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PHARMACOLOGY AND PHARMACODYNAMICS OF ANTIFUNGAL AGENTS (J AMSDEN, SECTION EDITOR)

Combination Therapy for Invasive Fungal Infections

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Abstract

Purpose of the Review The purpose of this review is to summarize and evaluate relevant literature on combination antifungal therapy for invasive fungal infections (IFIs).

Recent Findings Cryptococcal meningitis has the largest body and highest quality in support of combination therapy with amphotericin B and flucytosine. More recent data in treatment of invasive aspergillosis suggest combination therapy with voriconazole and echinocandins may be effective in select patients. Quality studies are needed to define combination therapy in rare mold infections.

Summary Multiple strategies have been employed to optimize treatment of the growing incidence of IFIs. With exceptions as noted above, justification for the use of combination antifungal therapy is most often based on uncontrolled and/or underpowered studies, in vitro data, and case reports.

Keywords Combination antifungals · Invasive fungal infections · Antifungal synergy · Aspergillosis · Cryptococcus · Candidiasis

Introduction

As modern medicine advances, invasive fungal infections (IFI) are increasing and result in significant morbidity and mortality [1]. The mean annual IFI incidence from 2006 to 2015 was 27.2 cases/100,000 patients in one US study, with an estimated increase of approximately 1% each year [1]. This study reported a crude mortality rate of 28.8% among patients with IFIs [1]. *Candida* species account for approximately half of all such infections [1]. These IFIs are most common in transplant recipients, a variety of immunocompromised hosts, and specifically patients with hematologic malignancies [2]. With a growing of the immunocompromised populations, IFI's incidence can be expected to continue to increase in the immediate future.

With a growing incidence of IFIs, several strategies have been employed in attempts to optimize antifungal therapy for deadly IFIs. These strategies include new antifungal drug development, pharmacodynamically optimized dosing, better diagnostics, and therapeutic strategies that may include the use of combination therapy [3, 4]. Rationale for the use of combination antifungal agents includes reducing risk for antifungal resistance against monotherapy, potential for dose reductions of agents associated with significant side effects (such as polyenes), and either additive or synergistic antifungal activity to increase potency of therapy and reduce lengths of treatment [5, 6]. Despite the lack of standardized testing methodology and interpretive criteria for examining combination antifungal activity, justification for most indications has initially been restricted to in vitro susceptibility or animal model studies. It is the purpose of this review to efficiently summarize the evaluation and relevant literature (in vitro, animal model, and clinical data) on combination antifungal therapy for today and tomorrow.

Challenges in Assessing the Value of Combination Therapy

Data regarding the use of combination therapy may come from in vitro, animal model, or clinical studies. Standards

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for in vitro testing for synergy and antagonism of antifungals are generally lacking correlation with clinical outcome. For instance, the checkerboard dilution technique is a form of microdilution which utilizes a two-dimensional array of serial concentrations of test compounds to measure susceptibilities [7]. This methodology is limited when testing synergy with antifungal agents (especially in molds) given the different patterns of growth inhibition exhibited by the different antifungal agents [7]. Limitations to in vitro susceptibility testing include difficulties predicting clinical outcomes and differing results depending on the specific test used [8]. The in vitro system does not take into consideration the varying pharmacokinetics and exposure of the multiple agents. While methods to model antibiotic combinations (such as the Loess method) have been published, the details of such methods are beyond the scope of this discussion [6]. While in vivo animal models are often used to predict safety and efficacy in humans, such data may not always translate into clinical outcomes due (in part) to differences in both pharmacokinetics and pharmacodynamics between such models and humans [9, 10]. Finally, in most cases, clinical data regarding the utility of combination therapy is poorly studied, due to the low incidence of IFIs, high rates of mortality, and costs. Retrospective studies often do not adjust for confounders, may be subject to selection bias, and are unable to establish causal effects [11]. Importantly, clinical trials of antifungal agents often suffer from uncertainty in diagnosis (requiring categories such as "possible," "probable," or "definite"), limited endpoints such as composite outcomes and difficulties enrolling patients, and, thus, major selection biases [12, 13]. Outcomes can be especially troublesome for antifungal drug trials, as mortality in the IFI patient population is particularly affected by multiple co-morbidities that increase the risk for mortality [14].

Pathogen-Specific Antifungal Combinations

Aspergillus spp.

Aspergillus is a significant cause of severe, invasive infections in immunocompromised patients, notably those on immunosuppressants, with hematological malignancy, and those undergoing either hematologic stem cell or solid organ transplantation [1, 7]. Mortality of invasive aspergillosis (IA) can be high, with up to 49% at 1 year in some studies [1]. Current IA treatment guidelines from the Infectious Diseases Society of America (IDSA) recommend voriconazole as the first-line agent. Alternative agents consist of liposomal amphotericin B and other amphotericin B lipid formulations [15••]. Subsequent to the publication of these guidelines, isavuconazole has been investigated and approved for the treatment of IA [16•].

Azole-Echinocandin

Synergy with azoles and echinocandins is thought to result from dual mechanisms of targeting the fungal cell membrane and wall, through ergosterol synthesis and 1,3 beta-D-glucan synthesis, respectively [17]. In vitro studies have shown isavuconazole to have synergy with micafungin for *Aspergillus* species [18]. Encouragingly, in animal models, isavuconazole combination with micafungin showed significant dose-dependent decline in residual fungal burdens, diminished pulmonary injury, and prolonged survival with the combination of agents [19].

Much of the clinical data regarding the combination of an azole with an echinocandin for the treatment of IA are generally observational, uncontrolled trials. For example, a retrospective cohort study in 53 adult patients with refractory IA reported on treatment outcomes utilizing a variety of combination therapies with caspofungin [2]. Data in the subset of patients treated with azoles and caspofungin reported 21 of 37 (56.8%; 95% CI 39.5–72.9) had success at the end of therapy, and 8 of 16 (50%; 95% CI 24.7–75.3) had success when treated with amphotericin B in combination. At the end of 84 days, mortality was similar between both groups (48.6% vs 50%). These data suggest azole-echinocandin combination is as effective as polyene-echinocandin.

A meta-analysis was conducted to compare outcomes of salvage therapy for IA with combination echinocandin therapy to non-echinocandin monotherapy [20]. This review analyzed 24 studies and looked at combination therapy with echinocandins and azoles or polyenes for 629 patients compared to monotherapy in 1204 patients. They concluded that 12-week survival (OR 1.80, 95% CI 1.08–3.01) and treatment success (OR 2.17, 95% CI 1.21–3.91) were significantly higher when the combination therapy regimen was used with an echinocandin and a triazole or a polyene compared to monotherapy.

Perhaps the most important study for evaluation of combination therapy for invasive aspergillosis is a randomized, prospective study in patients with hematologic malignancies and hematopoietic cell transplantation (Table 1) [21]. In this study, voriconazole monotherapy was compared to combination with anidulafungin in a prospective, randomized, double-blind study involving 277 patients with IA [21]. A nonsignificant difference in 6-week mortality of 27.5% vs 19.3% ($p = 0.087$) were reported in the two groups, respectively. However, a post hoc analysis did show significance differences in mortality among galactomannan-positive patients (27.3% vs 15.7% $p = 0.037$). Secondary outcomes showed a 12-week mortality of 29.3% for combination treatment and 39.4% for monotherapy (CI 21.4 to 1.1), and on the other hand, a 6-week successful global response of 32.6% for combination treatment and 43.0% for monotherapy (CI 21.6 to 1.2). Rate of adverse events were similar between both groups (96.1% vs 96.9%).

Table 1 Summary of clinical trials of combination antifungal therapy

Pathogen (disease)	Design	Population	Therapy	Findings	Comment	Reference
Aspergillus (invasive)	Randomized, double-blind, placebo-controlled trial	277 patients with IA and hematologic malignancies and HCT	Voriconazole (6 mg/kg every 12 h on day 1, followed by 4 mg/kg every 12 h) vs voriconazole + anidulafungin (200 mg on day 1, followed by 100 mg every 24 h)	Six-week mortality of 27.5% with monotherapy and 19.3% with combination (CI −19.0 to 1.5; $p = 0.087$) Six-week mortality in subgroup with galactomannan positive: 27.3% vs 15.7%, respectively (CI -22.7 to −0.4; $p = 0.037$)	Combination of voriconazole + anidulafungin showed mortality benefit in galactomannan positive patients. Safety was similar between groups	Marr et al. [21]
Candida (bloodstream)	Randomized, blinded, placebo-controlled trial	219 nonneutropenic patients with candidemia	FLU (800 mg/day) vs FLU + AmB deoxycholate	Thirty-day success rates were 57% with monotherapy and 69% with combination ($p = 0.08$) Bloodstream infection failed to clear in 17% and 6%, respectively ($p = 0.02$)	Trend toward better success with combination therapy with FLU and AmB	Rex et al. [29]
Cryptococcus (meningitis)	Randomized trial	20 patients with AIDs	FLU (400 mg/day) for 10 weeks vs AmB for 1 week, then three times weekly for 9 weeks combined with 5-FC	57% in FLU vs 0% AmB failed therapy ($p = 0.04$) Death in 28.6% with FLU vs 0% with AmB ($p = 0.27$)	Findings do not support the use of FLU over AmB; however, sample size was small	Larsen et al. [40]
Cryptococcus (meningitis)	Randomized trial	299 patients with HIV	AmB alone vs AmB + 5-FC vs AmB + FLU	Mortality at 70 days with AmB + 5-FC compared with AmB alone (30 vs 44 deaths, respectively, $p = 0.04$) AmB + FLU had lower mortality than AmB alone at 70 days (33 vs 44 deaths, $p = 0.13$)	AmB + 5-FC performed better than AmB + fluconazole	Day et al. [41, 43]
Cryptococcus (meningitis)	Randomized, open-label trial	721 patients with HIV	FLU (1200 mg/day) + 5-FC for 2 weeks - AmB and either 5-FC or FLU intravenously for 1 week followed by 1 week of fluconazole - AmB and either 5-FC or FLU intravenously for 2 weeks	AmB + 5-FC had a lower mortality than those with FLU (31.1 vs 45.0%, $p = 0.002$) Lowest with 1-week AmB combined with 5-FC (24.2%)	1 week of AmB plus 5-FC was the most effective option for induction therapy. Oral regimen was FLU and 5-FC was also proven to be effective	Molloy et al. [38•]
Cryptococcus (meningitis)	Randomized trial	40 patients with HIV	Step 1: FLU (1200 mg/day) ± 5-FC Step 2: FLU and AmB ± 5-FC	Faster clearance with addition of 5-FC and AmB ($p = 0.03$) Trend toward fewer early deaths with addition of 5-FC (4/41 vs 11/39, $p = 0.05$) Trend toward fewer deaths overall with addition of AmB (13/39 vs 20/40, $p = 0.1$)	Small sample size ($n = 40$) Supports the use of AmB and 5-FC as add-on therapy	Jackson et al. [45]
Cryptococcus (meningitis)	Randomized open-labeled controlled trial	90 patients with HIV	AmB + 5-FC vs AmB + 5-FC + IFN-gamma (3 or 6 doses)	Faster clearance with addition of IFN-gamma: Mean EFA (log CFU/ml/day) was −0.49 with standard treatment, −0.64 with IFN-gamma 2-doses, and − 0.64 with IFN gamma 6 doses ($p = 0.02$ and 0.006) No difference in mortality	IFN-gamma therapy may be effective in clearing infection, but no mortality data is present. Cost vs benefit must be weighed	Jarvis et al. [46]
Cryptococcus (meningitis)	Randomized open-labeled controlled trial	460 patients with HIV	AmB + FLU ± sertraline	18-week survival (52% in sertraline group vs 46% in placebo group; $p = 0.15$) Fungal clearance and adverse effects were similar between groups	Sertraline should not be used as an adjunctive treatment for cryptococcal meningitis	Rhein et al.[47•]
Mucormycosis	Randomized, double-blind, placebo-controlled trial	20 patients with proven or probable mucormycosis	Liposomal AmB vs Liposomal AmB + deferasirox (20 mg/kg/day for 14 days)	Death at 30 days (45% vs 11%, $p = 0.1$) and 90 days (82% vs 22%, $p = 0.01$) was higher in deferasirox group	Small sample size, but given increase in death in deferasirox group, would not recommend this adjunctive therapy	Spellberg et al. [55]

AmB amphotericin B, *EFA* early fungicidal activity, *5-FC* flucytosine, *FLU* fluconazole, *HCT* hematopoietic cell transplantation, *IFN* interferon

Current IDSA treatment guidelines for IA make a recommendation to consider combination therapy with voriconazole and echinocandins in the setting of documented invasive pulmonary aspergillosis [15••]. Therefore, it seems reasonable to consider combination therapy of IA with an azole (notably voriconazole, itraconazole, posaconazole, or isavuconazole) and an echinocandin in patients with a positive galactomannan, which is recommended as an accurate marker for diagnosis in the guidelines with high-risk hematologic patients, rapidly declining patients where treatment failure is suspected or in a seriously ill patient [15••, 22]. It is likely that the most benefit of combination therapy will be early in infection treatment and (hopefully) might impact length of therapy. However, this remains speculative and the optimal length of combination therapy before de-escalating to monotherapy is still uncertain.

Azole-Polyene

Based on their mechanisms, polyenes and azoles could have a presumed antagonistic combination. This presumption is due to azoles inhibition of the synthesis of ergosterol, which polyenes bind to in order to produce their fungicidal effects [6]. The initial animal studies suggested that the use of the azole before the polyene reduced activity of the polyene [23]. An antagonistic effect was observed when isavuconazole was used with amphotericin in vitro against *Aspergillus* isolates [18]. In contrast, the combination of voriconazole and amphotericin B reported concentration-dependent activity [24]. While the combination of low amphotericin B/high voriconazole concentrations showed synergy, antagonism was observed in high amphotericin B/low voriconazole concentrations. No robust clinical data reviewing the combination between azoles and polyenes was found, but for treatment of IA, it would likely be less desirable than azole-echinocandin combinations.

Echinocandin-Polyene

Combination therapy between caspofungin and polyenes may be effective for *Aspergillus* [25]. In a murine model study, amphotericin B combination therapy with an echinocandin was found to be no more effective than monotherapy with amphotericin B when treating mice with *A. fumigatus* [26]. Clinical data showing combination therapy with echinocandins and polyenes is reviewed above in the echinocandin-azole section, and it is generally favorable, but with no certainty that it is better than monotherapy [2, 20].

Candida spp.

Candida spp. are the most common cause of IFIs, specifically with *C. albicans* and *C. glabrata* (61 and 24%, respectively) as the leading *Candida* species [1]. However, many species of *Candida* have been shown to cause invasive disease. Crude mortality for bloodstream infections ranges between 27 and 40% [1]. Current IDSA Guidelines for the treatment of invasive candidiasis/candidemia recommend using an echinocandin as initial therapy for invasive *Candida* infections [27••]. Fluconazole can be used if patients are not critically ill and if the presence of a resistant organism is not suspected. A lipid-based formulation amphotericin B can also be used if an alternative monotherapy therapy is needed. Echinocandin monotherapy in studies of standard candidemia has a success rate high enough to make it difficult to prove combination therapy is better.

Polyene Combinations

In an in vitro study, 15 different antifungal combinations were tested against 5 species of *Candida*. Amphotericin B demonstrated synergy with both echinocandins and posaconazole [28]. A randomized clinical trial compared high-dose fluconazole (800 mg/day) plus placebo to fluconazole and amphotericin B deoxycholate in 219 nonneutropenic adult patients with candidemia [29]. Thirty-day success rates were 57% and 69%, respectively ($p = 0.08$). While not statistically significant, faster and higher rates of yeast clearance in the combination arm were observed. Considerations of the addition of a polyene to an azole or echinocandin to improve outcome in candidemia or invasive intra-abdominal candidiasis must ultimately include the severity of polyene-related toxicities.

In the case of invasive candidal infections involving the central nervous system, the current IDSA Guidelines recommend amphotericin B alone or in combination with flucytosine. The data to suggest flucytosine addition is limited to case reports and is based on flucytosine's excellent CNS penetration and its experience with cryptococcal meningitis [27••, 30, 31].

Azole-Echinocandin

Posaconazole has demonstrated synergy against *C. albicans* in vitro and in vivo with caspofungin [32]. No robust clinical data could be found for azole-echinocandin combination against *Candida*. However, retrospective study data regarding the use of multiple antifungal agents with or without surgery for the treatment of candidal endocarditis has been encouraging [33].

Other Combinations

Fluconazole has been tested and shown to have synergy in vitro with many non-antifungal medications (such as tetracyclines, linezolid, cyclosporine, tacrolimus, and amiodarone)

against *Candida* spp. [34–36]. Linezolid was further tested in vivo in larvae infected with *C. albicans* and was shown to produce a synergistic combination with fluconazole [36]. However, clinical data regarding these other combinations are insufficient to provide support for such a strategy at present.

Cryptococcus spp.

Cryptococcus spp. cause opportunistic infections in immunocompromised hosts and are the most common cause of fungal meningitis [37, 38•]. Crude mortality for cryptococcal infections was shown to be 33% at 1 year in the USA but is as high as 70% at 3 months in countries where antifungal therapies are limited and necessary healthcare for management are not widely available [38•, 1].

Cryptococcus guidelines recommend treating the meningoencephalitis of *Cryptococcus* with an induction phase consisting of amphotericin B and flucytosine for 2 weeks [39]. Amphotericin B and high-dose fluconazole can also be used for induction in resource-limited areas but are not optimal. After the induction phase, fluconazole can be used alone for consolidation. For non-meningeal *Cryptococcus*, which is primarily pulmonary, fluconazole monotherapy can be used for entire course.

Polyene-Flucytosine

Combination therapy for treatment of CNS infections is routinely recommended, with an induction phase consisting of amphotericin B or lipid formulations of amphotericin B and flucytosine for 2 weeks [39]. Synergy with polyenes and flucytosine is proposed to be due to the polyene's ability to damage the fungal cell wall membrane, allowing increased uptake of flucytosine [6]. One randomized, un-blinded trial published in 1990 compared oral fluconazole (400 mg/day) for 10 weeks to amphotericin B for 1 week and then three times weekly for 9 weeks combined with flucytosine (150 mg/kg/day) in 20 patients with acquired immunodeficiency syndrome (AIDS) [40]. Treatment failure was reported in 57% and none of the patients, respectively ($p = 0.04$). More studies with polyene-flucytosine combinations are discussed below in the polyene-azole, azole-flucytosine, and polyene-containing combinations section, but it is the most robustly recommended combination in all of medical mycology with multiple randomized supportive studies [41, 38•, 42].

Polyene-Azole

Amphotericin B, fluconazole, and flucytosine, alone and in combination, have also been tested in murine models. While the greatest activity was observed with amphotericin B and fluconazole when compared to amphotericin B and flucytosine, this has not been clinically replicated where the most fungicidal regimen and superior clinical outcome is with amphotericin B plus flucytosine [43].

As an alternative to amphotericin B and flucytosine, amphotericin B and high-dose fluconazole (greater than 800 mg/day) may be used for induction in resource-limited areas [41] A randomized trial was performed in 299 patients with human immunodeficiency virus (HIV) for treatment of cryptococcal meningitis with 3 groups: amphotericin B alone, amphotericin B plus flucytosine, and amphotericin B plus fluconazole. Amphotericin B plus flucytosine showed significance in reducing mortality at 70 days when compared with amphotericin B alone (30 vs 44 deaths respectively, $p = 0.04$) [41]. The combination of amphotericin B plus fluconazole also exhibited numerically lower mortality than amphotericin B alone at 70 days (33 vs 44, $p = 0.13$); however, the results were not statistically significant. When combination therapy with amphotericin B plus flucytosine was compared to amphotericin B plus fluconazole, only a difference in fungal clearance was significant in favor of the flucytosine combination (74 vs 60 patients, $p = <0.001$). More studies with polyene-azole combinations are discussed below in the azole-flucytosine and polyene-containing combinations section.

Polyene-Echinocandin

A recently published in vitro study showed synergy between amphotericin B and micafungin. The study also looked at anidulafungin and caspofungin each with amphotericin B, and while still showing synergy, it showed less synergy when compared to the micafungin combination [44]. However, echinocandins display limited antifungal activity against cryptococcus, making the relevance to clinical use questionable.

Azole-Flucytosine

The combination of an azole and flucytosine is thought to be a similar mechanism as between polyenes and flucytosine, with damage to the cell membrane allowing increased uptake of flucytosine [6]. A phase II randomized controlled trial was performed in Africa with 40 patients presenting with HIV and cryptococcal meningitis [45]. The study had 2 steps and compared patients receiving fluconazole ± flucytosine in step 1 and patients receiving fluconazole and amphotericin B ± flucytosine in step 2. Overall, the study showed that patients who received flucytosine or amphotericin B had faster clearance of infection with a trend toward decreased mortality with flucytosine (4/41 vs 11/39, $p = 0.05$) and fewer deaths overall with addition of amphotericin B (13/39 vs 20/40, $p = 0.1$).

Another randomized trial performed in 721 HIV-infected patients with cryptococcal meningitis compared the following induction regimens (each followed by fluconazole for

10 weeks): oral "high-dose" fluconazole (1200 mg/day) plus flucytosine for 2 weeks; amphotericin B and either flucytosine or fluconazole intravenously for 1 week followed by 1 week of fluconazole; or amphotericin B and either flucytosine or fluconazole intravenously for 2 weeks [38•]. Results for the trial showed amphotericin B groups with flucytosine had a lower mortality than those with fluconazole (31.1% vs 45.0%, $p = 0.002$). Lowest mortality was seen with 1-week amphotericin B combined with flucytosine (24.2%), while outcome in the oral flucytosine plus oral fluconazole group was comparable to amphotericin B plus fluconazole.

Polyene-Containing Combinations

An open-labeled randomized controlled trial evaluating the addition of interferon-gamma to standard therapy for cryptococcal meningitis in patients with HIV was performed [46]. The study found that patients who received interferon-gamma had quicker yeast clearance and similar adverse events to those who received standard therapy alone. However, no difference in mortality was seen. Despite these results, interferon-gamma has get to find wide acceptance in primary induction therapy regimens, likely due to concern for the immune reconstitution inflammatory syndrome (IRIS). In Uganda, a double-blind, randomized placebo-controlled trial was performed assessing the adjunctive use of sertraline, a repurposed drug, in HIV-infected patients with cryptococcal meningitis [47•]. The trial randomized 460 patients to receive amphotericin B and fluconazole with sertraline (400 mg/d) or placebo. The primary outcome of 18-week survival showed no difference with the addition of sertraline compared to placebo (52% vs 46%; 95% CI 0.93–1.57; $p = 0.15$).

Triple Drug Therapy

There have been several studies combining azole, flucytosine, and polyene. These studies have demonstrated mixed results, showing either no improvement over optimal two-drug therapy and one study demonstrating an increase in fungal clearance with triple drug therapy compared to amphotericin B and fluconazole (−0.50 log CFU/ml/d vs −0.38 log CFU/ml/d, $p = 0.03$) [43, 45]. Further studies are needed to assess the use of triple therapy compared to double therapy.

Mucormycosis

Mucormycosis is a rare fungal infection, with a mean incidence of 0.3/100,000 patients per year [1]. *Rhizopus* is the most common genus in the Mucorales order to cause disease. These infections are associated with mortality which may exceed 50% if disease is disseminated [48]. High-dose lipid formulations of amphotericin B are the mainstay of treatment for mucormycosis [49]. Isavuconazole has also been studied as an option, and posaconazole primarily as salvage therapy [50, 51].

Polyene-Containing Combinations

Studies performed in mice have shown synergy with liposomal amphotericin B and echinocandins therapy, but no synergy with posaconazole and liposomal amphotericin B against mucormycosis [52, 53]. A case series reviewed patients treated for rhino-orbital-cerebral mucormycosis with amphotericin B and caspofungin [54]. All 6 patients treated with the combination had treatment success, suggesting a need for more clinical data to assess the combination. These findings are particularly interesting, since echinocandins do not have intrinsic antifungal activity against Mucorales in vitro. Thus, it is unclear what is the mechanism for this potential positive interaction with the polyene.

Only one prospective, randomized clinical trial has ever been performed testing combination therapy for mucormycosis. This trial tested liposomal amphotericin B alone compared to combination therapy with liposomal amphotericin B and deferasirox. In the study, only 20 patients were randomized, and results showed an increase in mortality at 90 days in the combination group (82% vs 22%, $p = 0.01$) [55]. Unfortunately, the study was small and underpowered, and the results do not support adjunctive deferasirox therapy. Of note was the increased number cancer patients assigned to the combination arm. Thus, the value of iron chelation in combination with a polyene is still unresolved.

Azole-Echinocandin

The combination of isavuconazole and micafungin has been tested in vitro against *Rhizopus* and demonstrated no synergistic or antagonistic effect [48]. Despite the lack of supportive data, patients with mucormycosis often receive combination therapy with two or three drug therapy (polyene, echinocandin, and azole) sometime during their management. Triple combination therapy has not been critically assessed in mucormycosis.

Scedosporium and *Fusarium*

Hyaline mold infections are rare, occurring in 0.2/100,000 patients per year, with *Scedosporium* and *Fusarium* being the most common, and are usually pathogens infecting immunocompromised patients [1]. Mortality is high for *Scedosporium* and *Fusarium*, often greater than 50%. *Scedosporium* spp. are often resistant to polyenes, echinocandins, and some azoles [56, 57]. Voriconazole is the antifungal agent most often used and combination antifungal therapy is common, but its value is uncertain [57]. One in vitro study reported data from 35 different antifungal combinations

against 12 isolates of two different species of *Scedosporium* [58]. In the study, azole agents plus echinocandins were found to be synergistic against *S. apiospermum* in most combinations tested, with itraconazole and caspofungin showing the greatest synergy. *Lomentospora* (*Scedosporium*) *prolificans* is a more resistant mold, which demonstrated low susceptibility and synergy with the agents tested in the study. In previous studies, terbinafine plus voriconazole was found to be synergistic against *L. prolificans* and other *Scedosporium* species. However, this combination remains infrequently evaluated and value remains uncertain [58].

Like *Scedosporium*, *Fusarium* spp. is often highly resistant. This factor may encourage the use of combination antifungal therapy empirically when treating infected patients. One in vitro study tested dual and triple therapies with amphotericin B, voriconazole, and/or anidulafungin [59]. Against *Fusarium solani*, each dual therapy combination, as well as triple therapy, showed synergy. Data on combination therapy to treat *Fusarium* is limited to case reports and few in vitro studies, with most cases receiving polyenes with voriconazole or an echinocandin [57]. Against *Fusarium solani*, each dual therapy combination, as well as triple therapy, showed synergy. Combination of triazoles and polyenes may be a good choice, such as voriconazole and amphotericin B. It is important to note that while voriconazole does have an FDA approved indication for *Fusarium*, some species are resistant to this azole [57].

Discussion

Since invasive IFIs are rising and a high mortality rate is associated with these infections occur, a need for guidance on antifungal combinations is evident. The use of antifungal combinations may provide increased potency and broadened spectrum of antifungal activity while potentially reducing the development of fungal resistance. However, these theoretical benefits must be balanced by increased toxicity, drug-drug interactions, antagonisms, and costs. Except in cryptococcal meningitis, clinical data regarding the benefits of combination therapy are scarce, leaving conclusions to be drawn based on uncontrolled and/or underpowered studies, in vitro data, and case reports.

Given the risk of mortality in patients with IFI, it is common to entertain the idea of adding multiple antifungal agents to a patient's regimen. There are certainly fungal infections where combination therapy with two antifungal agents has been used and clinically proven to be effective, such as amphotericin B and flucytosine for cryptococcal meningitis [38•]. Combinations unproven in clinical trials or the addition of more than two antifungal agents will need careful consideration when used, and caution should be maintained. When making such decisions, there is a real concern for an increased likelihood of a medication adverse effect or even possibility of antagonism with certain antifungal combinations, such as polyenes and azoles [18]. In fact, more may not always be better. It has also been proposed that timing and the sequence of antifungals being given may impact their activity [60]. While clinical data may be lacking to support these claims, the possibility of such effects in a patient being treated for an IFI needs to be considered. Consideration of in vitro and in vivo data with sound clinical decision making must be employed when clinical data are lacking or absent.

For *Aspergillus fumigatus*, the clinical data present support the potential use of combination therapy with voriconazole and echinocandins [21]. In vitro and in vivo studies with other triazoles active against *Aspergillus* spp. combined with echinocandins also show similarly a synergistic effect. Polyenes in combination with echinocandins have also shown synergy in vitro and in in vivo models but lack strong clinical data for other hyaline molds; however this combinations use is frequent.

With the development of echinocandins and their clinical success, combination therapy for *Candida* may not seem as attractive as it once did. Fluconazole and polyene combinations do have clinical data to show trends toward better treatment success but was not statistically significant in the trial [29]. Guidelines also make a recommendation for consideration to add flucytosine if greater CNS penetration is needed, and frequently combination antifungal therapy is used in *Candida* endocarditis.

Cryptococcal infections have the most literature to support combination use. Based on the information presented, amphotericin B combination with flucytosine appears to be the most effective regimen to treat cryptococcal meningitis. High-dose fluconazole and flucytosine appear to be effective to treat cryptococcal meningitis as an all oral regimen, but at present, the data do not support its use over the amphotericin B and flucytosine combination. Addition of interferon-gamma showed quicker clearance of infection, but no mortality benefit was seen. Given the lack of mortality data, concern about IRIS, and the medication's high cost, interferon-gamma add-on therapy is not routinely recommended.

Data are scarce for combination therapy in the treatment of mucormycosis. Amphotericin B and echinocandin combinations have shown synergy in murine models and have been reported in a case series to treat rhino-orbital-cerebral mucormycosis. This combination appears favorable but needs more data for the routine support of its use. For *Scedosporium* and *Fusarium* infections, no recommendations can be made. Data for these IFIs are very limited and consists of in vitro studies and case reports but are frequently considered on the medical wards.

Conclusion

Combination treatment with voriconazole and echinocandins appears to be effective for *Aspergillus* and even holds a

recommendation in current treatment guidelines. Add-on therapies may not be needed for most *Candida* infections with the addition of echinocandins to the antifungal armamentarium, unless CNS penetration is needed or vegetation sterilization is a goal. Flucytosine has an established role as "add-on" therapy to amphotericin B in *Cryptococcus* and may show promise when added to fluconazole as well. Combination data for mucormycosis, *Scedosporium*, and *Fusarium* is lacking and needed for more studies or experience to define clear combination therapies for these difficult IFIs.

Compliance with Ethical Standards

Conflict of Interest John Perfect reports grants and being a member of the advisory board for Merck; grants from Astellas, serving on the advisory board for F2G, serving on the advisory board, and consulting for Scynexis; grants from Pfizer; grants from Amplyx, serving on the advisory board and consulting for Ampili; and grants from Minnetronix, serving on the advisory board for Matinas outside the submitted work. Richard Drew reports expenses for CME presentation from American Society of Microbiology, consultant fees from Paratek, and publication royalties from UpToDate. Spencer Livengood declares no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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