

Synergistic and antagonistic effects of immunomodulatory drugs on the action of antifungals against *Candida glabrata* and *Saccharomyces cerevisiae*

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ABSTRACT

Candidemia and other forms of invasive fungal infections caused by *Candida glabrata* and to a lesser extent *Saccharomyces cerevisiae* are a serious health problem, especially if their steadily rising resistance to the limited range of antifungal drugs is taken into consideration. Various drug combinations are an attractive solution to the resistance problem, and some drug combinations are already common in the clinical environment due to the nature of diseases or therapies. We tested a few of the common antifungal-immunomodulatory drug combinations and evaluated their effect on selected strains of *C. glabrata* and *S. cerevisiae*. The combinations were performed using the checkerboard microdilution assay and interpreted using the Loewe additivity model and a model based on the Bliss independence criterion. A synergistic interaction was confirmed between calcineurin inhibitors (Fk506 and cyclosporine A) and antifungals (fluconazole, itraconazole, and amphotericin B). A new antagonistic interaction between mycophenolic acid (MPA) and azole antifungals was discovered in non-resistant strains. A possible mechanism that explains this is induction of the Cdr1 efflux pump by MPA in *C. glabrata* ATCC 2001. The Pdr1 regulatory cascade plays a role in overall resistance to fluconazole, but it is not essential for the antagonistic interaction. This was confirmed by the *Cgpd1*Δ mutant still displaying the antagonistic interaction between the drugs, although at lower concentrations of fluconazole. This antagonism calls into question the use of simultaneous therapy with MPA and azoles in the clinical environment.

Subjects Microbiology, Drugs and Devices, Infectious Diseases, Public Health

Keywords *Candida glabrata*, *Saccharomyces cerevisiae*, Antagonistic mechanism, Drug interactions, Candidiasis, Drug resistance, Antifungal drugs, Immunomodulatory drugs

INTRODUCTION

The frequency and associated mortality of candidemia and other forms of invasive candidiasis have not decreased over the past two decades despite the introduction of several extended-spectrum triazole and echinocandin antifungal drugs for use in prophylaxis, empiric therapy, and targeted therapy (Pfaller & Diekema, 2007; Pfaller & Castanheira, 2016).

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page 14

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Candida albicans is the dominant pathogen, but the incidence of invasive infections caused by *Candida glabrata* has been steadily rising (Pfaller et al., 2012b; Pfaller et al., 2014). The most vulnerable populations include transplant patients, patients with AIDS or cancer, those on immunosuppressive therapy, patients receiving total parenteral nutrition, and premature infants (Pfaller & Diekema, 2010; Whaley & Rogers, 2016). In certain populations, *C. glabrata* even surpasses *C. albicans* as the leading pathogen; these include patients with hematologic malignancies, diabetes mellitus, and patients with an abdominal source of infection (Hachem et al., 2008; Segireddy et al., 2011; Khatib et al., 2016; Whaley & Rogers, 2016). The reasons for the rise of *C. glabrata* infections include the introduction of fluconazole in 1990 and its widespread prophylactic use against fungal infections (Berrouane, Herwaldt & Pfaller, 1999), a higher rate of antifungal use and intrinsic or acquired resistance of *C. glabrata* to both fluconazole and echinocandins (Silva et al., 2012; Pfaller et al., 2012a; Alexander et al., 2013; Pfaller & Castanheira, 2016; Colombo, Júnior & Guinea, 2017), and better identification of non-*albicans* species in the clinic (Liguori et al., 2009).

One of the main problems when dealing with *C. glabrata* is its intrinsically low susceptibility to azole antifungals (Vermitsky & Edlind, 2004) and its ability to develop resistance to several antifungal drug classes (Pfaller, 2012; Glöckner & Cornely, 2015). For example, resistance to azole antifungals in clinical isolates is mostly connected to mutations in the gene *PDR1*, which encodes the transcription factor for the pleiotropic drug response (Vermitsky & Edlind, 2004). Activating mutations in *PDR1* lead to distinct patterns of altered gene expression among Pdr1 targets, commonly leading to overexpression of efflux pumps that lower the bioavailability of the azoles, thus lowering their effectiveness (Whaley & Rogers, 2016). The efflux pumps most commonly associated with azole resistance in *C. glabrata* are the ATP-binding cassette (ABC) transporters Cdr1 (Sanglard, Ischer & Bille, 2001), Cdr2/Pdh1 (Miyazaki et al., 1998), and Snq2 (Torelli et al., 2008). Alternative azole resistance mechanisms include petite mutants with increased expression of *CDR1* and *CDR2* through Pdr1 induction and lower levels of ergosterol intermediates (Brun et al., 2004; Tsai et al., 2006; Whaley & Rogers, 2016). Namely, azoles inhibit Erg11 (lanosterol 14- α demethylase in ergosterol biosynthesis), causing disruption of the membrane and the accumulation of toxic sterol intermediate (Cowen, 2008). Documented mechanisms of azole resistance also include Upc2A regulated uptake of exogenic sterols with the Aus1 transporter (Nakayama et al., 2007; Nagi et al., 2011), and mutations or changes in the expression of target gene *ERG11* and genes involved in sterol intermediate synthesis (*ERG3*, *ERG24*) (Morio et al., 2012; Whaley et al., 2017). However, *C. glabrata* clinical isolates do not appear to utilize azole resistance mechanisms that involve mutations or changes in the expression of genes in the ergosterol biosynthesis pathway (Sanguinetti et al., 2005).

S. cerevisiae has resistance mechanisms to azole antifungals similar to those in *C. glabrata* through the increased activity of efflux pumps (Pdr5 homologue of Cdr1) induced by Pdr1 (Moye-Rowley, 2003), and the dysfunctional mitochondria of petite mutants (Kontoyiannis, 2000). *S. cerevisiae* is not usually associated with pathogenesis; however, instances of *Candida*-like infections (Aucott et al., 1990; Murphy & Kavanagh, 1999; Piarroux et al., 1999; Wheeler et al., 2003), often connected with its probiotic variant

Saccharomyces boulardii (nom. nud.), have been reported ([Lherm et al., 2002](#); [Cassone et al., 2003](#); [Enache-Angoulvant & Hennequin, 2005](#); [Roy et al., 2017](#)). Food-oriented *S. cerevisiae* and pathogenic *C. glabrata* therefore present an interesting link for observing the development of various aspects of adaptations to the human host and the mechanisms of evolution in the Saccharomycetaceae ([Wheeler et al., 2003](#); [Roetzer, Gabaldón & Schüller, 2011](#); [Bolotin-Fukuhara & Fairhead, 2014](#)). *S. cerevisiae* serves as a model organism, and so its regulatory networks and gene functions are extensively studied. A high degree of homology with *C. glabrata*, therefore, makes it possible to utilize the accumulated knowledge from the model organism and apply it to the pathogen.

Developing new antifungals agents is difficult and costly. Synergistic and additive drug treatments are potential strategies for controlling resistance development and evolution because the administration of multiple drugs may disrupt several mechanisms or processes in the pathogen and thus minimize the selection of resistant strains ([Yeh et al., 2009](#); [Bollenbach, 2015](#)). The combination of antifungals flucytosine (5FC) and amphotericin B (AMB) is recommended by the Infectious Diseases Society of America for *Candida* infections ([Pappas et al., 2015](#)) due to the high rate of resistance developed during 5FC monotherapy ([Barchiesi et al., 2000](#)). Other combinations between two antifungals have been put through clinical trials ([Scheven et al., 1992](#); [Ghannoum & Elewski, 1999](#); [Rex et al., 2003](#); [Pachl et al., 2006](#)), but so far only the 5FC + AMB combination therapy has a clinical role ([Pappas et al., 2015](#)). Common combinations studied are between a commercial antifungal and a specific inhibitor of a protein of interest; for example, antifungals combined with Hsp90 inhibitors ([Cowen, 2013](#); [Veri & Cowen, 2014](#)) or protein kinase C inhibitors ([LaFayette et al., 2010](#)). Several promising drug combinations against pathogenic fungi have recently been reviewed ([LaFayette et al., 2010](#); [Liu et al., 2014](#); [Cui et al., 2015](#); [Svetaz et al., 2016](#)).

Due to the nature of the therapy already employed in the clinical environment, drug combinations are often overlooked with regard to their effect on the pathogens, and treatments can become problematic because of unexpected interactions ([Henry et al., 1999](#)). Possible side effects and the toxicity of certain drug-drug interactions usually focus on the host, whereas the actual pathogens and their role in these interactions are rarely taken into consideration ([Nett & Andes, 2016](#)). Simultaneous therapy with several overlapping drugs often occurs in the clinic. Such instances often involve administration of antifungal and immunomodulatory drugs ([Pfaller & Castanheira, 2016](#)). Many immunomodulatory drugs have conserved targets in fungal pathogens; for example, calcineurin is crucial for the survival during membrane stress ([Cruz et al., 2002](#)), and calcineurin inhibitors (cyclosporine A (CsA) and Fk506) have antifungal properties ([Steinbach et al., 2007](#); [Li et al., 2015](#); [Denardi et al., 2015](#); [Yu, Chang & Chen, 2015](#)). Methotrexate (MTX), which blocks folic acid metabolism, also has antifungal properties, as it inhibits ergosterol production in *C. albicans* and makes it more susceptible to azoles ([Navarro-Martínez, Cabezas-Herrera & Rodríguez-López, 2006](#)). Mycophenolic acid (MPA) targets inosine-5'-monophosphate dehydrogenase, which is a crucial enzyme for the *de novo* synthesis of guanine nucleotides ([Shah & Kharkar, 2015](#)). MPA has antifungal properties and is synergistic with AMB in *C. albicans* ([Banerjee, Burkard & Panepinto, 2014](#)).

Table 1 Clinical isolates and their source.

Strain	Collection number	Species	Source
Sc1	ZIM 2558	<i>S. cerevisiae</i>	Throat swab
Sc2	ZIM 2566	<i>S. cerevisiae</i>	Sputum
Sc3	ZIM 2247	<i>S. cerevisiae</i>	Adrenal gland
Sc4	ZIM 2255	<i>S. cerevisiae</i>	Lung of man with immune deficiency syndrome
Sc5	ZIM 2260	<i>S. cerevisiae</i>	Bile tube
Sc6	ZIM 2269	<i>S. cerevisiae</i>	Sorghum beer
Cg1	ZIM 2344	<i>C. glabrata</i>	Urine
Cg2	ZIM 2365	<i>C. glabrata</i>	Sputum
Cg3	ZIM 2369	<i>C. glabrata</i>	Bronchoalveolar lavage
Cg4	ZIM 2382	<i>C. glabrata</i>	Urine taken from a permanent catheter
Cg5	ZIM 2385	<i>C. glabrata</i>	Intestine swab (anus, rectum)
Cg6	ZIM 2389	<i>C. glabrata</i>	Urine

The study's main goal was to observe whether drug combinations of immunomodulatory and antifungal drugs have any modulatory effects on the selected *C. glabrata* and *S. cerevisiae* isolates. Synergy between calcineurin inhibitors and antifungals was detected. Antagonism between MPA and fluconazole (FLC) in most non-resistant strains was detected as well. This antagonism was unexpected, and therefore the underlying mechanism was briefly investigated. Because FLC resistance is commonly connected to the activation of Pdr1 and subsequent overexpression of the Cdr1 efflux pump, their gene expression was analysed. MPA appears to induce overexpression of the *CDR1* efflux pump, but the central role of Pdr1 is questionable. This was further confirmed in a *Cgpd1Δ* mutant, in which an antagonistic interaction between FLC and MPA was observed, although at lower concentrations of FLC. This antagonistic interaction between FLC and MPA opens the potential to further explore the underlying mechanism and its impact in the clinical environment.

MATERIALS & METHODS

Strains

Five *Saccharomyces cerevisiae*, six *Candida glabrata* clinical isolates and one *S. cerevisiae* non-clinical isolate were selected from the Collection of Industrial Microorganisms (ZIM) at the Biotechnical Faculty, Slovenia (Table 1). These were selected from 96 clinical isolates (40 *S. cerevisiae* and 56 *C. glabrata*; full list of strains is in Supplemental Information 5) and nine non-clinical *S. cerevisiae* isolates based on their minimal inhibitory concentrations (MICs) obtained by the reference method for broth dilution antifungal susceptibility testing of yeasts (CLSI M27-A3) (CLSI, 2008). The criterion was to select strains with different MICs to cover several spectra of antifungal resistance. *S. cerevisiae* strain Sc6 from sorghum beer was selected because it displayed high tolerance (16 mg/l) towards fluconazole for a non-clinical isolate. *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were used as the control strains in the drug susceptibility and checkerboard assays. In addition, to explore the antagonistic mechanism of MPA and azole antifungals, we used *C. glabrata* ATCC 2001 and *Cgpd1Δ* (CAGL0K10780g Δ::*NAT1*) mutant, provided by Karl Kuchler's

laboratory, Medical University of Vienna, from their deletion library ([Schwarzmueller et al., 2014](#)). *Cgpd1Δ* is isogenic to *C. glabrata* ATCC 2001.

Media

Strains were preserved in storage media (10% glycerol, 1% NaCl, and 1% Tween 20) at -80°C . They were revitalized and routinely grown on yeast peptone dextrose (YPD) agar plates (2% Bacto Peptone, 1% yeast extract, 2% dextrose, and 2% Bacto agar) at 35°C , regularly sub-cultured before each experiment. Throughout the assays we also used YPD broth (2% Bacto Peptone, 1% yeast extract, 2% dextrose), Sabouraud dextrose agar (3% Sabouraud dextrose from Sigma-Aldrich, 2% Bacto agar), and RPMI (1.04% RPMI-1640 from Sigma-Aldrich, 3.453% morpholinepropanesulfonic acid from Sigma-Aldrich, pH adjusted to pH 7 with 10 M NaOH solution) ([Adams et al., 1998](#)).

Drugs

Immunomodulatory drugs used for the screening were methotrexate (MTX), mycophenolic acid (MPA) and its derivate mycophenolate mofetil, cyclosporine A (CsA), and tacrolimus (Fk506). We also included a β -lactam antibiotic amoxicillin trihydrate (AMX) because it is often administered simultaneously with immunomodulatory agents. Antifungal drugs used for the initial screenings were amphotericin B (AMB), itraconazole (ITC), and fluconazole (FLC). For further exploration of the antagonistic mechanism between MPA and azoles, we used posaconazole (POS), ketoconazole (KCT), and voriconazole (VRC). All of the drugs were obtained from Sigma-Aldrich, except Fk506, which was provided by Acies Bio.

Stock solutions of MPA, MTX, CsA, Fk506, AMB, ITC, POS, KCT, VRC, and CLO were prepared in dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO, USA), whereas AMX and FLC were diluted directly in the medium of choice for the assay. All the final drug concentrations were made in media (RPMI for drug susceptibility and checkerboard assay, YPD for further evaluation of the antagonistic interaction). List of stock solutions is in [Supplemental Information 2](#).

Checkerboard assay

To determine the susceptibility of the selected strains to these drugs and to observe the effects of the drug combinations on them, we used CLSI M27-A3 checkerboard microdilution assay ([CLSI, 2008](#)). Briefly, drug dilutions and combinations were prepared in RPMI medium in microtiter plates. Negative (only medium) and positive (strain and medium without drug) controls were included. Strains were grown on Sabouraud agar at 35°C and, after 24 h, one colony was transferred in 1 ml 0.85% saline solution. Inoculum was prepared by diluting yeast cells in RPMI medium to $3-5 \times 10^3$ cells/ml using the automatic ImageJ-counting technique ([Zupan et al., 2013](#)). A total of 100 μl of this cell suspension was then transferred to 100 μl of drug suspension prepared in microplates. When the DMSO was used for drug dilution, it comprised $<1\%$ of the final test volume in the microtiter well. Tested drug concentrations ranged from 200–2 mg/l for MTX, 400–6.25 mg/l for AMX, 120–2 mg/l for MPA, 400–0.25 mg/l for Fk506, 16–0.125 mg/l for CsA, 256–0.25 mg/l for FLC, 256–0.25 mg/l for ITC, 1–0.004 mg/l for AMB, 16–0.068 mg/l for KCT, 16–0.017 mg/l for VRC, and 16–0.25 mg/l for POS. After incubation at 37°C

for 24, 48, or 72 h, we measured the optical density at 600 nm (OD_{600}) with a microplate reader (Tecan, Männedorf, Switzerland). Background optical densities were subtracted from that of each well. *In vitro* susceptibility and drug combination tests were performed at least in biological triplicates.

When we further investigated the observed mechanism of the antagonism between MPA and additional azole antifungals versus *C. glabrata* ATCC 2001 and *Cgpd1* Δ mutant, we used a version of the assay described above but replaced SAB and RPMI media with YPD agar plates and broth, respectively. We have confirmed that the antagonistic effect in *C. glabrata* ATCC 2001 is present in both RPMI and YPD media, results are in [Supplemental Information 1](#).

Fractional inhibitory concentrations index

The data obtained from the checkerboard microdilution assays were analysed with the fractional inhibitory concentrations index (FICI) based on the Loewe additivity model ([Loewe, 1928](#)), using the following equation: $FICI = FIC_A + FIC_B$, where $FIC = MIC_{\text{combination}}/MIC_{\text{individual}}$. For azoles and immunomodulatory drugs MIC50 was used, and MIC90 for AMB. FICI is interpreted as synergistic when ≤ 0.5 , indifferent when >0.5 and <4 , and antagonistic when ≥ 4 ([Odds, 2003](#)). For calculation of the FICIs when the MIC resulted in an off-scale value, the next higher concentration (e.g., $>32 = 64$ mg/l) was used ([Moody, 2010](#)). $FICI_{\text{min}}$ was reported as the FICI in all cases unless the $FICI_{\text{max}}$ was greater than 4, in which case $FICI_{\text{max}}$ was reported as the FICI for that particular data set ([Meletiadis et al., 2005](#)).

Bliss independence

The expected effect of drug combinations was also calculated by a model based on the Bliss independence (BI) criterion, where we assume that the relative effect of a drug at a particular concentration is independent of the presence of the other drug ([Bliss, 1939](#); [Goldoni & Johansson, 2007](#); [Yeh et al., 2009](#)). We calculated the predicted decrease of relative growth ($E_{\text{predicted}}$) using the following equation: $E_{\text{predicted}} = 1 - E_A * E_B$, where E_A and E_B are individually measured relative growth inhibitions by drugs A or B, respectively. Positive or negative deviations ($\Delta E = E_{\text{measured}} - E_{\text{predicted}}$) from this predicted decrease of relative growth describe synergistic and antagonistic interactions, respectively ([Yeh et al., 2009](#)).

To interpret and summarize the entire interaction surface calculated by the BI criterion among several different drug combination concentrations, we used previously described interpretations ([Meletiadis et al., 2005](#)). Briefly, we summed all statistically significant ΔE (ΣSSI), determined the mean percentage (MSSI), and calculated the 95% confidence interval (CI). If it did not include 0 and was positive or negative, statistically significant synergy or antagonism, respectively, was claimed for the entire data set. In addition, we also calculated the ΣSSI and MSSI for all the significant synergistic (ΣSYN and $MSYN$, respectively) and antagonistic (ΣANT and $MANT$, respectively) ΔE separately. The absolute sum of all ΣSYN or ΣANT was considered to be a weak (0–100%), moderate (100–200%), or strong ($>200\%$) interaction.

The interaction between two drugs was considered significant, if it was confirmed with at least one model (either BI or FICI).

Gene expression

C. glabrata ATCC 2001 was grown in liquid YPD overnight at 37 °C. In the morning, we diluted it in fresh YPD to OD₆₀₀ 0.05 and let it grow at 37 °C back to OD₆₀₀ 0.1 (approximately 1.5 h). At that point, we added the following drug combinations: (a) untreated, (b) 5 mg/l FLC, (c) 5 mg/l MPA, and (d) 5 mg/l FLC and 5 mg/l MPA. The optimal concentrations were determined with preliminary growth tests and checkerboard assays. All of the samples received the same amount of DMSO.

At timepoints 0, 2, and 4 h we collected the samples with a 3 min spin down at 1,500 g, 4 °C, resuspended them in ice-cold water, and transferred them to 2 ml screw caps, where we performed a short spin down to remove the supernatant and froze the pellet in liquid nitrogen. Samples were stored at −80 °C. Time points were determined according to growth curve assays ([Supplemental Information 4](#)).

RNA isolation and qPCR analysis were performed as described previously ([Tscherner et al., 2012](#)). *PGK1* and *RIP1* were used as housekeeping genes ([Hnisz et al., 2010](#); [Li, Skinner & Bennett, 2012](#)).

The primers used were CgPGK1f (5'-ACGAAGTTGTCAAGTCCTCCA-3'), CgPGK1r (5'-TTACCTTCCAACAATTC CAAGGAG-3'), CgRIP1f (5'-CTTCATGGTTCGGTTCTCTAGG-3'), CgRIP1r (5'-ACAACAACGTTCTTACCCTCAG-3'), CgPDR1f (5'-TACCAATGTCTCAGATACCACCA-3'), CgPDR1r (5'-CTGTCTTTAGAATCCA ACTGCGT-3'), CgCDR1f (5'-AGACTTACGCTAGACATTTAACGG-3'), and CgCDR1r (5'-CACAAATAGACTTCAGCAATGG-3'). Amplification curves were analysed using the Realplex Software (Eppendorf) and relative mRNA quantification was performed using the efficiency corrected $\Delta\Delta C_t$ method ([Pfaffl, 2001](#)). Quantification was performed in Excel (Microsoft) and statistical analysis with GraphPad Prism (GraphPad Software; GraphPad, San Diego, CA, USA) using one-way ANOVA and Bonferroni's multiple comparison test.

RESULTS

Drug susceptibility

Each test had MICs of the control strains (*C. parapsilosis* ATCC 22019, *C. krusei* ATCC 6258) within the expected range for the tested antifungal. MICs for selected strains against individual drugs at 48 h are summarized in [Table 2](#). Strains showed resistance to certain antifungals; for example, Sc2 (32–64 mg/l), Cg5 (64–128 mg/l), and Cg6 (128 mg/l) against FLC and Sc1 (2 mg/l), Sc2 (2–4 mg/l), Sc3 (2–4 mg/l), Cg5 (4–16 mg/l), and Cg6 (128 mg/l) against ITC ([CLSI, 2008](#)). For most of the immunomodulatory drugs, the concentration range that was used here, and was based on the range expected in human blood after drug administration, did not obtain a MIC; exceptions were some strains with MPA (Sc3, Sc4, Sc5, Cg2 at 120 mg/l) and Fk506 (Sc4, Sc6 at 200 mg/l).

Interpretation of drug interactions

Results were obtained using the checkerboard microdilution assay. A summary of the interpretations for each strain and drug combination is found in [Fig. 1](#). The interaction was considered significant, if it was confirmed with at least one model (FICI or BI). Calculated FICI and BI values are located in [Supplemental Information 3](#).

Table 2 Susceptibility of clinical isolates against individual drugs.

Strain	MIC range (mg/l)							
	FLC	ITC	AMB	MPA	MTX	CsA	Fk506	AMX
Sc1	4–8	1–2 ^b	0.125–0.5	>120	>200	>16	>400	>400
Sc2	32–64 ^a	2–4 ^a	0.25	>120	>200	>16	>400	>400
Sc3	16–32 ^b	2–4 ^a	0.25	120	>200	>16	>400	>400
Sc4	1–2	0.5–1	0.5	120	>200	>16	200	>400
Sc5	4–8	1–2 ^b	0.25	120	>200	>16	>400	>400
Sc6	8–16 ^b	1	0.125–0.25	>120	>200	>16	200	>400
Cg1	2–4	1	0.25–0.5	>120	>200	>16	>400	>400
Cg2	2–4	0.5–1	0.5–1 ^b	120	>200	>16	>400	>400
Cg3	4–16 ^b	0.125–1	0.125–0.5	>120	>200	>16	>400	>400
Cg4	2–4	0.25–0.5	0.125–0.5	>120	>200	>16	>400	>400
Cg5	64–128 ^a	4–16 ^a	0.5	>120	>200	>16	>400	>400
Cg6	128 ^a	128 ^a	0.125–0.25	>120	>200	>16	>400	>400

Notes.^aResistant.^bSusceptible dose dependent according to *CLSI (2008)*.

The FLC + MPA combination was antagonistic in eight out of 12 strains (five *S. cerevisiae*, three *C. glabrata*) and synergistic in two *C. glabrata* strains. One synergistic and two indifferent interactions involved highly resistant strains (MIC of 64 mg/l or higher). The AMB + MPA combination had a synergistic effect in eight out of 12 strains (three *S. cerevisiae*, five *C. glabrata*) and antagonism in one *S. cerevisiae* isolate. The effect of ITC + MPA was not as uniform; four synergistic and three antagonistic interactions were observed, indicating that the response is strain-specific.

A strain-specific response was observed in the combination of antifungals and MTX or AMX as well. MTX + FLC had two synergistic and three antagonistic interactions, MTX + ITC four synergistic and one antagonistic, MTX + AMB two synergistic and five antagonistic, AMX + FLC three synergistic interactions, AMX + ITC two synergistic and one antagonistic, and AMX + AMB five synergistic and four antagonistic interactions.

Out of 72 interactions between antifungals and calcineurin inhibitors (CsA and Fk506), 67 were synergistic, even in the FLC and ITC resistant strains Cg5 and Cg6. Antagonistic interactions were observed only in the combination of CsA and FLC in three *S. cerevisiae* isolates.

The results from the FICI and BI models fitted well, and both showed a trend towards the same interpretation; if the interpretation with one model was synergy, the other model either showed synergy or indifference, but never the opposite, antagonism. Out of 180 combinations tested against selected strains, the FICI model showed 60 significant modulatory interactions and the BI model 124, in which most of the absolute sum values (Σ SYN or Σ ANT) indicated strong interactions (>200%).

Antagonistic interaction between MPA + azole antifungals

The strain *C. glabrata* ATCC 2001 was used to further explore the observed antagonism between MPA and azole antifungals (Fig. 2A). Azole antifungals included FLC, ITC, KCT,

Drugs Strain	MPA			MTX			CsA			Fk506			AMX		
	+ FLC	+ ITC	+ AMB	+ FLC	+ ITC	+ AMB	+ FLC	+ ITC	+ AMB	+ FLC	+ ITC	+ AMB	+ FLC	+ ITC	+ AMB
Sc1	4.25	4.25	0.38	0.41	0.66	1.00	1.02	0.52	0.16	0.14	0.25	0.25	1.01	0.51	8.78
	-875%	-443%	486%	841%	110%	31%	-142%	-561%	573%	694%	486%	875%	-17%	207%	-225%
Sc2	1.01	1.01	0.63	1.00	1.00	1.00	4.50	0.06	0.16	0.14	0.13	0.25	1.00	1.01	4.25
	98%	64%	146%	162%	162%	162%	807%	542%	355%	978%	917%	31%	52%	33%	-57%
Sc3	2.02	1.02	0.77	1.00	0.50	1.00	1.02	0.75	0.09	0.13	0.50	0.06	1.01	0.51	1.01
	-282%	-75%	115%	35%	50%	1.00	276%	439%	1470%	888%	682%	2091%	-36%	-325%	-291%
Sc4	2.02	0.52	1.00	1.00	1.00	0.52	0.52	0.51	0.38	0.16	0.25	0.09	1.01	1.01	1.01
	198%	231%	114%	141%	153%	66%	707%	490%	480%	753%	1223%	-79%	98%	18%	-244%
Sc5	0.66	1.01	0.58	1.01	1.08	1.00	4.25	0.28	0.16	0.14	0.25	0.25	1.01	1.01	1.01
	68%	44%	378%	55%	200%	200%	618%	1006%	641%	1078%	987%	44%	115%	50%	-21%
Sc6	1.01	1.03	1.03	0.66	1.00	1.00	0.53	0.56	0.28	0.26	0.75	0.08	1.02	1.00	1.01
	96%	169%	628%	51%	118%	140%	276%	1033%	865%	475%	1906%	-24%	-26%	-37%	334%
Cg1	1.01	0.17	0.63	0.50	1.00	0.66	1.00	0.14	0.28	0.25	0.13	0.52	0.64	0.07	0.13
	694%	1002%	474%	194%	126%	217%	885%	631%	423%	544%	788%	53%	300%	195%	-13%
Cg2	8.50	4.13	0.50	1.00	0.50	1.00	1.00	0.27	0.25	1.00	0.50	0.25	0.63	0.56	0.26
	61%	118%	659%	119%	248%	126%	956%	450%	221%	271%	336%	114%	378%	212%	-35%
Cg3	4.25	1.01	1.01	1.00	1.00	1.00	1.02	0.52	0.25	0.50	0.25	0.26	1.01	1.01	1.01
	120%	25%	163%	42%	19%	453%	1074%	760%	210%	373%	467%	109%	31%	22%	-68%
Cg4	4.25	0.51	0.50	1.00	1.00	1.00	0.63	0.51	0.50	0.50	1.00	0.25	1.01	1.01	0.51
	27%	119%	285%	-142%	-118%	-70%	569%	750%	490%	181%	202%	154%	80%	-69%	63%
Cg5	0.51	0.51	0.50	1.00	0.50	2.00	0.27	0.09	0.16	1.00	0.07	0.28	1.02	1.01	0.51
	105%	383%	329%	69%	54%	584%	1064%	613%	534%	571%	714%	242%	57%	219%	-146%
Cg6	0.53	0.51	0.53	0.50	1.00	1.00	0.52	0.13	0.28	0.25	0.06	0.26	1.02	1.01	0.51
	196%	483%	437%	40%	34%	102%	1022%	743%	800%	864%	97%	323%	84%	234%	-25%



Content of a square:  Color key:  Confirmed by 1 model
Confirmed by both models

Figure 1 Interpretation of the effects of drug interactions against each selected strain with FICI and BI. Each small square includes the calculated overall FICI and BI values of several experiments combined for each drug combination and strain; list of strains in Table 1. Yellow indicates synergy and blue antagonism. Darker colors signify confirmation of the effect by both FICI and BI, and a lighter color only by one model. The interaction was considered significant, if it was confirmed with at least one model. Interpretation of FICI: synergistic ≤ 0.5 , indifferent 0.5–4, antagonistic ≥ 4 (Odds, 2003). FICI_{min} was reported unless the value of FICI_{max} was greater than 4, in which case FICI_{max} was reported. Interpretation of BI (Σ SYN and Σ ANT): positive values are interpreted as synergy and negative as antagonism, where the absolute sum of all Σ SYN or Σ ANT was considered to be a weak (0–100%), moderate (100–200%), or strong ($> 200\%$) interaction (Meletiadis et al., 2005). FICI, fractional inhibitory concentration index; BI, Bliss independence; Σ SYN, sum of all significant positive values calculated by the BI model; Σ ANT, sum of all significant negative values calculated by the BI model.

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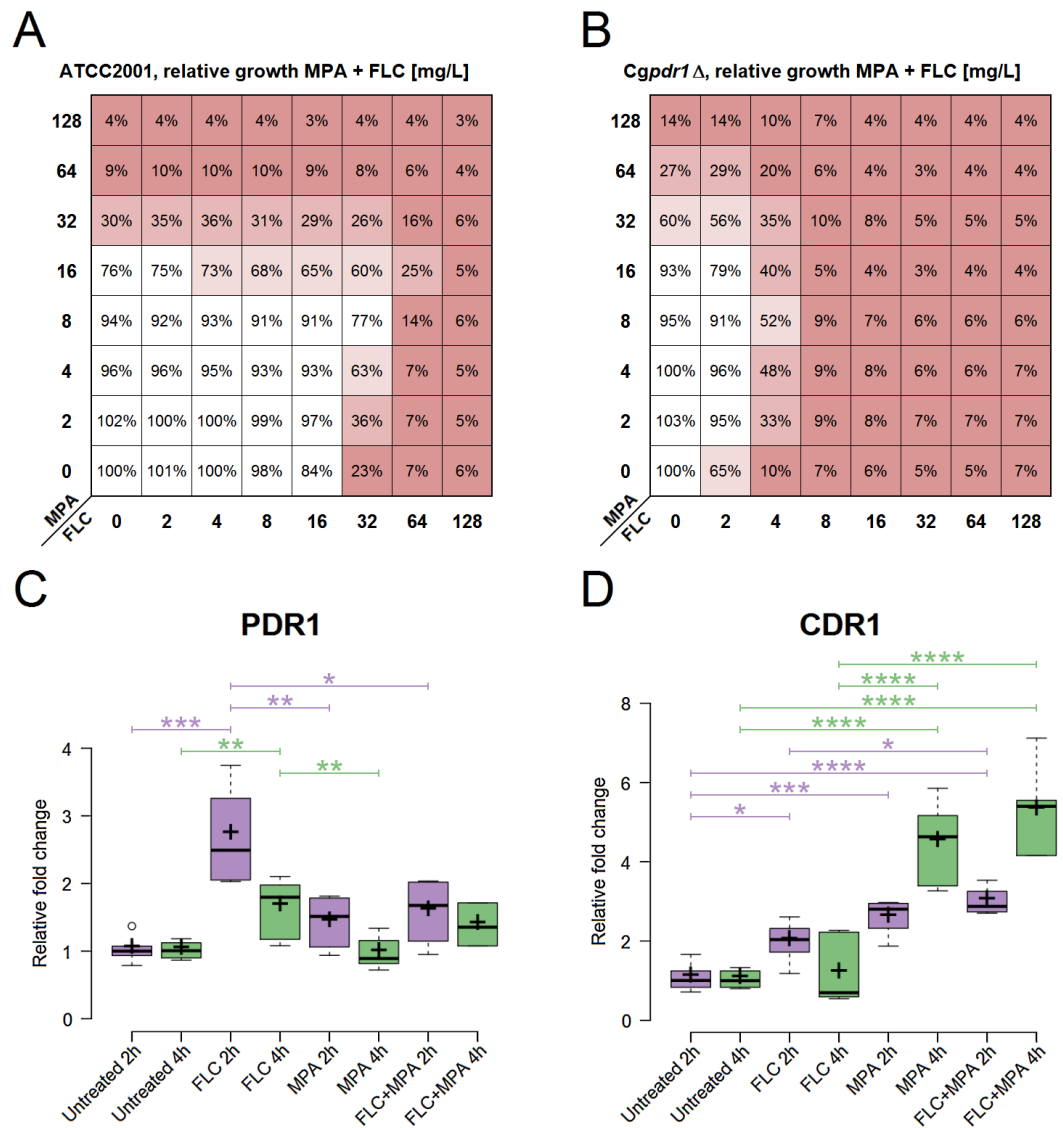


Figure 2 Role assessment of *PDR1* and *CDR1* for the antagonism in the drug combination of FLC + MPA against *C. glabrata* ATCC 2001. (A) Antagonistic interaction (interpreted by the BI model with Σ ANT of -266.9%) observed via relative growth in a checkerboard assay with *C. glabrata* ATCC 2001 against various combinations of MPA and FLC in YPD at 37°C . Red indicates lower growth. (B) Relative growth in a checkerboard assay with *Cgpd1* Δ against various combinations of MPA and FLC in YPD at 37°C . Antagonism is visible (interpreted by the BI model with Σ ANT of -320.79%), most emphasized at MPA 8 mg/l and FLC 4 mg/l. (C, D) Relative fold change of gene expression for *PDR1*, *CDR1*, respectively, in *C. glabrata* ATCC 2001 after 2 and 4 h of four different treatments at 37°C : YPD broth (untreated) and YPD broth with either 5 mg/l FLC, 5 mg/l MPA, or 5 mg/l FLC + 5 mg/l MPA. The figures show one representative experiment with three independent biological replicates for each strain tested. Statistical analysis was performed in GraphPad Prism using one-way ANOVA and Bonferroni's multiple comparison test. *P*-value of less than 0.05 was significant, statistically significant differences are marked with *.

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Table 3 Interactions of azole antifungals and MPA against *C. glabrata* ATCC 2001 interpreted by FICI and BI.

Drug	MIC (mg/l)	FICI (+ MPA)		BI (+ MPA)		
		(Range)	INT	Σ SYN (<i>n</i>)	Σ ANT (<i>n</i>)	INT
FLC	32	1.06–2.5	IND	6.42% (1)	–266.9% (15)	ANT
ITC	0.25	1.25–4.25	ANT	16.59% (2)	–477.41% (22)	ANT
KCT	0.5	1.25–4.125	ANT	0	–229.8% (12)	ANT
VRC	0.25	1.063–2.5	IND	53.1% (7)	–284.2% (11)	ANT
POS	0.25	1.5–4.125	ANT	0	–321.1% (9)	ANT

Notes.

INT, interpretation; IND, indifference; ANT, antagonism.

n, number of significant interactions (out of 49, which combine the entire interaction surface of a single experiment) in one representative experiment with three independent biological replicates for each drug combination tested.

VRC, and POS. Table 3 shows the MIC, FICI, and BI of this strain against azole antifungals combined with MPA (MIC for MPA ranges from 32 to 64 mg/l) in YPD at 37 °C after 48 h. All of the interactions were interpreted as antagonistic by at least one model. This confirms that an antagonistic interaction between MPA and all of the selected azole antifungals occurs in *C. glabrata* ATCC 2001.

The roles of *PDR1* and *CDR1*

Gene expression of *PDR1* and *CDR1* was analysed in *C. glabrata* ATCC 2001 at two different timepoints (Fig. 2). It was calculated relative to the untreated samples for each timepoint. All of the following differences described have statistical significance (p -value < 0.05).

At 2 h, *PDR1* expression was significantly higher in samples treated with FLC. It was higher than in all the other conditions, including the samples treated with the combination of FLC + MPA. At 4 h the expression of *PDR1* was still higher in samples treated with FLC compared to the untreated samples and samples treated with MPA.

For *CDR1*, at 2 h, all treated samples (FLC, MPA, FLC + MPA) had higher expression than the untreated samples. At 2 h FLC + MPA had higher expression of *CDR1* than the FLC-treated samples. At 4 h the expression of *CDR1* was even higher in MPA and FLC + MPA-treated samples and was significantly higher than in the samples treated only with FLC.

The expression patterns for *CDR1*, which seemed like a good candidate to explain the antagonism of the drug combination, and the low expression of *PDR1* in the MPA and FLC + MPA-treated samples questioned whether the *PDR1* regulatory cascade has a central role in the drug response mechanism responsible for antagonism between FLC and MPA. To test this, we performed a checkerboard assay with *Cgpd1* Δ mutant against the combination of FLC and MPA. Figure 2B shows the relative growth from this assay, in which we saw increased susceptibility to FLC (MIC at 4 mg/l) and the antagonistic pattern, which was confirmed by the BI model (Σ ANT = –320.79%). These results suggest that the typical *PDR1* drug response is not the only pathway required for this antagonistic interaction and that the induction of *CDR1* in this case could to be regulated by alternative pathways.

DISCUSSION

Drug combinations can present an attractive way to deal with antifungal resistance and the lack of antifungals, but there may be dangerous complications when there are adverse reactions in the host or antagonism between the drugs. In this study, the combination of MPA and FLC made eight out of twelve strains (five *S. cerevisiae*, three *C. glabrata*; all are less resistant strains) more tolerant to the antifungal. This could have consequences in the clinical environment, and it also opens a path to explore drug resistance mechanisms.

Synergism: calcineurin inhibitors + antifungals

Certain patterns were observed with different drug combinations (Fig. 1). The most striking one was a generally synergistic effect between most antifungals and calcineurin inhibitors (CsA and Fk506) against all strains tested. The synergistic effect of calcineurin inhibitors and antifungals is well documented (Cruz *et al.*, 2002; Li *et al.*, 2015; Denardi *et al.*, 2015; Yu, Chang & Chen, 2015). Our results differ to those of (Cruz *et al.*, 2002) who reported synergistic toxicity toward other fungal species but not *S. cerevisiae*. This may be due to a strain-specific response in *S. cerevisiae*, but further tests are required to clarify this.

Antagonism: MPA + azoles

Eight out of twelve antagonistic interactions between MPA and FLC were found. Antagonism was not observed in strains that had high resistance to FLC (Sc2 32–64 mg/l, Cg5 64–128 mg/l, and Cg6 128 mg/l), which indicates that the antagonism is probably the result of similar mechanisms that produce the high resistance. FLC resistance mechanisms include overexpression of the efflux pumps in most cases (Whaley & Rogers, 2016). The antifungal activity of MPA, which is due to the depletion of purine nucleotides (Banerjee, Burkard & Panepinto, 2014), made the antagonistic effect all the more surprising. We therefore explored whether the most common mechanism of azole resistance in *C. glabrata* and *S. cerevisiae* (Pdr1 induction of efflux pump Cdr1/Pdr5) had a role in this antagonistic interaction.

Antagonism: the roles of *PDR1* and *CDR1*

The central role of the transcriptional factor Pdr1 in azole resistance has been described in detail (Caudle *et al.*, 2011; Yibmantasiri *et al.*, 2014). It is usually linked to the induction of efflux pumps (Cdr1 as a dominant example) that remove the azole antifungals from the cell. In this study, relatively high expression values of *CDR1* were observed in the drug combination FLC + MPA and MPA alone compared to the untreated samples and even to the FLC-treated ones (Fig. 2D). This makes the induction of efflux pumps a good explanation for the increased resistance to azole antifungals. However, an interesting aspect arose when examining the expression values of *PDR1* (Fig. 2C) because their expression pattern did not match the *CDR1* induction. Normally, Pdr1 positively regulates the expression of *CDR1*, but this was not seen here. This suggests that Pdr1 is not the only regulatory mechanism enabling a higher expression of *CDR1* with MPA or the FLC + MPA combination. This was further demonstrated by an antagonistic effect in the *Cgpd1Δ* mutant, interpreted by the BI model with a Σ ANT value of -320.79% , although at lower

concentrations of FLC (Fig. 2B). Undoubtedly Pdr1 still plays a major role in overall resistance (because resistance to FLC did drop in the combination versus *Cgpd1Δ*), but the antagonistic pattern still existed. This opens up new and interesting questions regarding which mechanisms instead of the Pdr1 regulatory cascade are responsible for higher *CDR1* expression and the observed antagonistic effect. Alternative regulators of *CDR1* could include transcriptional factors associated with multidrug resistance (e.g., *RDR1*, *YRR1*, *YRM1*, and *STB5*) or *CAD1*, *MSN2*, and *MSN4* for general stress response, *YAP1* for oxidative stress, *CRZ1* from calcineurin-mediated stress response, or *ECM22* and *UPC2* for cell membrane composition (Monteiro et al., 2017; Teixeira et al., 2018). In addition to Cdr1, other efflux pumps associated with azole resistance should also be considered in the future, such as Cdr2 (Miyazaki et al., 1998), Snq2 (Torelli et al., 2008), Flr1 (Alarco et al., 1997), Qdr2 (Costa et al., 2013), Tpo1_2 (Pais et al., 2016), Ybt1 (Tsai et al., 2010), Yhk8 (Barker, Pearson & Rogers, 2003) and Yor1 (Vermitsky et al., 2006). Further studies at the systemic level would be required to obtain a better picture of the entire mechanism.

Antagonism: clinical environment

There are clinical implications to this discovery. MPA (and its prodrug mycophenolate mofetil) is widely used as a maintenance immunosuppressive regimen in solid organ transplant patients, for the prophylaxis and treatment of acute and chronic graft-versus-host disease, and to promote engraftment after hematopoietic stem cell transplantation (Zhang & Chow, 2016). There is no data on the frequency of clinical usage for the combination of FLC + MPA; however, antifungal prophylaxis with azoles (VRC, POS, ITC, and FLC) is commonly prescribed in immunocompromised populations, which also involve the use of MPA (Pappas & Silveira, 2009; Brizendine, Vishin & Baddley, 2011; Groll et al., 2014). Possible antifungal prophylaxis or actual treatments with azole antifungals in these cases should therefore be used with caution and considered for each individual case because induced resistance could result in a failed therapy. There is even a report of a statistically significant increase in fungal infections in the geriatric renal transplant population when receiving MPA versus azathioprine, but the specific organisms and sites of infection were not reported (Meier-Kriesche et al., 1999; Ritter & Pirofski, 2009). It can be speculated that this antagonism could be connected to this increase in fungal infections, because FLC and other azoles (VRC, POS and ITR) are used for the prophylaxis in solid organ transplantations and other therapies involving immunocompromised patients (Pappas & Silveira, 2009; Brizendine, Vishin & Baddley, 2011; Groll et al., 2014; Vazquez, 2016). The next steps should expand the number of tested strains and species against the combination of azoles and MPA, include an *in vivo* evaluation of the combination, and include other drugs involved in a certain therapy as well; for example, a typical cocktail of prednisolone, MPA, and cyclosporine in solid organ transplantations (Sollinger, 1995).

CONCLUSION

This study examined how combinations of immunomodulatory and antifungal drugs affect selected strains of *C. glabrata* and *S. cerevisiae*. It confirmed a strong synergistic toxicity between calcineurin inhibitors and antifungals, but also discovered an antagonistic

interaction between MPA and azoles in non-resistant strains. Based on observation of gene expression in *C. glabrata* ATCC 2001 this is probably due to increased expression of drug efflux pump Cdr1 by MPA. However, the mechanism of the induction is still unknown because the Pdr1 regulatory cascade was not essential for the antagonistic interaction, and this deserves further investigation. In addition, the combined use of MPA and azoles in the clinical environment should be carefully reevaluated. In particular, there is a need to recognize that drug combinations affect not only the host but also the pathogen.

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The authors declare there are no competing interests.

Author Contributions

- Miha Tome and Jure Zupan conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Zorica Tomičić conceived and designed the experiments, performed the experiments, analyzed the data, authored or reviewed drafts of the paper, approved the final draft.
- Tadeja Matos and Peter Raspor conceived and designed the experiments, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.

Data Availability

The following information was supplied regarding data availability:
The raw data are provided in the [Supplemental Files](#).

Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.4999#supplemental-information>.

REFERENCES

- Adams A, Gottschling D, Kaiser C, Stearns T. 1998.** *Methods in yeast genetics: a laboratory course manual*. New York: Cold Spring Harbor Laboratory Press.
- Alarco AM, Balan I, Talibi D, Mainville N, Raymond M. 1997.** Ap1-mediated multidrug resistance in *Saccharomyces cerevisiae* requires FLR1 encoding a transporter of the major facilitator superfamily. *Journal of Biological Chemistry* **272**:19304–19313 DOI [10.1074/jbc.272.31.19304](https://doi.org/10.1074/jbc.272.31.19304).
- Alexander BD, Johnson MD, Pfeiffer CD, Jiménez-Ortigosa C, Catania J, Booker R, Castanheira M, Messer SA, Perlin DS, Pfaller MA. 2013.** Increasing echinocandin resistance in *Candida glabrata*: clinical failure correlates with presence of FKS mutations and elevated minimum inhibitory concentrations. *Clinical Infectious Diseases* **56**:1724–1732 DOI [10.1093/cid/cit136](https://doi.org/10.1093/cid/cit136).
- Aucott JN, Fayen J, Grossnicklas H, Morrissey A, Lederman MM, Salata RA. 1990.** Invasive infection with *Saccharomyces cerevisiae*: report of three cases and review. *Clinical Infectious Diseases* **12**:406–411 DOI [10.1093/clinids/12.3.406](https://doi.org/10.1093/clinids/12.3.406).
- Banerjee D, Burkard L, Panepinto JC. 2014.** Inhibition of nucleotide biosynthesis potentiates the antifungal activity of Amphotericin B. *PLOS ONE* **9**:e87246 DOI [10.1371/journal.pone.0087246](https://doi.org/10.1371/journal.pone.0087246).
- Barchiesi F, Arzeni D, Caselli F, Scalise G. 2000.** Primary resistance to flucytosine among clinical isolates of *Candida* spp. *Journal of Antimicrobial Chemotherapy* **45**:408–409 DOI [10.1093/jac/45.3.408](https://doi.org/10.1093/jac/45.3.408).
- Barker KS, Pearson MM, Rogers PD. 2003.** Identification of genes differentially expressed in association with reduced azole susceptibility in *Saccharomyces cerevisiae*. *Journal of Antimicrobial Chemotherapy* **51**:1131–1140 DOI [10.1093/jac/dkg217](https://doi.org/10.1093/jac/dkg217).
- Berrouane YF, Herwaldt LA, Pfaller MA. 1999.** Trends in antifungal use and epidemiology of nosocomial yeast infections in a University Hospital. *Journal of Clinical Microbiology* **37**:531–537.
- Bliss CI. 1939.** The toxicity of poisons applied jointly. *Annals of Applied Biology* **26**:585–615 DOI [10.1111/j.1744-7348.1939.tb06990.x](https://doi.org/10.1111/j.1744-7348.1939.tb06990.x).
- Bollenbach T. 2015.** Antimicrobial interactions: mechanisms and implications for drug discovery and resistance evolution. *Current Opinion in Microbiology* **27**:1–9 DOI [10.1016/j.mib.2015.05.008](https://doi.org/10.1016/j.mib.2015.05.008).
- Bolotin-Fukuhara M, Fairhead C. 2014.** *Candida glabrata*: a deadly companion? *Yeast* **31**:279–288 DOI [10.1002/yea.3019](https://doi.org/10.1002/yea.3019).
- Brizendine KD, Vishin S, Baddley JW. 2011.** Antifungal prophylaxis in solid organ transplant recipients. *Expert Review of Anti-infective Therapy* **9**:571–581 DOI [10.1586/eri.11.29](https://doi.org/10.1586/eri.11.29).

- Brun S, Bergès T, Poupard P, Vauzelle-Moreau C, Renier G, Chabasse D, Bouchara J-PP, Bergés T, Poupard P, Vauzelle-Moreau C, Renier G, Chabasse D, Bouchara J-PP. 2004.** Mechanisms of azole resistance in petite mutants of *Candida glabrata*. *Antimicrobial Agents and Chemotherapy* **48**:1788–1796 DOI [10.1128/AAC.48.5.1788-1796.2004](https://doi.org/10.1128/AAC.48.5.1788-1796.2004).
- Cassone M, Serra P, Mondello F, Girolamo A, Scafetti S, Pistella E, Venditti M. 2003.** Outbreak of *Saccharomyces cerevisiae* subtype boulardii fungemia in patients neighboring those treated with a probiotic preparation of the organism. *Journal of Clinical Microbiology* **41**:5340–5343 DOI [10.1128/JCM.41.11.5340-5343.2003](https://doi.org/10.1128/JCM.41.11.5340-5343.2003).
- Caudle KE, Barker KS, Wiederhold NP, Xu L, Homayouni R, Rogers PD. 2011.** Genomewide expression profile analysis of the *Candida glabrata* Pdr1 regulon. *Eukaryotic Cell* **10**:373–383 DOI [10.1128/EC.00073-10](https://doi.org/10.1128/EC.00073-10).
- CLSI. 2008.** *Reference method for broth dilution antifungal susceptibility testing of yeasts; Approved standard, CLSI document M27-A3*. Third edition. Wayne: Clinical and Laboratory Standards Institute (CLSI).
- Colombo AL, Júnior JNDA, Guinea J. 2017.** Emerging multidrug-resistant *Candida* species. *Current Opinion in Infectious Diseases* **30**:528–538 DOI [10.1097/QCO.0000000000000411](https://doi.org/10.1097/QCO.0000000000000411).
- Costa C, Pires C, Cabrito TR, Renaudin A, Ohno M, Chibana H, Sá-Correia I, Teixeira MC. 2013.** *Candida glabrata* drug: H⁺ antiporter CgQdr2 confers imidazole drug resistance, being activated by transcription factor CgPdr1. *Antimicrobial Agents and Chemotherapy* **57**:3159–3167 DOI [10.1128/AAC.00811-12](https://doi.org/10.1128/AAC.00811-12).
- Cowen LE. 2008.** The evolution of fungal drug resistance: Modulating the trajectory from genotype to phenotype. *Nature Reviews Microbiology* **6**:187–198 DOI [10.1038/nrmicro1835](https://doi.org/10.1038/nrmicro1835).
- Cowen LE. 2013.** The fungal Achilles' heel: targeting Hsp90 to cripple fungal pathogens. *Current Opinion in Microbiology* **16**:377–384 DOI [10.1016/j.mib.2013.03.005](https://doi.org/10.1016/j.mib.2013.03.005).
- Cruz MC, Goldstein AL, Blankenship JR, Del Poeta M, Davis D, Cardenas ME, Perfect JR, McCusker JH, Heitman J. 2002.** Calcineurin is essential for survival during membrane stress in *Candida albicans*. *EMBO Journal* **21**:546–559 DOI [10.1093/emboj/21.4.546](https://doi.org/10.1093/emboj/21.4.546).
- Cui J, Ren B, Tong Y, Dai H, Zhang L. 2015.** Synergistic combinations of antifungals and anti-virulence agents to fight against *Candida albicans*. *Virulence* **6**:362–371 DOI [10.1080/21505594.2015.1039885](https://doi.org/10.1080/21505594.2015.1039885).
- Denardi LB, Mario DAN, Loreto ÉS, Santurio JM, Alves SH. 2015.** Synergistic effects of tacrolimus and azole antifungal compounds in fluconazole-susceptible and fluconazole-resistant *Candida glabrata* isolates. *Brazilian Journal of Microbiology* **46**:125–129 DOI [10.1590/S1517-838246120120442](https://doi.org/10.1590/S1517-838246120120442).
- Enache-Angoulvant A, Hennequin C. 2005.** Invasive *Saccharomyces* infection: a comprehensive review. *Clinical Infectious Diseases* **41**:1559–1568 DOI [10.1086/497832](https://doi.org/10.1086/497832).
- Ghannoum MA, Elewski B. 1999.** Successful treatment of fluconazole-resistant oropharyngeal candidiasis by a combination of fluconazole and terbinafine. *Clinical and Diagnostic Laboratory Immunology* **6**:921–923.

- Glöckner A, Cornely OA. 2015.** *Candida glabrata*—unique features and challenges in the clinical management of invasive infections. *Mycoses* **58**:445–450
DOI [10.1111/myc.12348](https://doi.org/10.1111/myc.12348).
- Goldoni M, Johansson C. 2007.** A mathematical approach to study combined effects of toxicants *in vitro*: evaluation of the Bliss independence criterion and the Loewe additivity model. *Toxicology in Vitro* **21**:759–769 DOI [10.1016/j.tiv.2007.03.003](https://doi.org/10.1016/j.tiv.2007.03.003).
- Groll AH, Castagnola E, Cesaro S, Dalle JH, Engelhard D, Hope W, Roilides E, Styczynski J, Warris A, Lehrnbecher T. 2014.** Fourth European Conference on Infections in Leukaemia (ECIL-4): Guidelines for diagnosis, prevention, and treatment of invasive fungal diseases in paediatric patients with cancer or allogeneic haemopoietic stem-cell transplantation. *The Lancet Oncology* **15**:e327–e340
DOI [10.1016/S1470-2045\(14\)70017-8](https://doi.org/10.1016/S1470-2045(14)70017-8).
- Hachem R, Hanna H, Kontoyiannis D, Jiang Y, Raad I. 2008.** The changing epidemiology of invasive candidiasis: *Candida glabrata* and *Candida krusei* as the leading causes of candidemia in hematologic malignancy. *Cancer* **112**:2493–2499
DOI [10.1002/cncr.23466](https://doi.org/10.1002/cncr.23466).
- Henry KW, Cruz MC, Katiyar SK, Edlind TD. 1999.** Antagonism of azole activity against *Candida albicans* following induction of multidrug resistance genes by selected antimicrobial agents. *Antimicrobial Agents and Chemotherapy* **43**:1968–1974.
- Hnisz D, Majer O, Frohner IE, Komnenovic V, Kuchler K. 2010.** The set3/Hos2 histone deacetylase complex attenuates camp/pka signaling to regulate morphogenesis and virulence of *Candida albicans*. *PLOS Pathogens* **6**:1–18
DOI [10.1371/journal.ppat.1000889](https://doi.org/10.1371/journal.ppat.1000889).
- Khatib R, Johnson LB, Fakhri MG, Riederer K, Briski L. 2016.** Current trends in candidemia and species distribution among adults: *Candida glabrata* surpasses *C. albicans* in diabetic patients and abdominal sources. *Mycoses* **59**:781–786
DOI [10.1111/myc.12531](https://doi.org/10.1111/myc.12531).
- Kontoyiannis DP. 2000.** Modulation of fluconazole sensitivity by the interaction of mitochondria and erg3p in *Saccharomyces cerevisiae*. *The Journal of Antimicrobial Chemotherapy* **46**:191–197 DOI [10.1093/jac/46.2.191](https://doi.org/10.1093/jac/46.2.191).
- LaFayette SL, Collins C, Zaas AK, Schell WA, Betancourt-Quiroz M, Leslie Gunatilaka AA, Perfect JR, Cowen LE, Gunatilaka AAL, Perfect JR, Cowen LE, Leslie Gunatilaka AA, Perfect JR, Cowen LE, Gunatilaka AAL, Perfect JR, Cowen LE. 2010.** PKC signaling regulates drug resistance of the fungal pathogen *Candida albicans* via circuitry comprised of mkc1, calcineurin, and hsp90. *PLOS Pathogens* **6**:e1001069
DOI [10.1371/journal.ppat.1001069](https://doi.org/10.1371/journal.ppat.1001069).
- Lherm T, Monet C, Nougère B, Soulier M, Larbi D, Le Gall C, Caen D, Malbrunot C. 2002.** Seven cases of fungemia with *Saccharomyces boulardii* in critically ill patients. *Intensive Care Medicine* **28**:797–801 DOI [10.1007/s00134-002-1267-9](https://doi.org/10.1007/s00134-002-1267-9).
- Li H, Chen Z, Zhang C, Gao Y, Zhang X, Sun S. 2015.** Resistance reversal induced by a combination of fluconazole and tacrolimus (FK506) in *Candida glabrata*. *Journal of Medical Microbiology* **64**:44–52 DOI [10.1099/jmm.0.081760-0](https://doi.org/10.1099/jmm.0.081760-0).

- Li QQ, Skinner J, Bennett JE. 2012.** Evaluation of reference genes for real-time quantitative PCR studies in *Candida glabrata* following azole treatment. *BMC Molecular Biology* **13**:22 DOI [10.1186/1471-2199-13-22](https://doi.org/10.1186/1471-2199-13-22).
- Liguori G, Di Onofrio V, Lucariello A, Gallé F, Signoriello G, Colella G, D'Amora M, Rossano F. 2009.** Oral candidiasis: a comparison between conventional methods and multiplex polymerase chain reaction for species identification. *Oral Microbiology and Immunology* **24**:76–78 DOI [10.1111/j.1399-302X.2008.00447.x](https://doi.org/10.1111/j.1399-302X.2008.00447.x).
- Liu S, Hou Y, Chen X, Gao Y, Li H, Sun S. 2014.** Combination of fluconazole with non-antifungal agents: a promising approach to cope with resistant *Candida albicans* infections and insight into new antifungal agent discovery. *International Journal of Antimicrobial Agents* **43**:395–402 DOI [10.1016/j.ijantimicag.2013.12.009](https://doi.org/10.1016/j.ijantimicag.2013.12.009).
- Loewe S. 1928.** Die quantitativen Probleme der Pharmakologie. *Ergebnisse der Physiologie* **27**:47–187 DOI [10.1007/BF02322290](https://doi.org/10.1007/BF02322290).
- Meier-Kriesche HU, Friedman G, Jacobs M, Mulgaonkar S, Vaghela M, Kaplan B. 1999.** Infectious complications in geriatric renal transplant patients: comparison of two immunosuppressive protocols. *Transplantation* **68**:1496–1502 DOI [10.1097/00007890-199911270-00012](https://doi.org/10.1097/00007890-199911270-00012).
- Meletiadis J, Verweij PE, TeDorsthorst DTA, Meis JFGM, Mouton JW, Te Dorsthorst DTA, Meis JFGM, Mouton JW. 2005.** Assessing *in vitro* combinations of antifungal drugs against yeasts and filamentous fungi: comparison of different drug interaction models. *Medical Mycology* **43**:133–152 DOI [10.1080/13693780410001731547](https://doi.org/10.1080/13693780410001731547).
- Miyazaki H, Miyazaki Y, Geber A, Parkinson T, Hitchcock C, Falconer DJ, Ward DJ, Marsden K, Bennett JE. 1998.** Fluconazole resistance associated with drug efflux and increased transcription of a drug transporter gene, PDH1, in *Candida glabrata*. *Antimicrobial Agents and Chemotherapy* **42**:1695–1701.
- Monteiro PT, Pais P, Costa C, Manna S, Sa-Correia I, Teixeira MC. 2017.** The PathoYeast database: an information system for the analysis of gene and genomic transcription regulation in pathogenic yeasts. *Nucleic Acids Research* **45**:D597–D603 DOI [10.1093/nar/gkw817](https://doi.org/10.1093/nar/gkw817).
- Moody J. 2010.** Synergism testing: broth microdilution checkerboard and broth macrodilution methods. In: Garcia L, ed. *Clinical microbiology procedures handbook*. 3rd Edition. Washington, D.C.: American Society of Microbiology, 140–162.
- Morio F, Pagniez F, Lacroix C, Miegerville M, pape PL. 2012.** Amino acid substitutions in the *Candida albicans* sterol δ 5,6-desaturase (Erg3p) confer azole resistance: characterization of two novel mutants with impaired virulence. *Journal of Antimicrobial Chemotherapy* **67**:2131–2138 DOI [10.1093/jac/dks186](https://doi.org/10.1093/jac/dks186).
- Moye-Rowley WS. 2003.** Transcriptional control of multidrug resistance in the yeast *Saccharomyces*. *Progress in Nucleic Acid Research and Molecular Biology* **73**:251–279 DOI [10.1016/S0079-6603\(03\)01008-0](https://doi.org/10.1016/S0079-6603(03)01008-0).
- Murphy A, Kavanagh K. 1999.** Emergence of *Saccharomyces cerevisiae* as a human pathogen Implications for biotechnology. *Enzyme and Microbial Technology* **25**:551–557 DOI [10.1016/S0141-0229\(99\)00086-1](https://doi.org/10.1016/S0141-0229(99)00086-1).

- Nagi M, Nakayama H, Tanabe K, Bard M, Aoyama T, Okano M, Higashi S, Ueno K, Chibana H, Niimi M, Yamagoe S, Umeyama T, Kajiwara S, Ohno H, Miyazaki Y. 2011. Transcription factors CgUPC2A and CgUPC2B regulate ergosterol biosynthetic genes in *Candida glabrata*. *Genes to Cells* 16:80–89 DOI 10.1111/j.1365-2443.2010.01470.x.
- Nakayama H, Tanabe K, Bard M, Hodgson W, Wu S, Takemori D, Aoyama T, Kumaraswami NS, Metzler L, Takano Y, Chibana H, Niimi M, Kumaraswami SN, Metzler L, Takano Y, Chibana H, Niimi M. 2007. The *Candida glabrata* putative sterol transporter gene CgAUS1 protects cells against azoles in the presence of serum. *Journal of Antimicrobial Chemotherapy* 60:1264–1272 DOI 10.1093/jac/dkm321.
- Navarro-Martínez MD, Cabezas-Herrera J, Rodríguez-López JN. 2006. Antifolates as antimycotics? Connection between the folic acid cycle and the ergosterol biosynthesis pathway in *Candida albicans*. *International Journal of Antimicrobial Agents* 28:560–567 DOI 10.1016/j.ijantimicag.2006.07.012.
- Nett JE, Andes DR. 2016. Antifungal agents: spectrum of activity, pharmacology, and clinical indications. *Infectious Disease Clinics of North America* 30:51–83 DOI 10.1016/j.idc.2015.10.012.
- Odds FC. 2003. Synergy, antagonism, and what the checkerboard puts between them. *The Journal of Antimicrobial Chemotherapy* 52:1 DOI 10.1093/jac/dkg301.
- Pachl J, Svoboda P, Jacobs F, Vandewoude K, Hoven Bvander, Spronk P, Masterson G, Malbrain M, Aoun M, Garbino J, Takala J, Drgona L, Burnie J, Matthews R. 2006. A randomized, blinded, multicenter trial of lipid-associated amphotericin B alone versus in combination with an antibody-based inhibitor of heat shock protein 90 in patients with invasive candidiasis. *Clinical Infectious Diseases* 42:1404–1413 DOI 10.1086/503428.
- Pais P, Costa C, Pires C, Shimizu K, Chibana H, Teixeira MC. 2016. Membrane proteome-wide response to the antifungal drug clotrimazole in *Candida glabrata*: role of the transcription factor CgPdr1 and the Drug:H⁺ Antiporters CgTpo1_1 and CgTpo1_2. *Molecular & Cellular Proteomics* 15:57–72 DOI 10.1074/mcp.M114.045344.
- Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, Reboli AC, Schuster MG, Vazquez JA, Walsh TJ, Zaoutis TE, Sobel JD. 2015. Clinical practice guideline for the management of candidiasis: 2016 update by the infectious diseases society of America. *Clinical Infectious Diseases* 62:e1–e50 DOI 10.1093/cid/civ933.
- Pappas PG, Silveira FP. 2009. *Candida* in solid organ transplant recipients. *American Journal of Transplantation* 9:S173–S179 DOI 10.1111/j.1600-6143.2009.02909.x.
- Pfaffl MW. 2001. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research* 29:e45 DOI 10.1093/nar/29.9.e45.
- Pfaller MA. 2012. Antifungal drug resistance: mechanisms, epidemiology, and consequences for treatment. *The American Journal of Medicine* 125:S3–S13 DOI 10.1016/j.amjmed.2011.11.001.

- Pfaller MA, Andes DR, Diekema DJ, Horn DL, Reboli AC, Rotstein C, Franks B, Azie NE. 2014.** Epidemiology and outcomes of invasive candidiasis due to non-albicans species of *Candida* in 2,496 patients: data from the Prospective Antifungal Therapy (PATH) registry 2004–2008. *PLOS ONE* **9**:e101510
[DOI 10.1371/journal.pone.0101510](https://doi.org/10.1371/journal.pone.0101510).
- Pfaller MA, Castanheira M. 2016.** Nosocomial candidiasis: Antifungal stewardship and the importance of rapid diagnosis. *Medical Mycology* **54**:1–22
[DOI 10.1093/mmy/myv076](https://doi.org/10.1093/mmy/myv076).
- Pfaller MA, Castanheira M, Lockhart SR, Jones RN. 2012a.** *Candida glabrata*: multidrug resistance and increased virulence in a major opportunistic fungal pathogen. *Current Fungal Infection Reports* **6**:154–164 [DOI 10.1007/s12281-012-0091-0](https://doi.org/10.1007/s12281-012-0091-0).
- Pfaller MA, Diekema DJ. 2007.** Epidemiology of invasive candidiasis: a persistent public health problem. *Clinical Microbiology Reviews* **20**:133–163
[DOI 10.1128/CMR.00029-06](https://doi.org/10.1128/CMR.00029-06).
- Pfaller MA, Diekema DJ. 2010.** Epidemiology of invasive mycoses in North America. *Critical Reviews in Microbiology* **36**:1–53 [DOI 10.3109/10408410903241444](https://doi.org/10.3109/10408410903241444).
- Pfaller M, Neofytos D, Diekema D, Azie N, Meier-Kriesche HU, Quan SP, Horn D. 2012b.** Epidemiology and outcomes of candidemia in 3648 patients: data from the prospective antifungal therapy (PATH Alliance??) registry, 2004–2008. *Diagnostic Microbiology and Infectious Disease* **74**:323–331
[DOI 10.1016/j.diagmicrobio.2012.10.003](https://doi.org/10.1016/j.diagmicrobio.2012.10.003).
- Piarroux R, Millon L, Bardonnnet K, Vagner O, Koenig H. 1999.** Are live saccharomyces yeasts harmful to patients?. *Lancet* **353**:1851–1852
[DOI 10.1016/S0140-6736\(99\)02001-2](https://doi.org/10.1016/S0140-6736(99)02001-2).
- Rex JH, Pappas PG, Karchmer AW, Sobel J, Edwards JE, Hadley S, Brass C, Vazquez JA, Chapman SW, Horowitz HW, Zervos M, McKinsey D, Lee J, Babinchak T, Bradsher RW, Cleary JD, Cohen DM, Danziger L, Goldman M, Goodman J, Hilton E, Hyslop NE, Kett DH, Lutz J, Rubin RH, Scheld WM, Schuster M, Simmons B, Stein DK, Washburn RG, Mautner L, Chu T, Panzer H, Rosenstein RB, Booth J. 2003.** A randomized and blinded multicenter trial of high-dose fluconazole plus placebo versus Fluconazole plus Amphotericin B as therapy for candidemia and its consequences in nonneutropenic subjects. *Clinical Infectious Diseases* **36**:1221–1228
[DOI 10.1086/374850](https://doi.org/10.1086/374850).
- Ritter ML, Pirofski L. 2009.** Mycophenolate mofetil: effects on cellular immune subsets, infectious complications, and antimicrobial activity. *Transplant Infectious Disease* **11**:290–297 [DOI 10.1111/j.1399-3062.2009.00407.x](https://doi.org/10.1111/j.1399-3062.2009.00407.x).
- Roetzer A, Gabaldón T, Schüller C. 2011.** From *Saccharomyces cerevisiae* to *Candida glabrata* in a few easy steps: important adaptations for an opportunistic pathogen. *FEMS Microbiology Letters* **314**:1–9 [DOI 10.1111/j.1574-6968.2010.02102.x](https://doi.org/10.1111/j.1574-6968.2010.02102.x).
- Roy U, Jessani LG, Rudramurthy SM, Gopalakrishnan R, Dutta S, Chakravarty C, Jillwin J, Chakrabarti A. 2017.** Seven cases of *Saccharomyces fungaemia* related to use of probiotics. *Mycoses* **60**(6):375–380 [DOI 10.1111/myc.12604](https://doi.org/10.1111/myc.12604).

- Sanglard D, Ischer F, Bille J. 2001.** Role of ATP-binding-cassette transporter genes in high-frequency acquisition of resistance to azole antifungals in *Candida glabrata*. *Antimicrobial Agents and Chemotherapy* **45**:1174–1183 DOI [10.1128/AAC.45.4.1174-1183.2001](https://doi.org/10.1128/AAC.45.4.1174-1183.2001).
- Sanguinetti M, Posteraro B, Fiori B, Ranno S, Torelli R, Fadda G. 2005.** Mechanisms of azole resistance in clinical isolates of *Candida glabrata* collected during a hospital survey of antifungal resistance. *Antimicrobial Agents and Chemotherapy* **49**:668–679 DOI [10.1128/AAC.49.2.668-679.2005](https://doi.org/10.1128/AAC.49.2.668-679.2005).
- Scheven M, Junemann K, Schramm H, Huhn W. 1992.** Successful treatment of a *Candida albicans* sepsis with a combination of flucytosine and fluconazole. *Mycoses* **35**:315–316 DOI [10.1111/j.1439-0507.1992.tb00886.x](https://doi.org/10.1111/j.1439-0507.1992.tb00886.x).
- Schwarz Müller T, Ma B, Hiller E, Istel F, Tscherner M, Brunke S, Ames L, Firon A, Green B, Cabral V, Marcet-Houben M, Jacobsen ID, Quintin J, Seider K, Frohner I, Glaser W, Jungwirth H, Bachellier-Bassi S, Chauvel M, Zeidler U, Ferrandon D, Gabaldón T, Hube B, D'Enfert C, Rupp S, Cormack B, Haynes K, Kuchler K. 2014.** Systematic phenotyping of a large-scale *Candida glabrata* deletion collection reveals novel antifungal tolerance genes. *PLOS Pathogens* **10**:e1004211 DOI [10.1371/journal.ppat.1004211](https://doi.org/10.1371/journal.ppat.1004211).
- Segireddy M, Johnson LB, Szpunar SM, Khatib R. 2011.** Differences in patient risk factors and source of candidaemia caused by *Candida albicans* and *Candida glabrata*. *Mycoses* **54**:e39–e43 DOI [10.1111/j.1439-0507.2009.01824.x](https://doi.org/10.1111/j.1439-0507.2009.01824.x).
- Shah CP, Kharkar PS. 2015.** Inosine 5'-monophosphate dehydrogenase inhibitors as antimicrobial agents: recent progress and future perspectives. *Future Medicinal Chemistry* **7**:1415–1429 DOI [10.4155/fmc.15.72](https://doi.org/10.4155/fmc.15.72).
- Silva S, Negri M, Henriques M, Oliveira R, Williams DW, Azeredo J. 2012.** *Candida glabrata*, *Candida parapsilosis* and *Candida tropicalis*: biology, epidemiology, pathogenicity and antifungal resistance. *FEMS Microbiology Reviews* **36**:288–305 DOI [10.1111/j.1574-6976.2011.00278.x](https://doi.org/10.1111/j.1574-6976.2011.00278.x).
- Sollinger HW. 1995.** Mycophenolate mofetil for the prevention of acute rejection in primary cadaveric renal allograft recipients. US Renal Transplant Mycophenolate Mofetil Study Group. *Transplantation* **60**:225–232 DOI [10.1097/00007890-199508000-00003](https://doi.org/10.1097/00007890-199508000-00003).
- Steinbach WJ, Reedy JL, Cramer RA, Perfect JR, Heitman J. 2007.** Harnessing calcineurin as a novel anti-infective agent against invasive fungal infections. *Nature Reviews Microbiology* **5**:418–430 DOI [10.1038/nrmicro1680](https://doi.org/10.1038/nrmicro1680).
- Svetaz LA, Postigo A, Butassi E, Zacchino SA, Sortino MA. 2016.** Antifungal drugs combinations: a patent review 2000–2015. *Expert Opinion on Therapeutic Patents* **26**:439–453 DOI [10.1517/13543776.2016.1146693](https://doi.org/10.1517/13543776.2016.1146693).
- Teixeira MC, Monteiro PT, Palma M, Costa C, Godinho CP, Pais P, Cavalheiro M, Antunes M, Lemos A, Pedreira T, Sá-Correia I. 2018.** YEASTRACT: an upgraded database for the analysis of transcription regulatory networks in *Saccharomyces cerevisiae*. *Nucleic Acids Research* **46**:D348–D353 DOI [10.1093/nar/gkx842](https://doi.org/10.1093/nar/gkx842).

- Torelli R, Posteraro B, Ferrari S, La Sorda M, Fadda G, Sanglard D, Sanguinetti M. 2008.** The ATP-binding cassette transporter-encoding gene CgSNQ2 is contributing to the CgPDR1-dependent azole resistance of *Candida glabrata*. *Molecular Microbiology* **68**:186–201 DOI [10.1111/j.1365-2958.2008.06143.x](https://doi.org/10.1111/j.1365-2958.2008.06143.x).
- Tsai HF, Krol AA, Sarti KE, Bennett JE. 2006.** *Candida glabrata* PDR1, a transcriptional regulator of a pleiotropic drug resistance network, mediates azole resistance in clinical isolates and petite mutants. *Antimicrobial Agents and Chemotherapy* **50**:1384–1392 DOI [10.1128/AAC.50.4.1384-1392.2006](https://doi.org/10.1128/AAC.50.4.1384-1392.2006).
- Tsai H-FF, Sammons LR, Zhang X, Suffis SD, Su Q, Myers TG, Marr KA, Bennett JE. 2010.** Microarray and molecular analyses of the azole resistance mechanism in *Candida glabrata* oropharyngeal isolates. *Antimicrobial Agents and Chemotherapy* **54**:3308–3317 DOI [10.1128/AAC.00535-10](https://doi.org/10.1128/AAC.00535-10).
- Tscherner M, Stappler E, Hnisz D, Kuchler K. 2012.** The histone acetyltransferase Hat1 facilitates DNA damage repair and morphogenesis in *Candida albicans*. *Molecular Microbiology* **86**:1197–1214 DOI [10.1111/mmi.12051](https://doi.org/10.1111/mmi.12051).
- Vazquez L. 2016.** Antifungal prophylaxis in immunocompromised patients. *Mediterranean Journal of Hematology and Infectious Diseases* **8**:e2016040 DOI [10.4084/MJHID.2016.040](https://doi.org/10.4084/MJHID.2016.040).
- Veri A, Cowen LE. 2014.** Progress and prospects for targeting Hsp90 to treat fungal infections. *Parasitology* **141**:1127–1137 DOI [10.1017/S0031182013002072](https://doi.org/10.1017/S0031182013002072).
- Vermitsky JP, Edlind TD. 2004.** Azole resistance in *Candida glabrata*: coordinate upregulation of multidrug transporters and evidence for a Pdr1-like transcription factor. *Antimicrobial Agents and Chemotherapy* **48**:3773–3781 DOI [10.1128/AAC.48.10.3773-3781.2004](https://doi.org/10.1128/AAC.48.10.3773-3781.2004).
- Vermitsky J-P, Earhart KD, Smith WL, Homayouni R, Edlind TD, Rogers PD. 2006.** Pdr1 regulates multidrug resistance in *Candida glabrata*: gene disruption and genome-wide expression studies. *Molecular Microbiology* **61**:704–722 DOI [10.1111/j.1365-2958.2006.05235.x](https://doi.org/10.1111/j.1365-2958.2006.05235.x).
- Whaley SG, Berkow EL, Rybak JM, Nishimoto AT, Barker KS, Rogers PD. 2017.** Azole antifungal resistance in *Candida albicans* and emerging non-albicans *Candida* species. *Frontiers in Microbiology* **7**:2173 DOI [10.3389/fmicb.2016.02173](https://doi.org/10.3389/fmicb.2016.02173).
- Whaley SG, Rogers PD. 2016.** Azole resistance in *Candida glabrata*. *Current Infectious Disease Reports* **18**(12):41 DOI [10.1007/s11908-016-0554-5](https://doi.org/10.1007/s11908-016-0554-5).
- Wheeler RT, Kupiec M, Magnelli P, Abeijon C, Fink GR. 2003.** A *Saccharomyces cerevisiae* mutant with increased virulence. *Proceedings of the National Academy of Sciences of the United States of America* **100**:2766–2770 DOI [10.1073/pnas.0437995100](https://doi.org/10.1073/pnas.0437995100).
- Yeh PJ, Hegreness MJ, Aiden AP, Kishony R. 2009.** Drug interactions and the evolution of antibiotic resistance. *Nature Reviews Microbiology* **7**:460–466 DOI [10.1038/nrmicro2133](https://doi.org/10.1038/nrmicro2133).
- Yibmantasiri P, Bircham PW, Maass DR, Bellows DS, Atkinson PH. 2014.** Networks of genes modulating the pleiotropic drug response in *Saccharomyces cerevisiae*. *Molecular BioSystems* **10**:128–137 DOI [10.1039/C3MB70351G](https://doi.org/10.1039/C3MB70351G).

- Yu SJ, Chang YL, Chen YL. 2015.** Calcineurin signaling: lessons from *Candida* species. *FEMS Yeast Research* **15**(4):fov016 DOI [10.1093/femsyr/fov016](https://doi.org/10.1093/femsyr/fov016).
- Zhang D, Chow DS-L. 2016.** Clinical pharmacokinetics of mycophenolic acid in hematopoietic stem cell transplantation recipients. *European Journal of Drug Metabolism and Pharmacokinetics* **42**(2):183–189 DOI [10.1007/s13318-016-0378-6](https://doi.org/10.1007/s13318-016-0378-6).
- Zupan J, Avbelj M, Butinar B, Kosel J, Šergan M, Raspor P. 2013.** Monitoring of quorum-sensing molecules during minifermentation studies in wine yeast. *Journal of Agricultural and Food Chemistry* **61**(10):2496–2505 DOI [10.1021/jf3051363](https://doi.org/10.1021/jf3051363).