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PERSPECTIVES

Optimising the Treatment of Invasive Candidiasis – A Case for Combination Therapy

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Invasive candidiasis is a rising global health threat with increasing incidence, persistently high mortality and diminishing treatment options. Antifungal resistance has rapidly emerged and spread, with multidrug resistant species deemed an urgent and serious threat (US CDC). While acknowledging the key role of antifungal stewardship and infection control in curbing spread, we examine the role of antifungal monotherapy in driving resistance and the potential for combination therapy to prevent stress adaptation and emergence of drug resistance. In addition to its role in mitigating resistance, combination treatment may improve drug penetration, expedite fungal clearance, and allow lower, less toxic doses of individual drugs to be used. A growing body of laboratory-based evidence suggests that antifungal combinations can yield synergistic activity against *Candida* spp, including against frequently multidrug resistant *Candida auris.* It is imperative to test these combinations in clinical trials, incorporating resistance endpoints as a marker of success.

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An estimated 750,000 *Candida* bloodstream infections occur per year, mostly among critically ill patients, associated with death in 10-47% of cases[1,2]. There are only three drug classes routinely used for treatment, and resistance is rising against azoles and echinocandins, while the use of the polyene AmB (deoxycholate (AmB) and liposomal formulations (L-AmB)) is limited by toxicity and cost. New agents are on the horizon, but potential environmental use and single agent clinical use threatens their longevity. Novel pharmaceutical approaches to optimise treatment and prevent antifungal resistance are essential to preserve the effectiveness of current and future antifungals.

The scope of the problem - Antifungal-Resistant *Candida* spp

Widespread use of antifungals has driven a global epidemiological shift toward *Candida* spp. with reduced susceptibility to azoles, rising secondary resistance, and the emergence of multi-drug resistant *C. auris* and *C. glabrata* (*Nakaseomyces glabrata*)[3]. Non-albicans species, including intrinsically and frequently azole-resistant *C. krusei* (*Pichia kudriavzevii*) and *C. glabrata* are on the rise globally, and are now responsible for more than half of invasive cases submitted to SENTRY surveillance (North America, Europe, Latin America and Asia-Pacific regions)[4]. Azole-resistant *C. parapsilosis* has emerged as a global threat, following extensive spread of clonal strains harbouring resistance mutations, with multiple outbreaks reported in Europe, the US, and Brazil[5]. A recent meta-analysis of 79 studies revealed fluconazole resistance has increased from 12% to 37% of *C. parapsilosis* isolates tested globally over the past 6 years[6]. *C. parapsilosis* is the most common cause of *Candida* bloodstream infections in South Africa, where around two-thirds are azole-resistant[7].

The rapid global spread of *C. auris* is particularly concerning. Although clade variations occur, and breakpoints are tentative, *C. auris* is almost always (>90%) azole-resistant, with resistance to AmB reported in up to 30%[8,9] and multidrug resistance (to azoles and polyenes) in 3-17%[9]. Echinocandin resistance is currently rare (0-3%),[8–10] but pan-resistance (to azoles, echinocandins and AmB) has been reported[8,11]. While outbreaks continue to be reported in the US[10] and Europe[12,13], *C. auris* has become a dominant pathogen elsewhere, now causing around a third of *Candida* bloodstream infections in South Africa[7].

With azole resistance rising, increasing reliance on echinocandins is threatening the final useful drug class. Echinocandin-resistance is reported in up to 10.6% of *C. glabrata*[4] and increasingly detected in *C. auris,* rising 3-fold in the US in 2021,[10] including in patients without prior echinocandin exposure[8,10].

The Role of Antifungal Monotherapy

Although nosocomial transmission is widely acknowledged,[8] prior antifungal exposure is a known risk factor for azole and echinocandin resistance[14,15]. Antifungal monotherapy is the recommended treatment for *Candida* bloodstream infections[2], initially with echinocandins, then azole stepdown if susceptible. Single antifungals are also used for treatment of non-invasive candidiasis and prophylaxis of yeast and mould infections in immune-suppressed populations e.g. second-generation azoles in haematological malignancies. Monotherapy promotes selection of intrinsic or acquired

resistance mechanisms and induces stress adaptation (tolerance), which may facilitate yeast persistence and result in treatment failure[16]. Monotherapy may also result in suboptimal penetration of some body sites and biofilm, causing resistance compartmentalisation, observed particularly in urine, abdominal cavity, on mucosal surfaces and biofilm-prone indwelling devices[9,14].

Emergence of phenotypic and genotypic resistance has been observed in *Candida* spp during antifungal exposure. Echinocandin-resistance associated with FKS hotspot mutations developed during micafungin treatment in patients with recurrent *C. auris* bloodstream infections in the US and South Africa[8,17]. A prospective study of 193 patients treated for candidaemia in Denmark demonstrated acquired resistance in 29.4% and 21.6% of *C. glabrata* isolated from oral swabs following ≥7 days of fluconazole or anidulafungin respectively[20]. Serial colonising *Candida* isolates from ICU patients in the UK (CandiRes ISRCTN14165977) revealed a ≥4-fold MIC increase to fluconazole in 6/39 (15%) and to anidulafungin in 8/88 (11%), following ≥7 days exposure to the respective drug[18].

In addition to changes in MIC, antifungal monotherapy induces other adaptive mechanisms in *Candida* spp. Heteroresistance is an intrinsic phenomenon whereby a fraction of the total population (usually <1%) grows at drug concentrations above the MIC[16]. This resistant subpopulation is selected for, becoming predominant during drug exposure, leading to treatment failure[16,19]. Size of heteroresistant populations in colonising *Candida* spp correlated with duration of antifungal exposure in the CandiRes study[20]. Heteroresistance has been described in *C. glabrata* following azole monotherapy,[19] and was associated with breakthrough *C. parapsilosis* infections in patients taking prophylactic echinocandin treatment[21]. Antifungal tolerance is also described in *Candida* spp, whereby phenotypically drug-susceptible subpopulations persist and grow slowly at supra-MIC concentrations. Tolerant cells adapt to drug via enhanced stress-response pathway signalling, decreased drug accumulation and cell wall remodelling[16], providing time for evolution of resistance-associated mutations.

The role of combination antifungal therapy

While stringent antifungal stewardship and infection, prevention and control measures are key, combination antifungal regimens have potential to prevent resistance whilst optimising the treatment of invasive candidiasis. Combination antifungals may enhance fungal clearance, potentially allow lower doses with reduced toxicity risk, optimise antifungal penetration to sanctuary sites where *Candida* persist, and mitigate the evolution of resistance and stress responses observed during antifungal monotherapy (Fig 1). Although theoretically lower doses could be used, risk of cumulative toxicity, and increased cost of drug combinations must be weighed against potential benefit.

Current research

Most research has focused on the nature of interactions of drug combinations against *Candida* using *in vitro* or animal experiments, with very limited study of resistance mitigation. Antifungal interactions vary between synergy, indifference and antagonism depending on the combination, concentration species/ isolate (*C albicans* predominant) and model used. Findings are not always

consistent across *in vitro-in vivo* studies (Table 1): the single randomised trial of fluconazole +/- AmB reported improved mycological clearance and higher success rates with combination, despite *in vitro* and murine studies frequently reporting antagonism (see Table 1 and Appendix).

Flucytosine (5-fc) combination therapy

Flucytosine (5-FC) is perhaps the most promising agent currently available for combination treatment of invasive candidiasis. *In vitro* studies report a range of interactions including synergy with every combination, including against *C. auris*[22–24]. Murine and rabbit studies also report synergy, improved survival and reduced tissue burden for AmB-5FC combinations vs monotherapy. A single study of micafungin-5FC combination in a *C glabrata* immunocompromised mouse model showed an indifferent effect[25] (see Table 1 and Appendix). Flucytosine has excellent oral bioavailability and penetration into peritoneal fluid, a common site of yeast persistence[26]. As an old, licensed drug, and an essential component of cryptococcal meningitis treatment, flucytosine is becoming globally accessible.

Although limited clinical data exist, flucytosine combination therapy is recommended for complicated candidiasis, including meningitis and endocarditis, in clinical guidelines[2]. However, recent experience demonstrating rapidly evolving flucytosine resistance[11] must heed caution in cases of already multidrug-resistant *C. auris* where flucytosine may be the only active drug; flucytosine monotherapy is known to predispose to rapid emergence of resistance. This case, and reports of rising MICs to flucytosine in the context of occasional use in New York (personal communication, *V. Chaturvedi*), emphasises that upfront combination treatment, to *mitigate* the evolution of resistance (as has been demonstrated in cryptococcal meningitis[27]) may be appropriate, rather than reserving combinations for salvage therapy. It also demonstrates the importance of meticulous pre-clinical and clinical evaluation of drug combinations to guide future management guidelines.

Novel antifungal agents

There are promising new antifungal drugs in the pipeline for candidiasis including fosmanogepix, ibrexafungerp, and the long-acting echinocandin, rezafungin[28]. The concern, as new agents reach clinical use, is that exposure to similar molecules both in the environment, and as monotherapy in patients, will see similar resistance emerge, limiting future utility. Our *In vitro* study revealed manogepix (active compound of fosmanogepix) to be the most synergistic of four agents tested in combination with anidulafungin against 15 *C. auris* isolates (clades I and III)[24]. Future drug development research must investigate combinations to optimise and future-proof novel agents.

Future research

Although evidence that particular drug combinations can enhance *Candida* clearance is mounting, very little is known regarding impact on antifungal resistance. Future research will be enhanced by collaborations to survey and study antifungal resistance, collate diverse isolate banks and build clinical networks. *In vitro* studies, must investigate whether exposure to two drugs can suppress resistance. However, even if resistance mitigation is observed in the laboratory, differential tissue

penetration may drive resistance compartmentalisation in patients; clinical studies must follow. Drug development at both pre-clinical and clinical trial stages must consider these factors, incorporating resistance endpoints (including compartmentalisation) and pharmacometrics, as well as mycological clearance, to define sustainable treatment approaches.

CONCLUSIONS

invasive candidiasis is a rising global health threat. Emergence and spread of resistant *Candida* spp demands an urgent and innovative response. Combination treatment must be explored for combating on-treatment resistance evolution whilst enhancing efficacy. Pre-clinical studies have identified promising combinations. It is now time to take these approaches to the bedside to evaluate whether combination treatment can improve clinical and resistance outcomes for patients, particularly in settings harbouring a high burden of antifungal resistance.

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Potential conflicts of Interest:

R.B has participated in Educational activity (lectures, webinars) for Gilead and Teva, and advisory board membership for MSD and GSK.

T.B has participated in advisory boards for and received speaker fees from Gilead, Pfizer and Mundipharma, and received research funding from MSD, Gilead and Pfizer.

Patient Consent Statement

This is a perspective piece and does not include factors necessitating patient consent.

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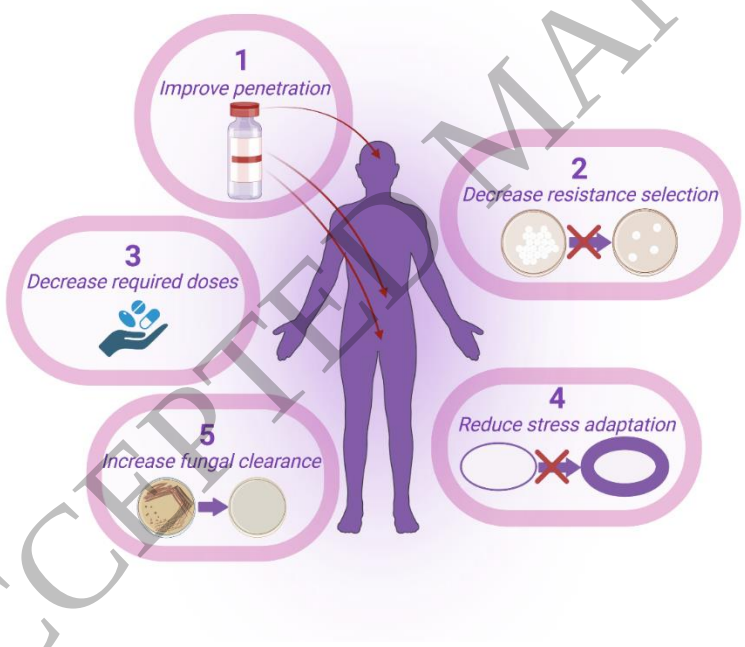

Figure 1 Mechanisms whereby combination therapy might optimise antifungal treatment of invasive candidiasis

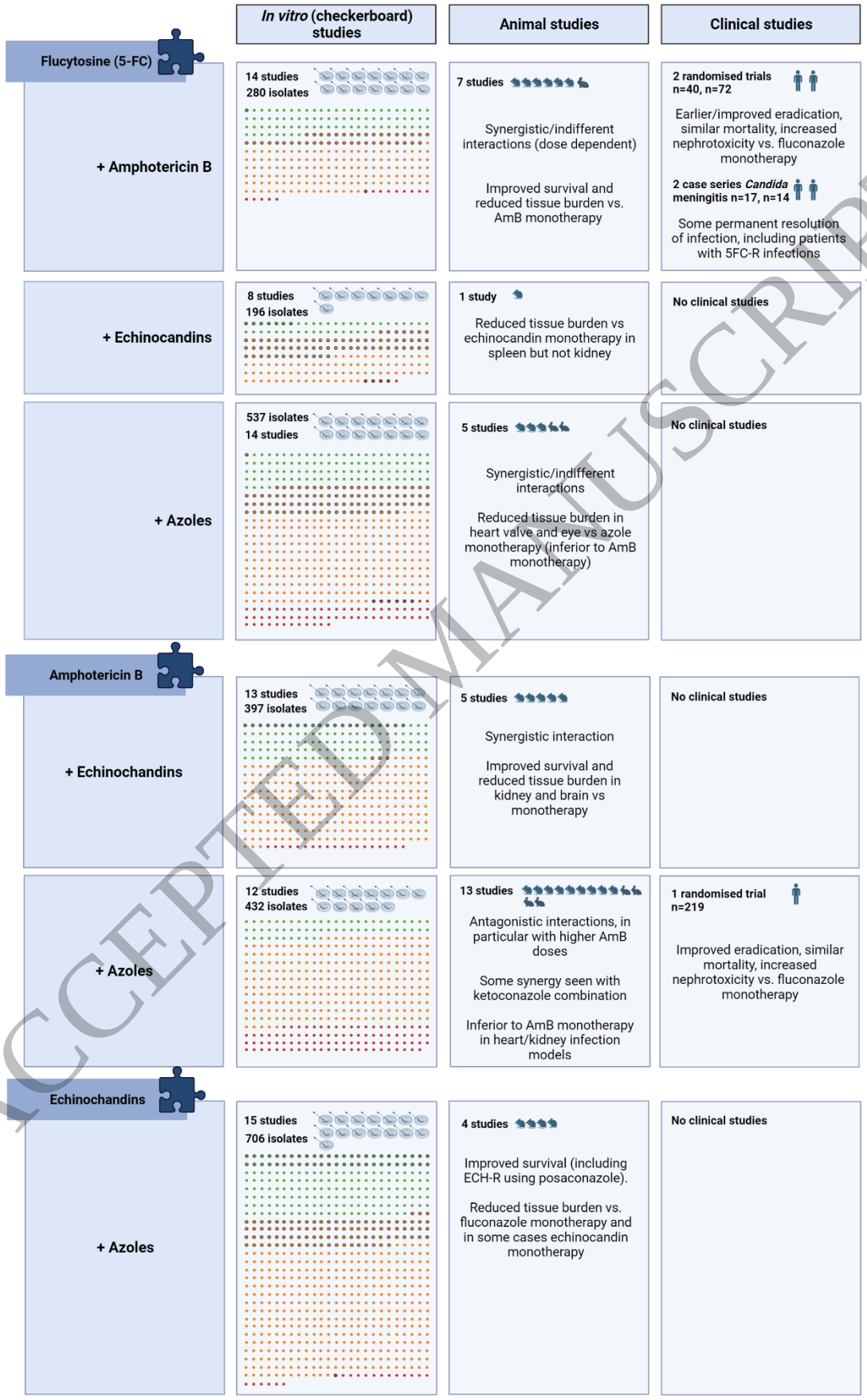

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Table 1 Summary of evidence from *In vitro*, animal and clinical studies of dual combination antifungals against *Candida* spp. Studies were included (see full table in Appendix 1) if they used antifungal drugs currently in use. *In vitro* studies were included if they used checkerboard techniques and reported fractional inhibitory concentration indices (FICI) defining synergy as FICI ≤0.5, additive/indifferent between 0.5 and 4.0, and antagonistic ≥4. Clinical studies were included if randomised trials. For *in vitro* studies, combinations are represented as synergistic, indifferent or antagonistic for each isolate by green, orange and red dots respectively. Black bordered dots represent *C. auris* isolates.

Abbreviatons: AmB = Amphotericin B; ECH = Echinocandin; 5FC = Flucytosine; -S, -R = sensitive, resistant.