Published** 16 November 2023

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in the culture collection proved to be very time-consuming, and the study period was limited.

## Fungal identification methods

We re-confirmed the identification of all the isolates by conventional PCR and sequencing of the internal transcribed spacer regions of the ribosomal gene before antifungal susceptibility testing. The mycelial phase on Sabouraud agar [Diagnostic Media Products (DMP), Johannesburg, South Africa] was converted to the yeast phase using brain heart infusion agar (DMP) and incubated at 37°C for up to 7 days. All testing was performed in a biosafety level 2 facility with the use of a class II biosafety cabinet and personal protective equipment, including N95 masks. We limited our work to the mold phase as far as possible. We worked primarily with the yeast phase because this is the pathogenic form in human disease and for laboratory safety reasons.

## Antifungal susceptibility testing

Manogepix susceptibility testing was conducted for the yeast phase using a broth microdilution (BMD) method. Manogepix powder was donated by Amplyx/Pfizer (NY, USA). We prepared BMD panels as per Clinical and Laboratory Standards Institute (CLSI) M27 recommendations with some modifications (8). Since there are no standardized methods for susceptibility testing of thermally dimorphic fungi, we followed CLSI methods but used a larger-than-recommended and previously validated inoculum (9) of 1 McFarland and a prolonged incubation time of 7 days to facilitate growth and visual endpoint determination (9). For test isolates, the minimum inhibitory concentration (MIC) was defined as the lowest concentration that inhibited growth by 50% relative to the positive (growth) control. We included the following quality control strains in each testing run: *Candida parapsilosis* American Type Culture Collection (ATCC) 22019 and *Candida albicans* ATCC 90028 as per the antifungal manufacturer's recommendations. For all test runs, MIC ranges for the quality control strains, read at 24 h, were within the recommended ranges.

## Activity of manogepix against *Emergomyces* and *Blastomyces* isolates

*E. africanus* manogepix MICs ranged from 0.0005 to 0.008 mg/L (Table 1). Of the 78 *E. africanus* isolates tested, 14 (18%) had an MIC of ≤0.0005 mg/L. The remaining isolates had an MIC that ranged from 0.001 to 0.008 mg/L. Only two *E. pasteurianus* isolates were tested and both had an MIC of <0.0005 and 0.004 mg/L, respectively. For the five *B. emzantsi* isolates tested, the MIC range was 0.0005–0.004 mg/L.

We conducted *in vitro* antifungal susceptibility testing in the yeast phase of 78 *E. africanus*, 2 *E. pasteurianus,* and 5 *B. emzantsi* isolates and found exceedingly low manogepix MICs ranging from <0.0005 to 0.008 mg/L for all three pathogens. While there are no established clinical breakpoints for manogepix, the very low MICs provide evidence of potent *in vitro* activity against these thermally dimorphic fungi. This suggests the potential clinical efficacy of manogepix for the treatment of emergomycosis and blastomycosis, which should be investigated in animal models of endemic mycoses and human clinical trials, as other indications are being investigated (10–15). *E. africanus* and *B. emzantsi* are currently known to be endemic only to Southern Africa, while *E. pasteurianus* is cosmopolitan.

**TABLE 1** Yeast-phase manogepix minimum inhibitory concentration distribution of 78 *Emergomyces africanus*, 2 *Emergomyces pasteurianus*, and 5 *Blastomyces emzantsi* clinical isolates

| No. of clinical isolates | Minimum inhibitory concentration (mg/L) | | | | | | |
|---|---|---|---|---|---|---|---|
| | <0.0005 | 0.0005 | 0.001 | 0.002 | 0.004 | 0.008 | ≥0.016 |
| *Emergomyces africanus* (*n* = 78) | 4 | 10 | 30 | 23 | 9 | 2 | 0 |
| *Emergomyces pasteurianus* (*n* = 2) | 1 | 0 | 0 | 0 | 1 | 0 | 0 |
| *Blastomyces emzantsi* (*n* = 5) | 0 | 1 | 1 | 1 | 2 | 0 | 0 |



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We have previously described the BMD MIC ranges of other antifungal classes against the yeast phases of *E. africanus* and *B. emzantsi*. For *E. africanus* (*n* = 50), the MIC ranges were: fluconazole 0.12–1 mg/L, voriconazole 0.008–0.015 mg/L, posaconazole 0.008–0.25 mg/L, itraconazole 0.008–0.12 mg/ L, 5FC 4–256 mg/L, caspofungin 0.06–8 mg/L, micafungin 0.12–8 mg/L, and anidulafungin 0.12–8 mg/L (9). In comparison with all other tested agents, manogepix had more potent activity against *E. africanus*. For *B. emzantsi* (*n* = 8), the MIC ranges were: fluconazole 0.25–4 mg/L, voriconazole 0.008–0.03 mg/L, posaconazole 0.008–0.008 mg/L, itraconazole 0.008–0.12 mg/ L, 5FC 16–128 mg/L, caspofungin 0.5–4 mg/L, micafungin 0.008–0.3 mg/L, and anidulafungin 0.5–2 mg/L (16). In comparison with all other tested agents, manogepix was also more potent against *B. emzantsi*.

Limitations of this study include (i) the lack of a standardized method for susceptibility testing, (ii) no breakpoints to interpret MICs, and (iii) a small number of tested *B. emzantsi* isolates. We did not test all fungal species that cause emergomycosis and blastomycosis, so these results cannot be generalized to other endemic areas of the world.

Fosmanogepix has been found to be safe in healthy volunteers (17) and is currently in Phase 2 clinical trials evaluating the efficacy and safety of both intravenous (IV) and oral formulations for the treatment of patients with invasive fungal infections (10). While there are no established clinical breakpoints for manogepix, the very low MICs provide evidence of potent *in vitro* activity against these thermally dimorphic fungi. This suggests the potential clinical efficacy of manogepix for the treatment of emergomycosis and blastomycosis, which could be further investigated in animal models and future clinical trials.

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## FUNDING


| Funder | Grant(s) | Author(s) |
|---|---|---|
| Pfizer Inc. \| Pfizer Pharmaceuticals (Pfizer Pharmaceuticals B.V.) | | Nelesh P. Govender |


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