

## REVIEW

# Combination antifungal therapy: what can and should we expect?

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**Invasive fungal infections are associated with significant morbidity and mortality among immunocompromised patients. Recent advances in antifungal development have afforded us more pharmacologic compounds to choose from when managing these fungal infections. The role of combination antifungal therapy has been well established for fungal infections such as cryptococcal meningitis. The availability of new antifungals, increased incidence of mould infections and high mortality among certain affected populations, such as hematopoietic stem cell transplant recipients, has stimulated interest in the clinical use of combination antifungal therapy. In this paper, we review supporting evidence for the use of combination antifungals in the treatment of cryptococcal meningitis, invasive candidiasis, invasive aspergillosis and zygomycosis. Several controlled clinical trials have demonstrated benefits of combination antifungal approaches for patients with cryptococcal meningitis and invasive candidiasis, but variable effects when using different agents in combination have been reported. Randomized prospective studies of combination antifungal therapy in mould infections are lacking but some series provide supportive evidence for this approach. We also describe limitations of the data and these study designs, including the fact that we still need randomized controlled multicenter studies of combination antifungal therapy for mould infections. Trials in this area should be performed with efficiency and economics in mind, and could potentially use surrogate markers as end points. Therefore, we suggest future investigations of combination antifungal therapy should include a randomized, comparative trial of primary therapy for invasive aspergillosis.**

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## Introduction

In discussion of antifungal therapies and strategies today, one of the most frequently asked questions is: when should we use antifungal combination therapy? This question is born of two critical factors: (1) new, safe options with several classes of antifungals; and (2) the extremely poor outcome of certain patients with invasive fungal infections. Mortality for patients infected with *Aspergillus* spp. is approximately 60% overall, but is as high as 87% among hematopoietic stem cell transplantation (HSCT) recipients.<sup>1,2</sup> This may be somewhat improved with use of voriconazole therapy, but in a recent trial overall response was only 32% among allogeneic HSCT recipients.<sup>3</sup> Mortality with zygomycosis in this population has been reported to be as high as 91%.<sup>4</sup> Furthermore, clinicians have become familiar with the concept of combination antimicrobial use from their experiences with antiretroviral therapy, treatment of mycobacterial infections, empirical sepsis management strategies and certain cases of bacterial endocarditis. In these infections, the primary motivators for combination therapy are limiting the impact and/or development of drug resistance, the desire for broad-spectrum antimicrobial coverage, and in some cases such as endocarditis, more potent antimicrobial activity (i.e. bactericidal rather than bacteriostatic antimicrobial action). On the other hand, in fungal infections, it is not common to select drug-resistant mutants during therapy unless flucytosine is used alone; there are already single antifungal agents for empirical therapy that offer broad-spectrum activity, and it is difficult to define *in vivo* that there is a more potent double or triple agent regimen over a single agent. Taken together, it is hard to initially justify widespread use of antifungal combinations unless we have strong evidence-based studies to support their use. Despite this assessment the clinician caring for seriously ill patients is occasionally confronted with the issue, and must consider if combination antifungal therapy will afford a better outcome for a patient with a life-threatening invasive fungal infection. In this discussion, we will attempt to provide opinions mixed with facts to approach the use of antifungal combination for the immediate future and what it means to the clinician.

## Challenges of managing invasive fungal infections in complicated patients: getting the balance right

It is first important to emphasize that combination antifungal therapy is but one issue that clinicians must

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deal with when they attempt to manage these refractory fungal infections. The other issues include rapid/accurate diagnosis, understanding/manipulating immune reconstitution, optimizing pharmacokinetics/pharmacodynamics, empiric/pre-emptive prevention strategies, surgical intervention and deciding between old and new antifungal agents (Table 1). Each of these issues could be discussed in an entire paper but to summarize quickly the following principles seem clear:

(1) Rapid and accurate diagnosis is intuitively important and recent studies continue to suggest that this is in fact true. For example, in two recent series early administration of antifungal therapy for invasive candidiasis (i.e. within 12 or 24 h of drawing the initial blood culture) was associated with better survival.<sup>5,6</sup> Earlier diagnosis will enable an opportunity for earlier appropriate therapy and therefore improve outcome. (2) In invasive fungal infections, clinicians are commonly caught in the 'Goldilocks' paradigm of host immunity (i.e. too much or too little of a host immune response can be catastrophic for the patient and clinicians have to get it just right). Clinical situations where antifungals cannot be successful without some restoration of host immunity include persistent neutropenia or monoclonal antibody-induced defects in cellular immunity that are not quickly reversible. However, restoration of host immunity using immunomodulators such as G-CSF, GM-CSF or HAART carries the risk of immune reconstitution inflammatory syndrome and clinical presentations mimicking antifungal drug failure.<sup>7-13</sup> Clearly, all immunodeficiency is not created equal, so a one-size-fits-all approach to immunomodulator therapy will probably not work.<sup>7</sup> Individualized approaches are likely necessary to restore the appropriate balance to any given host and maximize chances of successful outcome with antifungal drug therapy. (3) In clinical practice, issues related to drug bioavailability, pharmacokinetic variability, drug interactions and toxicities contribute to clinical failures.<sup>14</sup> While

these issues can be critical at the individual patient level, they can be difficult to encapsulate in the general description of treatment failures and frequently manifest after starting therapy. At times, there is little but clinical experience to guide clinicians in anticipating and managing such problems at the bedside. (4) Debulking or debriding infected tissue may be critical in achieving clinical success in patients with invasive mycoses, as it can reduce fungal burden and/or remove devitalized tissue. This is particularly important for management of those with invasive mould infections such as aspergillosis, zygomycosis and phaeohyphomycosis. However, its use is complicated by clinical stability of the patient, site of the infection and risks-benefits of the surgical intervention. A bedside decision regarding the value of surgery needs to be made one patient at a time, taking the above factors into consideration, along with identification of the invading fungus. (5) Prevention of an invasive fungal infection is always preferred to treating an established infection. Recent data in HSCT recipients with graft vs host disease and AML/MDS patients receiving chemotherapy demonstrated significant reductions in development of proven/probable invasive fungal infections (IFIs) with use of the new extended-spectrum azole, posaconazole.<sup>15,16</sup> Prevention of IFIs in study subjects was even associated with improved survival. Routine implementation of such preventative strategies will likely lead to challenges in selection of antifungal therapy for patients when they have suspected breakthrough fungal infections. While clinicians might favor a combination antifungal strategy for such patients, there are little or no data to support use of combination antifungal therapy after prophylaxis or for any empirical use. Without a definitive diagnosis of an IFI, combination antifungal therapy is not warranted in such situations and rather, a solid diagnosis should be aggressively and rapidly pursued. Spiraling empiricism meeting combination antifungal therapy is a prescription for ineffective, expensive

**Table 1** Challenges of managing invasive fungal infections

<i>Challenge</i>	<i>Recommendation</i>
Rapid and accurate diagnosis is important, but difficult to achieve	Clinicians should aggressively pursue an accurate diagnosis, but pre-emptive therapy based on clinical criteria and diagnostics can be helpful
Too much or too little of a host immune response can be problematic	Individualized approaches are necessary to balance restoration of host immunity with risks of immune reconstitution inflammatory syndrome
Poor drug bioavailability, pharmacokinetic variability, drug interactions and toxicities can contribute to clinical failures	Clinicians should anticipate and manage these problems proactively to reduce risks of drug failure, toxicity and resistance
Removal of infected tissue can be critical to achieving clinical success, particularly in mould infections	Not all patients are candidates for surgery, but debridement of devitalized tissue and debulking large fungal burdens may be helpful for patients with mould infections
For many patients, fungal infection is a catastrophic event that renders them unable to receive treatment for their underlying disease such as chemotherapy	Prophylaxis with antifungal agents should be considered for high-risk patients. If break-through infections occur, an accurate diagnosis should be aggressively pursued and consistent antifungal drug strategies employed
Combination therapy of invasive fungal infections is attractive from the perspective of synergistic potential, relative safety, and lack of overlapping toxicities. Randomized, controlled clinical trial data of combination antifungal therapy for mould infections are lacking	Single agents have been effective in treating the majority of invasive fungal infections if the patient's underlying disease can be controlled; combination therapy with amphotericin B and flucytosine should be routinely employed in patients with cryptococcal meningitis; combination therapies for other fungal infections should be considered on a case-by-case basis until additional studies demonstrate benefits of this approach

**Table 2** Select clinical studies of combination antifungal therapy in invasive fungal infections

Infection treatments	Population	Design	Results	Ref
<i>Cryptococcal meningitis</i>				
AmBd + 5FC	Non-AIDS patients	Prospective, randomized trial	Cure or improvement: 23/34 (68%) vs 15/32 (47%)	21
AmBd			Relapse: 1/24 (4%) vs 5/27 (19%)	
AmBd + 5FC	HIV-infected patients	Prospective, randomized trial	At 2 weeks: mycological and clinical response: 102/202 (50%) vs 76/179 (42%) ( $P=0.12$ )	23
AmBd			CSF sterilization: 122/202 (60%) vs 91/179 (51%) ( $P=0.06$ )	
5FC + FLU	HIV-infected patients	Noncomparative, prospective, open-label trial	Clinical success at 10 weeks: 63% (95% CI 48, 82%)	28
AmBd + 5FC	HIV-infected patients	Prospective, randomized, open-label trial	Fungicidal effect within first 14 days: $-0.54$ vs $-0.31$ , $-0.39$ , $-0.38$ log CFU/day (difference = 0.23 log CFU daily $P=0.001$ , 0.15 log CFU daily $P=0.03$ , log CFU daily 0.17, $P=0.01$ )	25
AmBd + FLU			Death at 10 weeks: 1/15 vs 3/16, 7/16, 3/16	
AMBd + 5FC + FLU				
<i>Candidiasis</i>				
AmBd + FLU	Non-neutropenic patients	Prospective, randomized trial	Time to failure: no significant difference	31
FLU			Success at 20 days: 69% vs 56% ( $P=0.043$ )	
			Failure to clear infection: 6% vs 17% ( $P=0.02$ )	
LFAB + Mycograb	ICU & non-ICU, primarily non-neutropenic patients	Prospective, randomized trial	Overall response: 84% vs 48% ( $P<0.001$ )	33
LFAB				
AmBd + 5FC	Non-neutropenic surgical patients	Prospective, randomized trial	Clearance of infection: 14/20 vs 12/20 ( $P=NS$ )	64
FLU			Time to elimination: 5.5 vs 8.5 days ( $P=0.03$ )	
<i>Aspergillosis</i>				
VRC + CAS	HSCT recipients ( $n=41$ ) or s/p chemotherapy ( $n=6$ )	Retrospective cohort study, salvage therapy	3-Month survival after start of salvage therapy higher with combination (HR 0.43, 95% CI: 0.17–1.1)	37
VRC				
VRC + CAS	Solid organ transplant recipients	Prospective cohort, compared to historical controls, primary therapy	90-Day survival 67.5% (27/40) vs 51% (24/47) ( $P=0.11$ )	39
LFAB				
MICA (mean dose 111 mg/day)	Immunocompromised patients	Prospective open-label study, primary or salvage	Primary therapy response: 29.4% vs 50%	65
MICA + other antifungals (mostly LFAB)	(80% HSCT recipients or hematological malignancy)		Salvage therapy response: 34.5% vs 40.9%	
CAS + L-AMB	Hematological malignancies (50% HSCT recipients, 63% neutropenic)	Primary (35%) or salvage (65%)	Overall response: 42%	38

management. (6) The newer antifungal agents including lipid formulations of amphotericin B, extended-spectrum azoles (voriconazole and posaconazole) and echinocandins (caspofungin, micafungin and anidulafungin) possess outstanding antifungal activity as single agents for well-documented infections, and in the vast majority of IFIs they can be successful as monotherapy if the patient's underlying disease can be controlled.

Having taken all of these issues into consideration, combination antifungal therapy may have a role in certain clinical situations, and more often than not, is discussed among other options at the bedside in managing any given patient with a rapidly progressive IFI or little hope of immediate immune reconstitution. Despite some intuitive feelings to the contrary, it cannot be assumed that two or more antifungal agents with different mechanisms of action in combination will actually result in better outcomes than a single antifungal agent. In fact, combinations of these agents could potentially result in reduced effects on fungal killing, reduced clinical efficacy, increased drug–drug interactions and more toxicities at higher economic costs.<sup>17</sup> Therefore, the use of combination antifungals should not

be taken lightly and a critical appraisal of its value needs to be assessed. The following discussion will describe combination antifungal therapy against the major fungal pathogens (*Cryptococcus* spp., *Candida* spp., *Aspergillus* spp. and zygomycetes). Extensive reviews have already been published summarizing *in vitro* and animal model studies, and highlighting their limitations and variable correlation with clinical experiences when combination antifungal therapy is employed in patients.<sup>18–20</sup> We will thus focus on recent clinical evidence in treating these infections (Table 2). This will allow the clinician to view the most evidence-based support for use of antifungal combinations as well as the most evidence-challenged data that leads to recommendations based simply on opinions.

#### Evidence for use of combination antifungals in invasive fungal infections

**Cryptococcosis.** To date, treatment of cryptococcal meningitis has been the most carefully studied area of combination antifungal therapy. The first human studies compared the combination of amphotericin B plus

flucytosine to amphotericin B alone in 1979.<sup>21</sup> In this study, amphotericin B (0.3 mg/kg/day) was combined with flucytosine for 6 weeks and compared to 10 weeks of amphotericin B (0.4 mg/kg/day). This strategy was attractive for several reasons: (1) doses of the toxic amphotericin B could be lower when used in combination; (2) combination use limited development of flucytosine-resistance; and (3) investigators observed reliable CSF yeast sterilization within 2 weeks of induction with combination therapy. This strategy resulted in an overall mortality benefit for those receiving the combination compared to amphotericin B alone (24 vs 47%,  $P < 0.05$ ). Follow-up studies in less-severely immunosuppressed individuals showed that induction therapy with amphotericin B and flucytosine together could treat cryptococcal meningitis for even shorter periods of time (i.e. 4 weeks).<sup>22</sup> However, with the advent of HIV infection and its severe immunosuppression, a new strategy was adopted, which included induction, clearance and suppression phases of treatment. In the initial induction phase, it was found that the combination of amphotericin B plus flucytosine reproducibly reduced CSF yeast counts over 2 weeks of treatment and also reduced the numbers of relapse cases.<sup>23</sup> Therefore, combination therapy has been adopted as the treatment of choice for initial therapy of cryptococcal meningitis.<sup>24</sup> A recent study with the use of quantitative CSF yeast counts convincingly showed that the combination of amphotericin B plus flucytosine was even more fungicidal than amphotericin B alone, amphotericin B plus fluconazole, and the three-drug combination.<sup>25</sup> This is a creative study which used a specific biomarker end point (yeast colony counts in CSF) to validate that a combination antifungal therapy has benefit and similarly, that more drugs do not always equate with more potency. For instance, three drugs were not better than two drugs. The concept that more is better is not necessarily true.

Present work in cryptococcal meningitis combination antifungal therapy has continued to examine a polyene and azole together.<sup>26</sup> There have been favorable animal model studies with this combination and certainly an azole following polyene induction therapy in sequence has been widely and successfully used.<sup>27</sup> However, the combination of an azole plus polyene for induction therapy still represents second-line therapy. Similarly, the all oral combination regimen of fluconazole plus flucytosine has been studied in cryptococcal meningitis. Although successful, this combination has yet to be shown either equivalent or superior to a polyene-containing regimen for initial treatment of cryptococcal meningitis despite its attractive pharmacokinetics with both oral bioavailability and high CSF penetration of both drugs.<sup>28,29</sup> Finally, in cryptococcal meningitis even a combination of an antifungal agent and immunomodulator agent has been studied. The use of induction therapy with amphotericin B with the addition of recombinant  $\gamma$ -interferon had a tendency toward more rapid CSF yeast clearance but it is likely that small numbers of patients contributed to a trend but not significant mycological improvement with adjunctive  $\gamma$ -interferon.<sup>30</sup> Therefore, the use of this combination is not routinely used but could be considered when the CSF is difficult to sterilize with standard therapies. In summary,

combination therapy in cryptococcal meningitis has a substantial evidence-based platform for its use and it has become a standard antifungal regimen. However, even in this well-validated clinical situation, it remains difficult to show substantial outcome improvements in the use of the combination regimen over single agent except when used early in infections having a high burden of organisms.

**Invasive candidiasis.** In *Candida* infections, the largest and most comprehensive study to examine combination therapy vs a single agent was a candidemia study comparing amphotericin B plus fluconazole vs fluconazole alone.<sup>31</sup> In this randomized trial of 219 evaluable non-neutropenic patients with largely *C. albicans* infections, amphotericin B or placebo was added to high-dose fluconazole (12 mg/kg/day) for an average of 5.6 days and then therapy was continued with fluconazole alone. Although the combination resulted in better sterilization of the bloodstream (94% combination, 83% monotherapy,  $P = 0.02$ ), there was only a small difference in successful clinical outcome between groups (69% combination, 56% monotherapy,  $P = 0.043$ ). In addition, even this short exposure to amphotericin B resulted in substantial nephrotoxicity, with 23% of amphotericin B plus fluconazole recipients vs 3% of placebo plus fluconazole recipients requiring reductions in study drug dosage due to renal dysfunction. This combination is now one of the options mentioned by IDSA as therapy for invasive candidiasis.<sup>32</sup> A more recent study among patients with invasive candidiasis showed that the use of a combination of an antibody to HSP 90 (Mycograb) plus lipid formulations of amphotericin B (LFAB) was superior to LFAB alone.<sup>33</sup> This is particularly impressive given the rapid fungicidal activity of polyenes against *Candida* spp., and should be validated in well-controlled future studies. Other new antifungal agents such as the echinocandins have fungicidal activity against *Candida* spp. as well, and have demonstrated excellent and safe activity in clinical trials of invasive candidiasis when used alone.<sup>34,35</sup> Similarly, broad-spectrum triazoles have also demonstrated potent clinical activity with an excellent safety profile.<sup>36</sup> It is likely that these new single antifungal agents would routinely be comparable to any combination of drugs on a clinical basis for invasive candidiasis. Therefore, there is little enthusiasm and probable need for combination antifungal therapy in routine cases of invasive candidiasis. However, most of the clinical trials to date excluded neutropenic hosts, and more potent and creative approaches may be required in the profoundly immunosuppressed patient. For these patients, as well as those with more severe manifestations of candidiasis (meningitis, endophthalmitis, endocarditis and hepatosplenic disease) combination therapies may offer some additional fungicidal benefits but this fact remains to be demonstrated in clinical trials.

**Invasive mould infections.** The biggest controversy that leads to therapeutic confusion and erratic recommendations with the use of combination antifungal therapy is in the management of invasive mould infections. As the most common mould infection, aspergillosis has been the 'ground zero' of antifungal combination contention.

The infrastructure around this therapeutic dilemma of when and where to use combination therapy in aspergillosis is solidly based in a variety of *in vitro* and animal studies which either suggest synergism, additive, antagonistic or no impact for a variety of combinations of antifungals.<sup>19,20</sup> Studies range from the antagonism of polyenes by pretreatment with azoles to the apparent improved antifungal activity of an echinocandin added to an extended-spectrum azole. Even in the controlled setting of animal models, the success of these interactions can be very tricky to tease out. How do clinicians expect to translate combination therapy for mould infections into humans?

Our initial insights into combination therapy for human aspergillosis treatment have been an attempt to manage refractory disease. In one study, HSCT or cytotoxic chemotherapy recipients ( $n=16$ ) with proven or probable invasive aspergillosis refractory or intolerant to  $\geq 7$  days of an amphotericin formulation were managed with a combination of voriconazole plus caspofungin.<sup>37</sup> This was compared to a historical group of patients ( $n=31$ ) at the same center who had received voriconazole alone as salvage therapy. Three-month survival was significantly better among patients who received the combination of voriconazole and caspofungin compared to voriconazole alone. Among allogeneic HSCT recipients, combination antifungal therapy was similarly associated with a reduction in mortality (HR 0.25, 95% CI 0.057–1.1) in a multivariable model. This is a challenging group of patients which include patients who appear to gain some benefit to those whose underlying diseases will not allow any antifungal treatment success. Studies such as this may help reassure us that we are not doing any harm to patients but on the other hand, it is impossible to appreciate in these salvage studies which single or combination regimens are most successful.<sup>37,38</sup> The next step has been to study prospectively combination antifungal therapy for primary disease and this has been reported for an azole plus echinocandin, but there have been no blinded or randomized comparative trials and thus accurate insights into success of combination therapies are still uncertain.<sup>39</sup> In this study, the combination of voriconazole plus caspofungin as primary therapy for invasive aspergillosis in solid organ transplant recipients was compared to historical controls managed with a lipid formulation of amphotericin B. Ninety-day survival was 67.5% (27/40) among those who received combination antifungal therapy compared to 51% (24/47) of controls (HR 0.57, 95% CI 0.29–1.1). Even a recent small comparative study of liposomal amphotericin B plus caspofungin vs liposomal amphotericin B alone, which showed a more favorable response for the combination at the end of antifungal therapy (67% 3 mg/kg/day L-AmB plus caspofungin vs 27% 10 mg/kg/day L-AmB alone), was severely under powered for any definitive conclusions.<sup>40</sup>

Zygomycetes infections represent another group of mould infections associated with particularly devastating consequences in the immunosuppressed patient. These infections have increased in recent years and are associated with mortality among 41–91% of infected patients.<sup>4</sup> In a recent review of 929 published cases of zygomycetes infections, mortality was greatest (91%) among HSCT recipients.<sup>4</sup> Since voriconazole lacks activity against zygo-

mycetes, centers routinely using this extended-spectrum azole may see zycomycosis emerge as a breakthrough infection. Facing such high and rapid mortality, clinicians often consider combination antifungal therapy for these patients. Unfortunately, no randomized controlled clinical trials of combination antifungal therapy have been performed in this area. However, recent experience with posaconazole is instructive. In an open-label study, posaconazole was initiated in 91 patients with proven (76%) or probable (24%) zygomycosis.<sup>41</sup> The majority of these patients were refractory to 7 or more days of prior antifungal therapy. After 12 weeks, 60% of patients had complete (14%) or partial (46%) response, with failure among 17%. Subjects (13 of 91) received lipid formulations of amphotericin B in combination with posaconazole. Partial response was experienced among 46% of these patients with 31% experiencing failure. Although this was not a randomized trial and could be biased by clinicians administering combination antifungal therapy to sicker patients or those with more extensive infection, these data certainly do not suggest that combination therapy was associated with better outcomes than posaconazole monotherapy for zygomycosis. Additional studies are needed to better define the role of combination antifungal therapy for patients with these devastating mould infections.

#### *Considerations for future clinical trials of combination antifungal therapy*

To make robust, evidence-based conclusions regarding the benefits or risks of combination therapy, clinicians desperately need a randomized, comparative, clinical trial. At this time, *Aspergillus* is still the most common cause of mould infections, and we lack data from large, well-controlled trials of combination antifungal therapy in this setting. Considering the high mortality and relative frequency of these infections, a clinical trial of combination antifungal therapy as primary treatment for invasive aspergillosis would be of great value. That said, proponents of such a study need to consider the practical implications of this endeavor. First, a combination of agents will probably need to be compared to standard initial therapy with voriconazole. From a previous landmark study in invasive aspergillosis, voriconazole will have an approximately 55% success rate at 12 weeks.<sup>3</sup> If it is assumed that with the underlying disease approximately 25% of the patients will be lost, then the best possible success rate of combination therapy will be 75%. Therefore, if the combination works extremely well, approximately 20% improvement is expected. With a power of 80% the study will require 176 evaluable patients (88 in each arm). To detect a 15% difference in the two treatment groups (i.e. 70% success with combination), approximately 324 subjects are required. For a 10% difference, 752 evaluable subjects would be required. Similar estimates have been published by representatives of the Food and Drug Administration.<sup>42</sup> A recent study took  $4\frac{1}{2}$  years to enroll and follow 391 subjects with proven or probable invasive aspergillosis in 95 medical centers from 19 countries, underscoring the enormous effort it takes to perform a clinical trial in this critically ill patient population.<sup>3</sup> Costs

of conducting such a trial are substantial. In a recent pharmacoeconomic analysis based on this randomized trial, the average costs of managing a patient receiving voriconazole were \$30 664.<sup>43</sup> Costs for survivors were even higher (approximately \$43 000). Although more than half of these costs were attributed to costs of hospitalization, drug therapy costs were substantial and would be increased further in a study of combination antifungal therapy. Thus performing a combination trial in the clinical setting will be expensive and challenging. Second, adoption of more routine antifungal prophylaxis in high-risk patients with the recent FDA-approval of posaconazole could reduce the incidence of documented aspergillosis (proven and probable), making it even harder to perform such a trial. However, despite these realistic concerns it should not dissuade the resolve to push forward and attempt to perform these critical evidence-based studies. If combination antifungal therapies are better, then we desperately need to know it.

Furthermore, development and validation of reliable surrogate markers, such as viral load response in studies of antiretrovirals for HIV infection or CSF colony counts for cryptococcal meningitis, might enable us to complete such a study on a more rapid, and possibly smaller scale. The measurement of galactomannan (Platelia *Aspergillus* EIA, Bio Rad Laboratories, Hercules, CA, USA) in serum could potentially serve as a surrogate marker in invasive aspergillosis. Although it has been used successfully as a screening tool for invasive aspergillosis among HSCT recipients and leukemics in several centers, the reliability of serum galactomannan can be affected by use of antifungal prophylaxis,  $\beta$ -lactam antibiotics such as piperacillin/tazobactam and dietary mannan ingestion in children.<sup>44–49</sup> Measurement of galactomannan in other fluids such as BAL is not currently performed on a routine basis nor is it FDA-approved, but may support a diagnosis of aspergillosis in the appropriate clinical context.<sup>50–53</sup> Additional studies are necessary to help define the role and significance of galactomannan assay results. Other new diagnostics assays have been explored, including the (1 $\rightarrow$ 3)- $\beta$ -D-glucan assay (GlucateLL, Associates of Cape Cod, Falmouth, MA, USA) for a variety of fungi including *Candida* and *Aspergillus* spp. PCR-based methods have been developed but none are currently commercially available.<sup>46,54–61</sup> For all of these assays, sensitivity has varied widely in published reports. Novel diagnostic methods may prove to be useful, and a combination of these diagnostics assays may be more reliable than a single test performed in isolation.<sup>57</sup> Since diagnostic delays are so common for infections owing to yeasts and in particular moulds, discovery and implementation of novel diagnostic strategies are critically needed and could possibly be used to help determine efficacy of drug therapy.

#### *Practical implications of combination therapy at the bedside*

Wishing for future studies to define properly successes of combination therapy for mould infections, however, does not help our patients today. Considerations for studying as well as managing these infections among HSCT recipients

are summarized in Table 3. How do we manage these infections in the present? First, what drives our optimism regarding combinations? Is it the success of HAART in HIV infection or is it the hematologists/oncologists who are familiar with the successes of combination chemotherapies for malignancies? Is it as simple as if one drug works, then two or three drugs might work better in this very sick patient? Whatever the thought process driving the clinician, it is a prominent force that must be examined. HAART is driven by development of direct drug resistance for single drug therapy. In fungal infections this has only been consistently documented with flucytosine therapy. Furthermore, it is not clear that an established fungal infection with high burden of organisms can be completely eliminated without some immune reconstitution or activation. Finally, the concept that 'more is better' is not consistently proven and in fact, in cryptococcal meningitis a three-drug regimen was not more fungicidal than a two-drug regimen.<sup>25</sup> Therefore, most of the superficial defenses for combination therapy are unfounded. Does that stop clinicians from considering combination therapy of an azole/echinocandin/polyene when they are confronted with a patient with invasive aspergillosis who is at high risk for drug failure? We suspect it does not. Furthermore, most recently, the combination strategy has been extended to include patients with invasive zygomycosis, since they too have such a poor prognosis. Should these patients now receive both a polyene and posaconazole together? Where are the data? Is there antagonism of azole and polyene together?

Questions abound without answers but clinicians cannot wait at the bedside, and with safer antifungals the common justification for their use is 'maybe we can do better and the added drugs won't harm the patient'. Well, can we do harm? Theoretically, we can. First, the pharmacy budget is adversely affected. A course of voriconazole for invasive aspergillosis is estimated to cost approximately \$21 000.<sup>42</sup> Addition of caspofungin would increase costs by more than \$30 000 for a 12-week course. This strategy is already resulting in dramatic increases of antifungal expenditures at most major medical centers.<sup>62</sup> A second potential for harm lies in increasing the hazards of the patient experiencing toxicities or drug interactions. Addition of drugs to any regimen increases complexity and carries risk of adverse effects. Third, the possibility of antifungal antagonism cannot be ignored. This was recently illustrated by the antagonism between ravuconazole and liposomal amphotericin B *in vitro* and in an animal model.<sup>63</sup> Therefore, it is a balancing act for the clinician in the haze of data deficiency and yet real, acute complications. There is simply no cogent answer to define the right way for combination antifungal use in invasive mould infections. That said, there can be definite criticisms of the use of combination antifungal therapy in an empirical strategy. Pouring extra drugs into patients because they are doing poorly without a solid diagnosis is probably doomed to failure for the majority of patients and should be discouraged.

As uncertain as the value of combination therapy remains in many invasive mycoses, there has been a subtle change in the medical management of invasive mycoses as the ability to use multiple antifungal classes is now

**Table 3** Special considerations for use and study of combination antifungal therapy in stem cell transplant patients

Rapid and accurate diagnosis	Routine screening with galactomannan has been helpful in some institutions; screening strategies are most effective when performed 2–3 times per week in high-risk patients. False-positive results with serum galactomannan measurements have been reported in the setting of $\beta$ -lactam use (piperacillin/tazobactam, amoxicillin, amoxicillin/clavulanic acid, etc.). Serial CT scans have been beneficial in some institutions, but organisms in addition to <i>Aspergillus</i> can cause a 'halo' or crescent sign on chest CT. Bronchoalveolar lavage (BAL) fluid should be cultured in those with pulmonary infections whenever possible to help make a solid diagnosis. Fungal PCR testing has not been routinely implemented in most clinical microbiology laboratories
Confounding issues in interpreting clinical signs and symptoms	Engraftment syndromes (ES) associated with fever and rash can mimic fungal infection, and these patients often receive empirical antifungal therapy. Presence of graft-versus-host-disease (GVHD) and GVHD-associated therapy can increase risk of fungal infections. Steroid treatment for ES and GVHD can increase risk of opportunistic infections. Idiopathic pneumonia syndrome following pre-HSCT radiation conditioning can initially present with symptoms similar to a pulmonary fungal infection, and given the high risk of IFI in this setting requires diagnostic work-up to rule out infection
Pharmacokinetics, pharmacodynamics, and drug interactions	Conditioning regimens, radiation, and presence of GVHD affect the gut mucosa among HSCT recipients, resulting in decreased ability to absorb enterally-administered medications. Azoles are often administered orally and may require monitoring to ensure adequate serum concentrations in this population. Antifungals may interact with tacrolimus, cyclosporine, and steroids. In addition, interactions leading to toxicity between azoles and antineoplastic therapy have been reported
Surgical intervention	Debulking or debriding infected tissue is particularly important for mould infections, especially in the sinus, CNS or lung. Soft-tissue infections isolated in the extremities may also benefit from surgical intervention and local application of antifungal therapy. Not all patients may be candidates for surgery, especially those with severe thrombocytopenia or those that are clinically unstable. However, surgery should be considered as soon as is medically safe and timing is a bedside decision
Prophylaxis	Routine use of combination antifungals in the setting of prophylaxis is not warranted, based on available data. Single antifungals have proven effective in this setting. Care must be taken to carefully diagnose and manage break-through infections that occur despite antifungal prophylaxis. Antifungal prophylaxis can substantially reduce the sensitivity of diagnostic assays such as galactomannan
Efficacy of single agents	Single antifungal agents have been efficacious in managing cases of invasive candidiasis, invasive aspergillosis, and other invasive mould infections. These approaches are most effective when underlying disease is controlled. More rapid initiation of antifungal therapy may be associated with better outcomes. Randomized, comparative trials for these infections have not demonstrated superiority of combination antifungal therapy compared to monotherapy
Excessive costs	Use of combination antifungal therapy increases medication treatment costs compared to single agents alone. For example, addition of caspofungin to voriconazole for a 12-week treatment course for invasive aspergillosis would more than double pharmacy costs compared to voriconazole alone
Emergence of resistance	With the exception of flucytosine, secondary resistance that develops while receiving antifungal therapy is rare. There are no data to suggest that administration of combination antifungal therapy (except in the case of flucytosine) will prevent the emergence of resistant fungal pathogens in a given patient. Resistant pathogens may cause breakthrough infections in patients receiving azole prophylaxis or treatment

available. With experience of other disciplines such as hematology/oncology and HIV care, we talk about phases of antifungal treatment such as induction, clearance and suppressive phases. Within these phases we may use different antifungal agents in sequence. This strategy allows us to control and suppress infection during the ongoing management of the underlying disease. This sequence strategy of combining antifungals is elegantly described in cryptococcal meningitis, frequently used in candidemia and even in mould infections. For instance, in cryptococcal meningitis induction therapy with amphotericin B plus flucytosine and then clearance/suppression therapy with fluconazole are used.<sup>24</sup> In candidemia, initial therapy with an echinocandin is followed by oral fluconazole to complete a course of therapy.<sup>34</sup> In fact, we suspect zygomycosis will initially be treated with a lipid formulation of amphotericin B and then switched to oral posaconazole to finish a course

of treatment. Therefore, the sequence strategy of combining antifungal agents is likely to be used frequently in clinical practice.

### Conclusions

In our opinion there have been substantial advances in new antifungal agents over the last decade. If these agents are given early in infection while gaining some control of immune status and underlying disease, present antifungal agents on their own will successfully manage most invasive fungal infections. On the other hand, high-risk patients or those with resistant isolates associated with poor outcomes do reside on the fringes of standard guidelines and strategies. In these patients, clinicians must practice the art of medicine and combination antifungal therapy is a therapeutic tool, which will likely be used until robust

studies are performed for better guidance. Our primary focus must be to cure optimally or control these opportunistic infections in very fragile patients. In some patients use of more than one antifungal agent will simply be considered necessary at the bedside one patient at a time.

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