Manogepix and amphotericin B combination is synergistic against *Candida glabrata* as evidenced by a new pharmacodynamic-modelling approach

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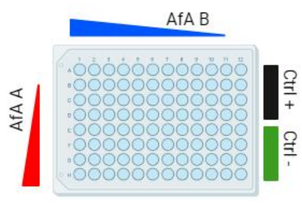
Introduction

Candida glabrata is the second most common yeast species involved in candidemia. It presents high MICs for azoles and several strains have showed resistance or tolerance to echinocandins. **Manogepix** (MGX) is an innovative antifungal agent that disrupts the GPI-anchor biosynthesis pathway by inhibiting the GWT1 enzyme. MGX could be an alternative to treat multi-resistant *C. glabrata* strains. The aim of this study was to evaluate the potential of MGX in **combination** with other antifungal agents, amphotericin B (AmB), micafungin (MCF), fluconazole (FLU) and voriconazole (VRZ), to **improve efficacy and/or limit the development of resistance** against *C. glabrata* using a **new pharmacodynamic-modelling approach** to overcome the problems encountered (varied definitions of MIC, 90% or 50% growth inhibition) with FIC index analysis.

Materials and methods

1 Study of the *in vitro* activity of antifungal combinations by checkerboard assays



OD measurement after 24h of incubation

Checkerboard assays were performed using RPMI medium and 4 different antifungal combinations :
- MGX/AmB
- MGX/FLU
- MGX/MCF
- MGX/VRZ

12 isolates of *C. glabrata* (including 2 echinocandin-resistant strains, 2 azole-resistant strains and the ATCC 2950 reference strain) were studied.

2 Interpretation of checkerboards using pharmacodynamic-modelling approach

Growth inhibition was modelled using the GDPI model[1] (Eq.1). The effect of combination was modelled with Bliss independence (Eq.2). A new index was developed, named effect improvement (EI) (Eq.3), which compares the effect of the combination with the effect of the best monotherapy at equivalent concentration.

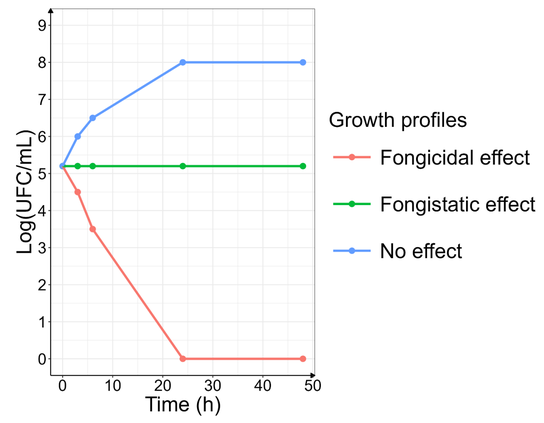
1 $$E_{A|B} = \frac{Emax_A * C_A^{H_A}}{\left(EC50_A * \left(1 + \frac{INT_{AB} * C_B^{H_{INT,AB}}}{EC50_{INT,AB}^{H_{INT,AB}} + C_B^{H_{INT,AB}}}\right)\right)^{H_A} + C_A^{H_A}}$$

2 $$E_{AandB} = E_{A|B} + E_{B|A} - E_{A|B} * E_{B|A}$$

3 $$Effect\ improvement = E_{AandB} - \max(E_{A|B=0}, E_{B|A=0})$$

Mathematical methods and software:
Modelling and simulation were performed with R² version 4.3.2.

3 Time kill curves of MGX/AmB combination



Time kill curves (TKC) assays were performed using RPMI medium and MGX/AmB combination.

6 isolates of *C. glabrata* (including 2 echinocandin-resistant strains, 2 azole-resistant strains and the ATCC 2950 reference strain) were studied.

Results

We developed a model that successfully described the effect of the four combination therapies against the 12 strains.

We were able to quantify the **improvement of the antifungal effect** (named **effect improvement**) **due to the combination** in each well of the checkerboard in comparison to **the most effective monotherapy** at equivalent concentrations.

Comparing the best effect improvement for each strain, the combination with the higher effect improvements was **MGX/AmB** (Figure 1).

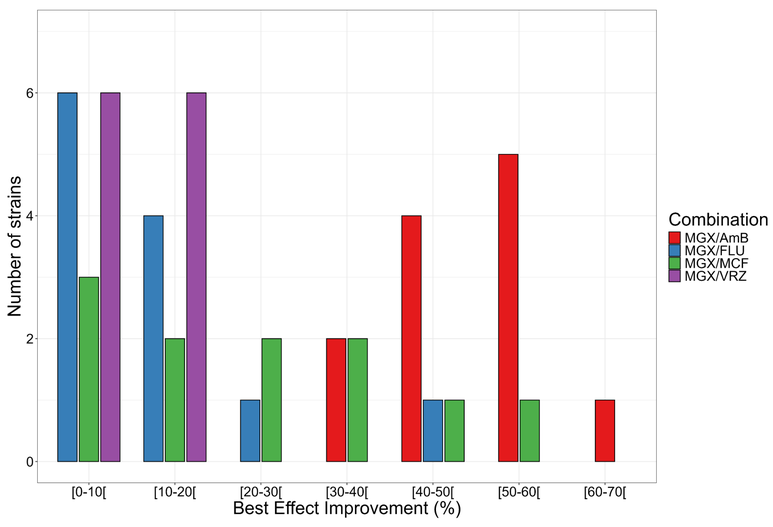


Figure 1: Cumulative representation of the best effect improvement for all strains for each combination of antifungals.

The best effect improvements were observed for combinations of MGX concentrations **between 1/8 of its MIC and its MIC** and AmB concentrations **between 1/2 and 1/8 of its MIC** (Figure 2).

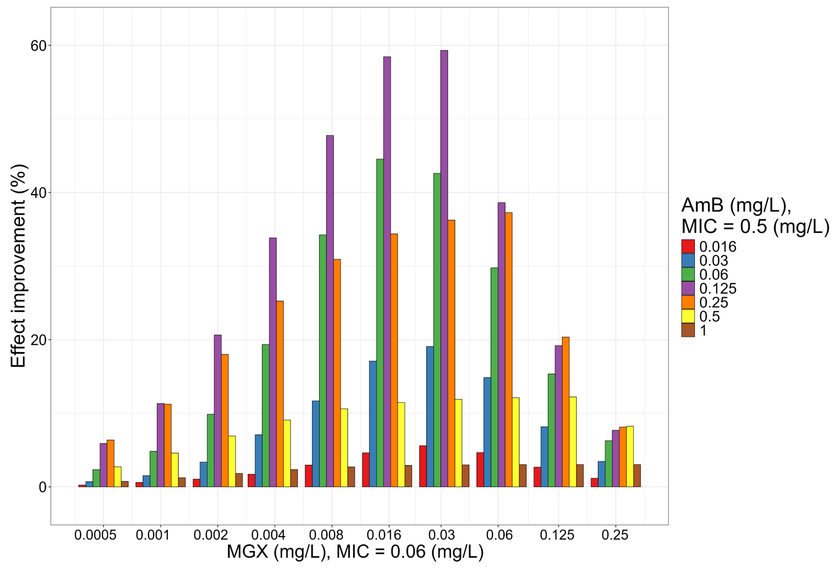


Figure 2: Effect improvement values for each well of the MGX/AmB checkerboard against one strain of *C. glabrata.*

The **MGX/AmB combination synergy** was confirmed in TKC. Use of **AmB concentrations 8 to 4 fold lower than its MIC** improved the efficacy of MGX compared to monotherapy.

For example, against one of the strains, use of **0,125 mg/L of AmB (1/4 of its MIC)** in combination with MGX at its MIC reduced the fungal load in the TKC by more than **99%** (Figure 3).

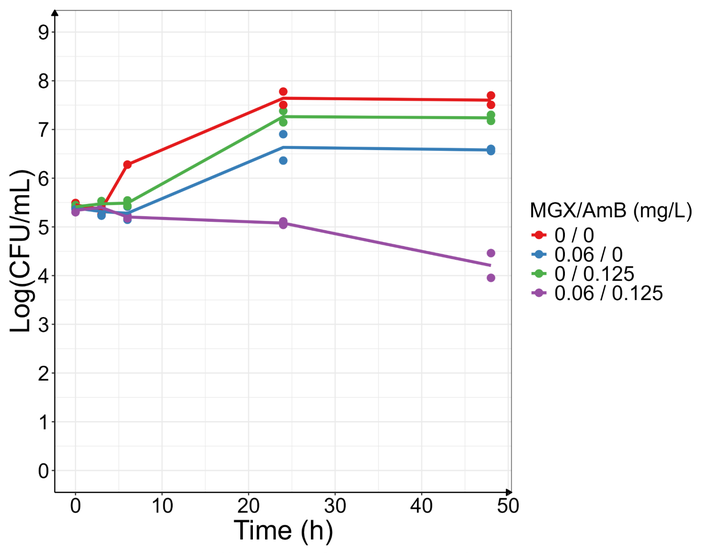


Figure 3: Time kill curve for the combination of 0.06 mg/L of MGX and 0,125 mg/L of AmB against one strain of *C. glabrata* (n=2).

Conclusion

We have developed **an innovative and robust checkerboard analysis model** for antifungal agent combinations analysis. The analysis showed that **MGX/AmB combination** appears to be the best combination on the twelve tested strains of *C. glabrata*. The model supposed that the use of **low doses of AmB and MGX** could improve the effect of both monotherapies. Theses results were confirmed by the TKC assays in which the use of **low doses of AmB** improved the effect of MGX at its MIC concentration. The MGX/AmB combination appears very promising **to treat *C. glabrata*** and reduce the risk of resistance development.

1) Wicha et al. 10.1038/s41467-017-01929-y

2) R Core Team (2023) https://www.R-project.org/

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