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Antifungal Combination Therapy
Clinical Potential

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Abstract

Combination antifungal therapy has been an area of research and clinical interest since systemic antifungals became available decades ago. *In vitro* and clinical data were generated for some of the more common invasive fungal infections, especially candidiasis, but until very recently few clinical studies were performed. The first invasive fungal infection to be examined in clinical trials with adequate statistical power was cryptococcal meningitis and several of these trials stand out as classical studies in the clinical evaluation of combination antifungal therapy. More recently, since the availability of the newer antifungal agents, including the echinocandins and extended-spectrum triazoles, there has been a growing interest in examining combination antifungal therapy for invasive fungal disease, especially invasive aspergillosis.

This is by no means a comprehensive review of all existing experimental data. Instead, the focus is on the clinical data that have been generated to date and on providing insights into potential future clinical directions. For instance, recent clinical data for cryptococcosis confirm that amphotericin B plus flucytosine is the most active combination for patients with cryptococcal meningitis. A recently completed clinical trial in candidaemia suggests a trend towards improved outcomes among patients receiving amphotericin B plus fluconazole versus fluconazole alone. In aspergillosis, several experimental models suggest benefit of a variety of antifungal combinations, but have not been confirmed in prospective clinical trials. Ultimately, the goal is to provide the reader with a comprehensive but useful review to this complicated and often confusing therapeutic dilemma.

Combination antimicrobial therapy has long been used as a successful treatment paradigm for various infectious diseases[1,2] and the use of combination antifungal therapy has been described for at least 2 decades.[3] Most clinical experience with combination antifungal therapy has been gained from the treatment of cryptococcosis.[4-6] Early studies of combination therapy were designed after it was discovered that rapid development of resistance occurred with use of flucytosine monotherapy for cryptococcal pneumonia.[7,8] The use of amphotericin B plus flucytosine for the treatment of cryptococcosis has resulted in higher rates of cure or improvement and more rapid cerebrospinal fluid (CSF) sterilisation when compared with amphotericin B alone.[4-6] Because of an increased incidence of invasive fungal infections due to *Candida* and *Aspergillus* spp. over the past several decades, and the associated high morbidity and mortality despite advances in patient care, clinicians are searching for

better therapeutic options. Fortunately, there has been a recent increase in the number of systemic antifungal agents commercially available, including the echinocandin class with an antifungal effect at a different site of action than other available antifungal agents. The use of combination antifungal therapy in the hope of achieving synergy and reduced antifungal resistance to combat invasive mycoses is an exciting yet unproven possibility; however, efficacy, toxicities and economics must be thoroughly evaluated.

This review discusses the rationale for combination therapy and the clinical potential of combination antifungal therapy, with a focus on cryptococcosis, candidaemia and aspergillosis. Although the underlying immune system is of extreme importance in the ability of patients to recover from invasive fungal infections, this review does not focus on combination immunotherapy. This is by no means a comprehensive review of all existing experimental data. Instead, the focus is on the clinical data that have been generated to date and providing insights into potential future clinical directions. To obtain data regarding combination antifungal therapy, we undertook a MEDLINE search of English-language articles, using keywords including 'combination antifungal therapy', 'synergy' and 'antifungal'. In addition, reference lists of identified articles and abstracts from recent international meetings were reviewed.

1. Pharmacological Principles of Combination Antifungal Therapy

Ideally, the overall goal of combination antifungal therapy is to achieve increased clinical efficacy and to avoid toxicity to the patient. There are several advantages and reasons to consider combination therapy: (i) potential increased potency and extent of fungal killing (synergy); (ii) broader spectrum of activity, targeting potentially resistant pathogens; (iii) to prevent the emergence of resistance; and (iv) combination therapy may allow for reduced dosages of individual antifungal drugs that may minimise toxicities. This review focuses on combination therapy in order to increase potency and fungal killing.

The interactions of antifungals in combination therapy *in vitro* and in animal models are best described by the terms synergy, antagonism, additivity and indifference.[9-11] Synergy is defined as improvement in activity by a magnitude greater than the expected sum of individual activities of individual agents. What must be avoided is antagonism, when the antifungal activity of the combination of agents is less than that of the least active antifungal when given alone. An additive effect is seen when an improvement in efficacy is no greater than the sum activity of individual drugs. Indifference refers to combinations that do not exhibit greater antifungal activity than the single most active antifungal agent used alone. For clinical studies, the terms synergy, additivity, antagonism and indifference are not commonly used and are poorly defined. The clinical importance is whether two antifungal drugs (when compared with one) will result in improved or worsened outcomes. It is important to note that the definitions of synergy, additivity, antagonism and indifference have been a topic of debate by investigators.[12] Clearly, while 'synergy' or 'antagonism' seem to be quite intuitive, the terms 'indifferent' and 'additive' are more nebulous and represent a neutral result. For the purposes of this review we have used these terms, but the inherent definition limitations must be considered when evaluating *in vitro* and animal data.[12]

In addition to the advantages and reasons to consider combination antifungal therapy listed previously, there are several important strategies of combination antifungal therapy to discuss, each of which depends on a complex interaction between the antifungal agent, the host and the pathogen. Pharmacokinetics generally refers to the disposition of drugs in the body, including absorption, distribution and elimination. These properties can be exploited with combination therapy. For example, a combination of an antifungal and another drug (not an antifungal) that decreases the metabolism of the antifungal would allow increased antifungal drug concentrations and potentially increased efficacy. This concept is illustrated with the administration of protease inhibitors (i.e. nelfinavir, ritonavir) and

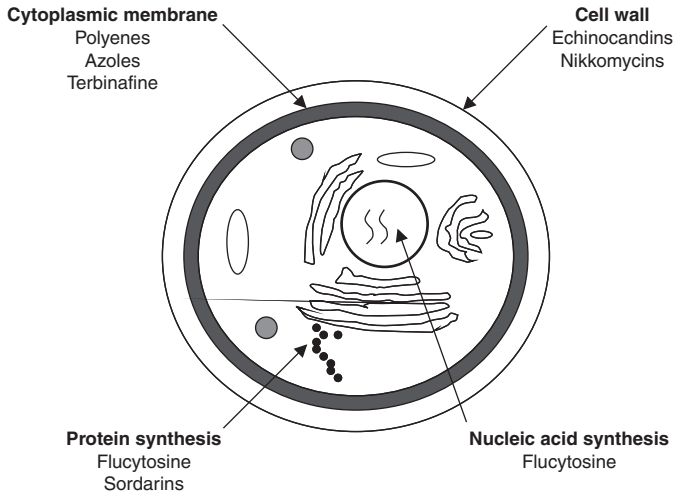

Fig. 1. Diagram of the fungal cell showing sites of action of antifungal drugs.

fluconazole. Protease inhibitors decrease hepatic metabolism of fluconazole, resulting in increased fluconazole concentrations.[9,13] More frequently, combinations of azoles and drugs that are metabolised through the cytochrome P450 (CYP) system (i.e. rifamycins) are antagonistic because of induction of azole metabolism leading to decreased azole concentrations. This pharmacokinetic combination therapy strategy, although important and sometimes clinically useful, is not discussed further.

Pharmacodynamics generally refers to the relationship between serum concentrations and the pharmacological and toxicological effects of the drug, and is important when discussing combination therapy.[13,14] Two antifungals that exert an effect on different sites of the fungal cell may have increased efficacy as a result of one agent affecting local concentrations of the other drug. An example of this is the combination of a polyene (amphotericin B) and flucytosine, where amphotericin B may allow increased cellular uptake of flucytosine resulting in increased flucytosine concentrations.[13,15] An analogous situation is the interaction of amphotericin B and rifampicin (rifampin), an agent without intrinsic antifungal activity.[12,16,17] In contrast, azoles, by their action on ergosterol synthesis, may deplete the cytoplasmic membrane of ergosterol and reduce binding sites for amphotericin B, ultimately causing antagonism.[9,18,19] Although we assume with combination therapy that two drugs should be administered at the same time, it is important to remember that peak tissue concentrations and optimal killing may vary between two antifungal drugs of different classes, depending on the antimicrobial activity pattern. For example, amphotericin B and echinocandins exhibit concentration-dependent killing,[14] whereas azoles and flucytosine have a time-dependent pattern of killing.[14]

2. Antifungal Drugs

To understand the theoretical basis for combination antifungal therapy, a review of the site and mechanism of antifungal drugs is helpful (figure 1). The main sites of action of antifungal drugs (directly or indirectly) are the: (i) cytoplasmic membrane (azole, polyenes); (ii) cell wall (echinocandins, nikkomycins); and (iii) DNA and protein synthesis machinery (flucytosine, sordarins). Using a combination of drugs that act at different sites theoretically may result in synergistic killing of fungal pathogens. Potentially significant combinations that may allow for synergistic effects include: (i) a cytoplasmic membrane agent (amphotericin B or azole) plus an agent that acts on DNA or protein synthesis (flucytosine) in the hope that the cytoplasmic agent would increase the penetration or uptake of the

second drug (this mechanism may explain the synergism of antifungals and other antibacterials such as rifampicin or quinolones);[13,15,20] (ii) a combination of agents that inhibit a step in a particular biochemical pathway, such as terbinafine and an azole;[10,13,21] and (iii) a cell wall-active agent (echinocandin) plus a cytoplasmic membrane agent (azole or amphotericin B), in the hope that two sites of action would lead to improved and more rapid killing.[12,22]

Equally important to consider are mechanisms for potential antagonism, particularly regarding the interaction of azoles and amphotericin B. Azoles result in depletion of ergosterol in the cytoplasmic membrane. Therefore, in combination with amphotericin B, fewer binding sites for amphotericin B are available and subsequently result in decreased binding and antifungal effect of amphotericin B.[9,18,19] However, on the basis of *in vitro* and *in vivo* models, not all azoles are similar in their antagonistic properties with amphotericin B. An explanation for this variability resides in the lipophilic properties of azoles. Lipophilic azoles such as itraconazole and ketoconazole, by accumulation on the cell membrane, are purported to block the interaction of amphotericin B with sterol components on the fungal cell membrane.[23,24] Fluconazole, because it is more hydrophilic, does not accumulate in the membrane and interact with amphotericin B, resulting in reduced antagonism or neutral effect. Indeed, no clinical or mycological antagonism between the combination of fluconazole and amphotericin B were observed in a recent clinical trial of candidaemia.[25] An additional method of antagonism, more related to sequential than combination therapy, is important to note. Recent laboratory studies have shown that pre-exposure to an azole compound causes changes in the membrane ergosterol component, to which amphotericin B may then bind less easily, and result in antagonism.[12,18,26,27]

Amphotericin B desoxycholate, lipid preparations of amphotericin B, flucytosine, ketoconazole, fluconazole, itraconazole, voriconazole, terbinafine and caspofungin are among the drugs currently available for the therapy of systemic fungal diseases. These drugs, in addition to investigational azoles, echinocandins, nikkomycin Z and sordarins, have been studied as combination therapy *in vitro* and in animal models. The drugs, listed by site or mechanism of action (figure 1), are reviewed briefly in sections 2.1 to 2.3.

2.1 Cytoplasmic Membrane

The polyene antifungals have been in clinical use since the 1950s. Amphotericin B, the most frequently used polyene, has a lipophilic structure and binds avidly to ergosterol, the principal sterol in fungal cytoplasmic membranes.[28,29] This results in a disruption of membrane integrity with subsequent leakage of intracellular contents and eventual cell death.[28,30] This fungicidal agent has typically been the 'gold standard' of antifungal therapy.

The azole antifungals were developed in the 1970s and since then have been utilised as effective and less toxic alternatives to amphotericin B for the treatment of fungal infections. The azole antifungals inhibit CYP-dependent 14-α-demethylase, thereby interrupting the conversion of lanosterol to ergosterol, the major component of the fungal cell membrane.[30,31] This results in increased membrane permeability with leakage of intracellular contents and, ultimately, cell death. The most commonly used systemic azoles include the triazoles fluconazole, itraconazole and voriconazole. Posaconazole is an extended-spectrum triazole in advanced clinical development.

Terbinafine is a synthetic allylamine derivative that exerts its antifungal effect by inhibiting squalene epoxidase, an enzyme involved in the synthesis of membrane ergosterol. Terbinafine has been primarily used in the US as therapy for superficial dermatophyte infections, but results from *in vitro* experiments in combination with polyenes and azoles against *Candida* and *Aspergillus* spp. support further evaluation in animal models.[21,32-34]

2.2 Cell Wall-Active Agents

The echinocandin class of antifungals was discovered in the 1970s as naturally occurring metabolites of fungal fermentation.[35] Echinocandins are

semisynthetic lipopeptide antifungals that act on the fungal cell wall by inhibiting 1,3-β-D-glucan.[36] Glucans are essential components of the cell wall in many pathogenic fungi but are not present in mammalian cells. Depletion of 1,3-β-D-glucan synthesis at the plasma membrane leads to depletion of cell wall glucan, osmotic instability and lysis of the fungal cell wall.[37] Caspofungin is the only echinocandin commercially available in the US; however, others in advanced development include micafungin and anidulafungin.

The nikkomycins are a class of drugs that are structurally similar to a precursor for chitin, a component of the fungal cell wall. Nikkomycins act as competitive inhibitors of fungal chitin synthase enzymes, and lead to cell wall defects and osmotic swelling of the fungal cell.[38] *In vitro* studies of nikkomycin Z combined with echinocandins or triazoles have demonstrated synergy against *Candida*, *Cryptococcus* and *Aspergillus* spp.; these observations warrant evaluation in animal models.[39,40]

2.3 DNA or Protein Synthesis

Flucytosine is an orally administered antifungal agent whose mechanism is based on antimetabolite properties. It is available as an intravenous formulation in some countries. After uptake by fungi, flucytosine is converted by intracellular deamination to fluorouracil, and then converted to 5-fluorouridine triphosphate (FUTP). FUTP is incorporated into fungal RNA and inhibits protein synthesis.[41] A second mechanism of action occurs when fluorouracil is converted to fluorodeoxyuridine monophosphate, which interferes with DNA synthesis by inhibiting thymidylate synthetase.[41,42] Resistance to flucytosine occurs rapidly when it is used as a single agent, and it is used principally in combination with amphotericin B for the treatment of cryptococcal meningitis and disseminated or invasive forms of candidiasis.[7,8]

The sordarins are a class of drugs that act by inhibiting protein synthesis in pathogenic fungi by acting on elongation factor 2.[43] Several sordarin compounds have been found to be synergistic or additive with amphotericin B or azoles against *Candida*, *Aspergillus* or *Scedosporium* spp.,[44] but these compounds remain in preclinical development.

3. Combination Therapy: Testing for Synergy

Finding the ideal testing conditions to determine synergy or antagonism, whether *in vitro* or *in vivo* animal models, is a formidable task.[12] The results of *in vitro* laboratory experiments are often highly dependent on methodology. Combination antifungal susceptibility testing is commonly performed using the 'checkerboard' method because of its relative ease.[10] This method uses the calculation of the fractional inhibitory concentration index to evaluate combinations. It also assumes that there is accuracy of *in vitro* minimum inhibitory concentrations and also that there is biological relevance. What is often overlooked in synergy testing is the importance of time of antifungal administration and individual pharmacokinetics of antifungal drugs. Typically, two drugs are tested simultaneously, even though drugs may require different times to become fungicidal or fungistatic.[13,14] (More information can be found in previous extensive reviews.[10,12,45]) Additional methods for synergy testing include time-kill studies, E-test and the response surface model.[9,10,13,45] Depending on the test method employed, authors may observe very disparate results due to differences in species or strains of fungi, different media, non-standardised incubation times and temperatures, or use of different measures of fungal growth.[46] Moreover, different methods may have differing reproducibility. A recent study of three methods concluded that the 'checkerboard' methodology may not be as reliable at detecting antagonism among *Candida* isolates when compared with the time-kill methodology.[47] In addition, time-kill methodologies are able to provide more detailed descriptions of antifungal activity over time.[9] The lack of a standardised method has made multicentre studies difficult because of problems with interlaboratory reproducibility. An important goal for future combination antifungal therapy research should be to develop a reproducible

model by which results are predictive of clinical outcomes.

Animal models have been helpful in elucidating appropriate combination antibacterial therapy and are preferred for initial evaluation of antifungal compounds.[10,13] Moreover, animal models lack some of the common methodological problems encountered with *in vitro* testing. Animal models also allow for a determination of toxicity and pharmacokinetics.[13,46] Studies of antifungal combination therapy are typically carried out with rabbits, mice or rats, and manipulation of the species to achieve immunosuppression is standardised and easily performed. Other than methodological problems, animal models often do not simulate human infections and may not be powered to determine subtle differences in outcome measures.[13] It is important to note that results of *in vitro* and *in vivo* combination studies have not been predictive of clinical outcomes. Indeed, susceptibility testing for one antifungal drug and demonstration of correlation to outcome is extremely problematic. With these important limitations in mind, selected *in vitro*, *in vivo* and clinical data of combination therapy are summarised for *Candida*, *Cryptococcus* and *Aspergillus* spp. in sections 4 to 6. This summary highlights areas where combination therapy is of potential and warrants further study.

4. Candidiasis

4.1 *In Vitro* Studies

In vitro studies of combination antifungal therapy for candidiasis have demonstrated variable results depending on the specific isolate and experimental conditions.[47-50] Generally, the combination of amphotericin B plus flucytosine has demonstrated at least an additive effect, and it is suggested that these findings have been most dramatic in the setting of flucytosine-resistant strains. Furthermore, it is suggested that the combination of flucytosine and amphotericin B reduces the emergence of flucytosine resistance.[51] Combinations of an azole and flucytosine have demonstrated less encouraging results, frequently resulting in either indifference or antagonism.[47,48,52] Some of the more consistent observations among *in vitro* studies of antifungal combinations for *Candida* spp. have been with amphotericin B and azole combinations.[53-55] Most of the *in vitro* studies with combinations of amphotericin B and fluconazole, itraconazole or the early azoles (e.g. ketoconazole and miconazole) have demonstrated antagonism. Moreover, prior exposure to an azole may result in reduction of amphotericin B activity in these *in vitro* studies through depletion or alteration of the sterol target.[27,56,57] Other *in vitro* data examining other combinations, especially terbinafine and an azole, have generally demonstrated either synergy or indifference; antagonism has been rarely reported in these circumstances.[21,58] Results from combinations of echinocandins with azoles or polyenes have generally been disappointing.[59,60]

4.2 *In Vivo* Studies

Murine and rabbit models of combination therapy with amphotericin B and flucytosine have generally resulted in positive results demonstrated by improved survival or tissue sterilisation,[51,61] consistent with *in vitro* findings. Animal models of combinations with amphotericin B and azoles have not always been consistent with *in vitro* data. For example, whereas *in vitro* studies of combinations with amphotericin B and azoles generally suggested antagonism, Sugar and colleagues[62] demonstrated no evidence of clinical or mycological antagonism in their murine model of invasive candidiasis using the combination of fluconazole and amphotericin B. Indeed, in this model of immunocompetent and immunocompromised mice, these authors demonstrated that the combination was at least as effective as amphotericin B and more effective than fluconazole alone.[62] The encouraging findings that were demonstrated in this model led to the development of a combination therapy clinical trial described in detail in section 4.3.[25] However, other models have yielded conflicting data, suggesting worse outcomes and/or 'clinical' antagonism.[63,64]

In vivo studies of combination therapy with an echinocandin and another antifungal agent have

generally yielded favourable results. In a recent study of caspofungin and fluconazole in a murine model, renal tissue burden was reduced in mice receiving combination therapy compared with those receiving fluconazole alone, but there was no difference when compared with those receiving caspofungin alone.[65] A summary of *in vitro* and animal model combination therapy studies for *Candida* spp. can be seen in table I.

4.3 Clinical Studies of Invasive Candidiasis

The first clinical application of combination therapy was for invasive candidiasis, and involved the use of parenteral amphotericin B and flucytosine administered orally. This combination has been used in various clinical settings for decades, and has included difficult clinical settings such as candidal endocarditis, meningitis and other life-threatening deeply invasive infections, but its use was based largely on anecdotal reports of successful therapy.[66,67] No randomised clinical trial was formally conducted until recently, when the combination of amphotericin B plus flucytosine was compared with fluconazole in a randomised study of non-neutropenic patients with invasive candidiasis in intensive care units.[68] In this randomised study, the combination was more effective than fluconazole in successfully treating peritonitis and sterilising tissues. The remaining reports of efficacy for this combination have been limited to small series and case reports among patients with prosthetic joint infections, native valve endocarditis, and candidal meningitis among HIV and non-HIV infected patients.[66,67,69]

Table I. Selected antifungal drug interactions for *Candida* spp.[a]

Combination	*In vitro*	*In vivo*
Amphotericin B + flucytosine[47-51,54]	S, Add, I	S, Add
Amphotericin B + itraconazole[55]	Ant	I, Ant
Amphotericin B + fluconazole[47,48,55,57,62,63]	Add, I, Ant	I, Ant
Amphotericin B + terbinafine[21]	S, Add	ND
Amphotericin B + echinocandin[60]	S, Add, I	I
Amphotericin B + rifampicin[16,61]	S	I
Fluconazole + echinocandin[65]	I	ND
Flucytosine + fluconazole[63]	S, I, Ant	S, Add, I

a Findings listed are a summary from the referenced studies. Different results for a particular combination may result from different methodologies or definitions.

Add = additive; **Ant** = antagonistic; **I** = indifferent; **ND** = insufficient data available; **S** = synergistic.

In a recently published study, the combination of conventional amphotericin B and high-dose fluconazole (800mg daily) was compared with high-dose fluconazole (800mg daily) alone for the treatment of non-neutropenic patients with candidaemia.[25] This study, involving 219 evaluable patients, is one of the largest published randomised and double-blind studies of candidaemia performed to date, and was designed not only to determine the efficacy of an amphotericin B and fluconazole combination compared with fluconazole alone, but also to determine if high-dose fluconazole offered a better therapeutic option than standard therapy with fluconazole 400mg daily when compared with historical controls. Patients with neutropenia and *Candida krusei* infections were excluded from this study, but it was open to most other non-neutropenic patients. The two study regimens were as follows: amphotericin B 0.6–0.7 mg/kg together with fluconazole 800mg administered daily for the first 5–7 days, followed by fluconazole 800 mg/day, versus fluconazole 800mg alone administered daily. Both regimens were administered for 14 days beyond the last positive culture and/or resolution of signs and symptoms attributable to candidaemia. The study was double-blinded and patients on fluconazole monotherapy received a placebo amphotericin B solution.[25]

Results of this study demonstrated a 69% versus 56% successful outcome in favour of combination therapy with amphotericin B plus fluconazole ($p = 0.043$) over monotherapy; however, success rates utilising a Kaplan-Meier time-to-failure analysis were not significantly different ($p = 0.08$). An important observation in the study was that only 6% versus 17% of patients receiving combination therapy versus fluconazole monotherapy, respectively, remained candidaemic on therapy ($p = 0.02$). An important confounding factor of this study was that the Acute Physiology and Chronic Health Evaluation (APACHE II) scores were significantly different for the two groups (16.8 and 15.0 for monothera-

py and combination therapy, respectively; p = 0.039). There were no differences in catheter management or duration of therapy (16.7 vs 15.0 days for monotherapy and combination, respectively; p = 0.123) to account for these differences. Finally, there were no significant differences or trends in outcome according to *Candida* spp., and mortality within 90 days of study initiation was approximately 40% in each group.[25] In the final analysis there was no significant difference in successful outcome between the two study arms, although there was clearly a trend in favour of combination therapy with amphotericin B and fluconazole.

Other important observations from the study relate to the fact that there was no clinical or mycological evidence of antagonism among patients treated with combination amphotericin B and fluconazole, regardless of whether they had previously received fluconazole. Moreover, the rate of persistently positive blood cultures in the combination (6%) was lowest among any randomised candidaemia study, indicating a potential benefit of combination therapy early in the course of treatment.[25]

Refractory oral pharyngeal candidiasis in patients with AIDS has received some attention as an area of potential study with combination antifungal therapy but there are few published data in this patient population. In one report, fluconazole and terbinafine were coadministered to an HIV-infected patient with persistent fluconazole-resistant oropharyngeal candidiasis, leading to a successful outcome.[70]

The recently revised Infectious Diseases Society of America guidelines for the treatment of candidiasis advocate the coadministration of amphotericin B and flucytosine among patients in selected situations, including those with serious and deep-seated candidal infections involving the CNS, endovascular infections and serious intra-abdominal candidiasis.[71] As noted earlier, the clinical data supporting this approach is limited to a few anecdotal reports and is supported by *in vitro* and animal data. Combination therapy with amphotericin B and higher-dose fluconazole is provided as an option for the therapy of uncomplicated candidaemia on the basis of the recently published randomised trial.[25,71] There are no clinical trials and little clinical experience involving the use of an echinocandin in combination with either amphotericin B or an azole for treatment of invasive candidiasis.

5. Cryptococcosis

5.1 *In Vitro* Studies

In vitro studies have examined several antifungal combinations against *Cryptococcus neoformans*, with most of the data suggesting either indifference or additive effects with various experimental combinations. Antagonism has only rarely been demonstrated in these studies. The 'classic' combination of amphotericin B and flucytosine has yielded inconsistent results in the experimental setting; in many of these studies, synergy has not been demonstrated.[48,49,72-74] Most recent studies examining flucytosine and azole combinations have demonstrated either synergy or indifference.[48,75-77] Amphotericin B and azole combinations, with and without flucytosine, have generally revealed either indifferent or synergistic results in *in vitro* studies of activity versus *C. neoformans*.[48,78-80] Compared with *in vitro* studies of the amphotericin B and azole combination for *Candida* spp., these results have been much more favourable. Some studies have demonstrated that prior exposure to azoles may lead to diminished activity of amphotericin B against *C. neoformans* isolates *in vitro*.[78] Echinocandins have been studied in combination with other antifungal agents and have demonstrated some positive interactions,[81,82] but because of their poor *in vitro* activity against *C. neoformans* as single agents the echinocandins have not been studied extensively in experimental settings.

5.2 *In Vivo* Studies

Animal models of cryptococcosis are well described, and the mouse and rabbit models of cryptococcal meningitis are the two of the most well established animal models for the pathogenesis and treatment of cryptococcal meningitis. Most of these studies have utilised improved survival and more

rapid clearance of brain tissue and spinal fluid as indicators of efficacy.[72,83-86] Studies with combinations of amphotericin B and flucytosine in these models have generally demonstrated trends towards better outcomes with combination therapy than with amphotericin B alone.[72,83-86] Flucytosine and azole combinations also appear to have beneficial effects on survival, fungal tissue burden and body weight in murine models of cryptococcal meningitis.[75,87-90] Most recently, Larsen and colleagues[91] demonstrated significantly improved survival and reduction in fungal tissue burden among mice receiving combination therapy with amphotericin B and fluconazole compared with amphotericin B, fluconazole or flucytosine alone. Other investigators have demonstrated less encouraging outcomes when examining amphotericin B and azole combinations.[86] A summary of *in vitro* and animal model combination therapy studies for *Cryptococcus* spp. can be seen in table II.

5.3 Clinical Studies of Cryptococcosis

The treatment of cryptococcal meningitis is perhaps the most well studied indication for combination antifungal therapy in the arena of clinical mycology.[92] The first large-scale study of the combination of amphotericin B plus flucytosine was performed among patients with cryptococcal meningitis in the pre-AIDS era.[4] The design of this study reflected the prevailing concern at the time about poor clinical outcomes and significant amphotericin B-associated nephrotoxicity. Thus, the addition of flucytosine to a therapeutic regimen containing amphotericin B was a manoeuvre to limit toxicity as well as to determine if combination therapy offered superior efficacy. At the time of its publication this was the largest randomised antifungal trial ever performed, enrolling 51 patients over a 4-year period. The regimens included amphotericin B 0.4 mg/kg/day for 70 days versus amphotericin B 0.3 mg/kg plus flucytosine 150 mg/kg, both administered daily for 6 weeks.[4] This landmark study demonstrated improvement in outcome for combination therapy compared with monotherapy with amphotericin B ($p < 0.05$). Combination therapy was also associated with fewer relapses, more rapid sterilisation of the spinal fluid and significantly less nephrotoxicity. On the basis of this study, combination regimen with amphotericin B plus flucytosine for 6 weeks became the 'gold standard' for the treatment of cryptococcal meningitis in non-HIV infected patients for the next 2 decades.[4]

A follow-up to the Bennett study published several years later compared a standard regimen of amphotericin B plus flucytosine for 6 weeks compared with a 'short course' of this combination for 4 weeks.[5] Efficacy and toxicity of antifungal therapy were compared among 91 patients in this randomised, open-label trial. Patients were eligible for randomisation into the study if they had successfully completed an initial 4-week course of therapy. Transplant recipients were excluded from the study because of excessive early relapses among this group. Among those patients who met criteria for randomisation, 85% of those treated with the standard regimen versus 75% of those receiving short-course therapy had a successful outcome, a difference that was not statistically significant. Relapses were higher with short-course therapy (27% vs 16%) and significant amphotericin B-associated toxicity was no different (44% vs 43%). This study demonstrated that, among carefully selected patients, combination therapy with amphotericin B plus flucytosine for 4 weeks was an acceptable alternative to a conventional 6-week course of therapy.[5]

With the development of the AIDS pandemic, subsequent studies of cryptococcal meningitis examined variations of standard therapy compared

Table II. Selected antifungal drug interactions for *Cryptococcus* spp.[a]

Combination	*In vitro*	*In vivo*
Amphotericin B + flucytosine[48,49,72-74,83,84,86]	S, I	S, I
Amphotericin B + itraconazole[48,78-80,83]	I	S, I
Amphotericin B + fluconazole[48,78-80,83]	S, I	A, I
Flucytosine + fluconazole[46,75-77,83]	S, Add, I	S, I

a Findings listed are a summary from the referenced studies. Different results for a particular combination may result from different methodologies or definitions.

Add = additive; **I** = indifferent; **S** = synergistic.

with monotherapy with fluconazole. The first trial in the AIDS era compared combination therapy with amphotericin B and flucytosine with fluconazole as monotherapy in 20 patients with cryptococcal meningitis, including 6 who received combination therapy and 14 who received fluconazole.[93] In this small open-label trial, combination therapy led to more rapid sterilisation of CSF (15.6 vs 40.6 days) and led to more successful outcomes (100% vs 43%, p = 0.04) compared with fluconazole monotherapy. Furthermore, four patients receiving fluconazole monotherapy versus no patients receiving combination therapy died. Small numbers of patients and inconsistent management of increased intracranial pressure were limiting factors in this study.[93]

In a subsequent study, Saag and colleagues[94] conducted a randomised, open-label study among 194 evaluable HIV-positive patients with acute cryptococcal meningitis, including 131 receiving fluconazole 400 mg/day alone compared with amphotericin B 0.4–0.5 mg/kg/day for 10 weeks. Among amphotericin B recipients, flucytosine was administered at the discretion of the investigator. In this study, the majority of amphotericin B recipients received flucytosine, and there were no significant differences in overall mortality and treatment success between treatment groups. The median length of time to the first negative CSF culture was 42 days in the amphotericin B group and 64 days in the fluconazole group (p = 0.25). The authors concluded that fluconazole was an effective alternative to amphotericin B, with or without flucytosine, as primary therapy for cryptococcal meningitis among patients with AIDS despite the slower rate of CSF culture conversion in the fluconazole group.[94] Clinicians expressed concern over these conclusions and few utilised fluconazole as primary therapy in this setting.

In a follow-up to the Saag et al.[94] study, another large randomised trial was conducted. In this trial, induction therapy with amphotericin B was compared with amphotericin B plus flucytosine, both regimens administered for the first 2 weeks of therapy, followed by therapy with an azole (either fluconazole or itraconazole).[6] In this double-blind study, 381 patients with AIDS and acute cryptococcal meningitis were randomised. In the primary analysis, 60% of 202 patients receiving combination therapy versus 51% of the 179 patients receiving monotherapy with amphotericin B were CSF culture-negative at 2 weeks (p = 0.06). Clinical outcome did not differ between the two groups. In the second phase of the study patients were randomised to receive either fluconazole or itraconazole to complete a course of 10 weeks total antifungal therapy. There were no differences in outcome at completion of study and overall mortality during the study period in both arms was <10%.[6] These results demonstrated that combination therapy with amphotericin B and flucytosine was associated with a trend towards more rapid sterilisation of CSF at 2 weeks. In a *post hoc* analysis among patients who were randomised to azole therapy and followed for several months, it was further noted that relapses occurred much more commonly among patients who received itraconazole when compared with fluconazole recipients (p < 001).[95] Moreover, relapses were more common among the patients who received initial therapy with amphotericin B alone compared with combination therapy with amphotericin B and flucytosine (p < 0.05).[95]

The use of fluconazole combined with flucytosine as an all-oral regimen for the treatment of cryptococcal meningitis was first espoused by Larsen and colleagues.[96] In an open-label pilot study, 32 patients received fluconazole 400mg combined with flucytosine 150 mg/kg, both orally administered daily for 10 weeks. At the completion of the study, 63% of patients had demonstrated clinical success. The median time to CSF culture negativity was 23 days. Both the overall success and the conversion to CSF culture negativity were superior to published experience of monotherapy with either fluconazole or amphotericin B in this patient population.[96] A subsequent randomised study in Uganda comparing combination therapy with fluconazole and flucytosine with monotherapy with fluconazole 200mg, both administered daily, demonstrated a trend towards a more favourable outcome at 6 months for combination (32% survival) versus

monotherapy (12% survival at 6 months, p = 0.22). In this study the daily dose of fluconazole (200mg) was lower than what is routinely administered.[97] Finally, there are anecdotal reports of cryptococcosis successfully treated with an all-oral regimen of fluconazole and flucytosine.[98] In aggregate, these data suggest that the combination of fluconazole and flucytosine has beneficial clinical and mycological effects.

In a recently published study, Brouwer and colleagues[99] demonstrated the benefit of combination antifungal therapies for AIDS-associated cryptococcal meningitis. In this open-label study the authors administered amphotericin B alone, amphotericin B plus flucytosine, amphotericin B plus fluconazole, or triple combination therapy with amphotericin B, flucytosine and fluconazole to 64 Thai patients with HIV. There were 16 patients in each treatment arm and quantitative CSF cultures were obtained four times during the first 2 weeks of treatment. The study clearly demonstrated mycological benefit with any combination compared with amphotericin B alone as measured by quantitative cultures and the rate of CSF culture negativity. The combination of amphotericin B plus flucytosine rendered CSF cultures negative earlier than amphotericin B alone ($p = 0.0006$), but the other combinations also rendered CSF cultures negative earlier than amphotericin B alone ($p < 0.05$). Toxicity was not a major confounding issue in this study. The authors noted that the small numbers of patients in the study made it impossible to determine the clinical significance of these findings, but this study clearly demonstrates an ability to perform quantitative CSF fungal cultures in humans cryptococcal meninigitis as a measure of mycological success.[99]

An innovative approach to therapy for cryptococcal meningitis involves the use of adjunctive immunotherapy combined with conventional antifungal therapy. In the only randomised study to date, interferon (IFN)-γ or placebo was administered three times weekly along with conventional antifungal therapy for the first 10 weeks for acute cryptococcal meningitis among patients with AIDS.[100] Conventional therapy in this study involved the use of amphotericin B 0.7 mg/kg/day for 2 weeks, with or without flucytosine, followed by fluconazole 400 mg/day for 8 weeks. Seventy patients were available for efficacy analysis. These results demonstrated an important but nonsignificant trend towards more rapid sterilisation of CSF at 2 weeks among IFNγ recipients compared with placebo (38% vs 18%) and more rapid decline in CSF cryptococcal antigen. There was no obvious survival benefit among those receiving adjunctive IFNγ compared with placebo, but there was in important trend in combined clinical and mycological success among IFNγ recipients compared with placebo recipients.[100]

In summary, there are strong data among laboratory and clinical studies supporting the use of combination amphotericin B plus flucytosine for initial therapy of patients with acute cryptococcal meningitis. Unfortunately, the use of flucytosine in clinical practice is sporadic because of its limited availability, potential toxicity and the need to monitor levels with extended use. Other forms of cryptococcosis (e.g. pneumonia) have been proven difficult to study and subsequently there are no randomised trials among patients with non-CNS cryptococcosis. There are also promising clinical data involving the use of amphotericin B combined with fluconazole, and for fluconazole combined with flucytosine. A study is currently underway in the US and Thailand examining the potential benefit of amphotericin B combined with fluconazole for the initial treatment of patients with acute CNS cryptococcosis. Finally, the use of adjunctive therapy with IFNγ, although not formally approved for use in cryptococcosis, may be appropriate among selected patients with refractory disease.

6. Aspergillosis

6.1 *In Vitro* Studies

Because of the increasing incidence of invasive aspergillosis and the high associated mortality, there is a need for better therapeutic approaches for the treatment of invasive aspergillosis. An important advance in the antifungal armamentarium is the

recent availability of voriconazole and caspofungin, both of which have proven efficacy in invasive aspergillosis.[101,102] These and similar agents in development have stimulated interest in combination antifungal therapy for aspergillosis.

Generally, combinations of amphotericin B with flucytosine against *Aspergillus* spp. have shown activity ranging from synergy to indifference, and findings are similar among different species of *Aspergillus*.[54,103-105] As discussed in section 2, the effect of amphotericin B on the fungal cell membrane may result in enhanced penetration of flucytosine.[15] Amphotericin B in combination with rifampicin commonly results in a synergistic effect.[17,104] Combination of amphotericin B with azoles (fluconazole, itraconazole, voriconazole) against *Aspergillus* spp. frequently shows indifference or antagonism;[22,103,104,106] however, synergistic activity has been reported.[54] Amphotericin B in combination with echinocandins has ranged from indifference to synergy.[107-109] Combinations of the echinocandins with triazoles range from synergistic activity to indifference and, importantly, no antagonism has been reported.[22,110,111] Other combinations such as terbinafine with amphotericin B[33] and nikkomycin Z with azoles or echinocandins[39,40] have shown promise in preliminary studies, but these data are limited.

6.2 *In Vivo* Studies

Animal models generally confirm results predicted by *in vitro* combination tests for *Aspergillus* spp. The combination of amphotericin B and flucytosine or rifampicin in mouse or rat models has resulted in synergy to indifference, but no antagonism has been described.[86,112,113] Antagonism is seen for the combination of amphotericin B plus itraconazole.[46,86] Pre-treatment with azoles prior to amphotericin B administration has resulted in decreased fungal activity against *Aspergillus* spp. in a murine model.[106,114] No antagonism was seen with amphotericin B plus fluconazole,[115] an observation also described in animal models of candidiasis.[19] As was previously discussed in section 2, this may result from differences in cytoplasmic membrane accumulation of the azoles.[23,24]

Several important *in vivo* studies evaluating newer azole and echinocandin combinations have been performed.[116-118] Petraitis and colleagues[116] tested the combination of ravuconazole (a newer triazole in development) and micafungin in a neutropenic rabbit model of pulmonary aspergillosis due to *Aspergillus fumigatus*. Combination therapy was superior to single-drug therapy in all outcome measures, including residual fungal burden, survival, total lung weight, pulmonary infarct score and galactomannan antigenaemia. Kirkpatrick and colleagues[117] also studied an azole-echinocandin combination using caspofungin and voriconazole in a neutropenic guinea pig model of invasive aspergillosis. This combination significantly reduced colony counts of lung, kidney, liver and brain tissue when compared with control or either drug alone; however, reduction in mortality with the combination was no different than with voriconazole alone.[117] Sabatelli[118] studied the combination of posaconazole with caspofungin in various dosages *in vitro* and for the treatment of infections with *A. fumigatus* and *A. flavus* in immunocompetent mice. *In vitro* activity with the combinations ranged from synergy to indifference but no antagonism was observed. *In vivo* data showed increased survival with combination therapy when compared with either drug alone.[118] Importantly, no antagonism was demonstrated in any of these studies.

Other studies have evaluated amphotericin B and echinocandin combinations with favourable results.[119-121] One recent study examined the combination of amphotericin B plus caspofungin in a neutropenic murine model of chronic disseminated infection with *A. fumigatus*.[119] Using real-time polymerase chain reaction to demonstrate *A. fumigatus* kidney burden, the combination therapy resulted in reduced kidney burden to levels less than or equal to that seen with either agent alone. There was an overall trend towards an additive effect and there was no antagonism.[119] In another murine model, Nakajima and colleagues[120] tested the combination of micafungin plus amphotericin B compared with

either agent alone. They demonstrated increased survival, reduction in lung fungal burden and improved histopathological findings with combination therapy. In an additional study, Petraitis and colleagues[121] studied the combination of micafungin and amphotericin B in a neutropenic rat model of invasive aspergillosis and found neither synergy nor antagonism with the combination. These *in vivo* studies demonstrate that combinations of an extended-spectrum triazole plus an echinocandin, or amphotericin B plus an echinocandin, may have clinical potential and warrant clinical investigation. A summary of *in vitro* and animal model combination therapy studies for *Aspergillus* spp. is demonstrated in table III.

6.3 Clinical Studies of Aspergillosis

Treatment of invasive aspergillosis is challenging and the disease is associated with high mortality.[122,123] Patients with invasive aspergillosis treated with amphotericin B, the traditional 'gold standard', often have response rates much less than 40%[122] and treatment is commonly associated with significant adverse events. The recent trial of voriconazole for the treatment of primary invasive aspergillosis demonstrated significantly better response rates (53%) when compared with amphotericin B (32%) after 12 weeks of therapy;[101] survival was also significantly improved. Data from the salvage study with caspofungin demonstrating a response rate of 43% among 66 patients with invasive aspergillosis are also encouraging.[102] These results utilising newer agents with a novel mechanism of action have heightened the interest in combination therapy for aspergillosis. Unfortunately, there are limited data from clinical trials on the use of combination antifungal therapy for aspergillosis.[124-128] The majority of information on combination therapy for the treatment of aspergillosis is derived from retrospective case series and reviews, which do not allow for appropriate critical analyses.[34,125,129] A recent review by Steinbach and colleagues[34] evaluated 6281 patients with aspergillosis from 1966 to 2001. Patients were included if they had invasive aspergillosis, systemic antifungal use of ≥14 days and well defined antifungal therapy. This review described 249 patients treated with combination therapy.[34] Combinations most commonly used were amphotericin B plus flucytosine (49%), amphotericin B plus itraconazole (16%) and amphotericin B plus rifampicin (11%); however, a total of 27 different combinations were reported amongst these patients.[34]

Table III. Selected antifungal drug interactions for *Aspergillus* spp.[a]

Combination	*In vitro*	*In vivo*
Amphotericin B + flucytosine[54,86,103-105,112,113]	S, Add, I	S, Add, I
Amphotericin B + itraconazole[46,86,103,104,106]	Ant	Ant
Amphotericin B + fluconazole[103,104,106,115]	I, Ant	I
Amphotericin B + terbinafine[33,34]	Add, I	I
Amphotericin B + echinocandin[107-109,119-121]	S, Add, I	S, Add, I
Amphotericin B + rifampicin[34,86,104,112]	S, I	Add
ExS triazole + echinocandin[110,111,116-118]	S, Add	S, Add
Amphotericin B + ExS triazole[22]	I	ND
Itraconazole + nikkomycin Z[39]	S	ND

a Findings listed are a summary from the referenced studies. Different results for a particular combination may result from different methodologies or definitions.

Add = additive; **Ant** = antagonistic; **ExS triazole** = extended-spectrum azole (posaconazole, voriconazole or ravuconazole); **I** = indifferent; **ND** = insufficient data available; **S** = synergistic.

The combination of amphotericin B plus flucytosine has been used to treat invasive aspergillosis since the 1970s, with scattered reports of success.[130,131] Denning and Stevens[129] reviewed 63 patients, all of whom received at least 14 days of antifungal therapy with amphotericin B plus flucytosine. Approximately two-thirds (68%) of these patients responded but an analysis of patients receiving <14 days of therapy demonstrated very poor outcomes.[132] In a subsequent study, Steinbach and colleagues[34] reviewed an additional 60 patients who received amphotericin B plus flucytosine from 1990 to 2001, all of whom received at least 14 days of therapy.[34] The combined analysis of 123 patients demonstrated overall improvement in 84 (68%) and death in 36 (29%) patients.[34] The only prospective, randomised trial of combination therapy for invasive aspergillosis to date compared amphotericin B plus flucytosine (nine patients) with amphotericin B alone (nine patients).[124] The study was terminated early because of poor response in both arms. There

was no significant difference in outcome in this small trial but the trial was not adequately powered to detect a difference.

Rifampicin has no intrinsic antifungal therapy when used alone, but the combination of amphotericin B plus rifampicin has shown synergy *in vitro* against *A. fumigatus* and *A. flavus*.[17,104] Results of animal models have been less convincing but have ranged from synergistic activity to indifference.[86,112] This combination in patients has been used relatively little over the past decade, probably because of issues with drug interactions due to induction of the CYP enzyme system. In a recent review, among 27 patients who received amphotericin B plus rifampicin for at least 14 days, improvement was seen in 18 (67%) of 24 patients, with 9 (33.3%) deaths;[34] however, a meaningful conclusion cannot be determined because no clinical comparison exists against other single or combination regimens.

With the introduction of the itraconazole in 1992, an alternative therapy for aspergillosis became available.[122] Because of its different mechanism of action (inhibition of ergosterol synthesis), combination therapy with amphotericin B and itraconazole was examined. Concern for antagonism notwithstanding, combination therapy with amphotericin B plus itraconazole has been reported in several case series.[123,125,129,132] In a recent multicentre review of the treatment and outcomes of invasive aspergillosis among 595 patients,[123] the combination of amphotericin B and itraconazole was used in 19 (3.2%) patients, but outcomes among this group were not specified. The only study to suggest benefit for this combination was a 3-year retrospective comparative series reported by Popp and colleagues,[125] which included 11 patients who received amphotericin B plus itraconazole compared with 10 patients who received amphotericin B alone. Improvement was seen in 89% of patients who received combination therapy and 50% of patients who received monotherapy. In addition, survival among patients who received combination therapy was improved. Importantly, no clinical antagonism was evident. However, these data must be interpreted with caution as the small number of patients does not allow for meaningful conclusions.[125] In contrast, in a 4-year retrospective review of 67 patients with definitive invasive aspergillosis at the MD Anderson Cancer Center (Houston, TX, USA), Hachem and colleagues[133] found that combination of amphotericin B formulations plus oral itraconazole resulted in no improved outcomes compared with amphotericin B monotherapy. In this study, the failure rate was approximately 85%. In the review of cases by Steinbach and colleagues,[34] 41 patients received amphotericin B plus itraconazole. Of these patients, improvement was seen in 20 (49%) and <50% survived. Because of potential for antagonism and lack of clinical data supporting synergy, the combination of amphotericin B and itraconazole has become less attractive to further investigation.

There are only a small number of clinical reports of combination therapy with extended-spectrum triazoles and echinocandins for invasive aspergillosis.[126-128,134] The combination of amphotericin B preparations with echinocandins has been studied in several small series.[126,127,134] Aliff and colleagues[126] treated 30 patients with amphotericin B-refractory fungal infections with the combination of amphotericin B (1 mg/kg/day or liposomal amphotericin B 3–5 mg/kg/day) plus caspofungin (70mg on day 1, followed by 50 mg/day), including 26 (87%) with acute leukaemia.[126] The majority of patients had 'possible' fungal infections based on recent criteria[135] and the causative agent in most cases was *Aspergillus* spp. There was a favourable response among 18 (60%) of 30 patients who received combination therapy and 66% survived to time of discharge. The authors concluded that combination antifungal therapy could be safely administered to the high-risk patients with haematological malignancies; however, the lack of a comparative monotherapy group makes interpretation of results difficult. O'Connor and colleagues[134] retrospectively evaluated a cohort of 36 immunocompromised patients who received combination therapy with an amphotericin B formulation plus caspofungin or micafungin, comparing them with a group who received monotherapy with an amphotericin B formu-

lation for proven or probable invasive aspergillosis. Patients who received combination therapy experienced increased survival (32.8% vs 15.4%) but this difference was not statistically significant ($p = 0.43$). No severe toxicity was seen in either group. Kontoyiannis and colleagues[127] recently described 48 patients with haematological malignancies who received combination therapy with liposomal amphotericin B (5 mg/kg/day) plus caspofungin (70mg loading dose on day 1 followed by a 50mg daily dose) for invasive aspergillosis.[127] Most received combination therapy for disease refractory to amphotericin B but 17 (35%) received the combination as primary therapy. Patients were evaluable for outcome if they received at least 7 days of combination therapy. The overall response rate was 42% and no significant toxic effects were seen. Response rates were higher among patients who received primary combination therapy (53%) compared with salvage therapy (35%), but were not statistically significant.[127]

Marr and colleagues[128] recently reported their experience with voriconazole plus caspofungin combination therapy for aspergillosis among patients who received haematopoietic stem cell transplants. They evaluated patients with proven or probable disease who had failed initial therapy with amphotericin B formulation and had received either voriconazole (31 patients) or voriconazole plus caspofungin (16 patients) as salvage therapy.[128] Dosages administered were voriconazole 6 mg/kg followed by 4 mg/kg twice daily, and caspofungin 70mg on day 1 followed by 50 mg/day. The study was not randomised and the main outcome measure studied was survival rate at 3 months after diagnosis. The survival rate at 3 months after diagnosis was higher in patients who received combination therapy ($p = 0.048$); moreover, the probability of death due to aspergillosis (after excluding patients who died of relapsing leukaemia) was lower in patients who received combination therapy ($p = 0.024$). The safety of the two salvage regimens, assessed by laboratory parameters of hepatic and renal function, did not significantly differ between the two groups.[128]

These recent studies using extended-spectrum triazoles, amphotericin B and echinocandins in various combinations for aspergillosis are important in that antagonistic clinical responses were not observed. Because most of these studies are retrospective, underlying biases exist and responses with combination therapy must be interpreted with caution. Importantly, these studies suggest that combination therapies are relatively safe, but the impact of drug interactions and healthcare costs associated with combination antifungal therapy have not been fully elucidated. Considering the *in vitro*, *in vivo* and available clinical data for amphotericin B plus echinocandins and triazoles plus echinocandins, there is potential to examine these important clinical research questions.

7. Conclusions and Future Directions

In the last several years there are few areas in anti-infective therapeutics that have engendered as much interest as combination antifungal therapy. The availability of newer agents with unique antifungal spectra and mechanisms of action has created enormous research opportunities coupled with significant challenges. Most current interest is focused on invasive aspergillosis and other emerging fungi such as zygomycosis, scedosporiosis and fusariosis. These infections have in common that they have substantial associated morbidity and mortality, occur largely among immunocompromised hosts and are relatively resistant to existing antifungal therapy. The successful study of these emerging disorders will depend on sound fundamental *in vitro* and *in vivo* data, but also on innovative approaches to the clinical evaluation of therapy. Large, well powered clinical trials are very unlikely among these less common fungal infections. The study of combination therapy for invasive aspergillosis is possible, but will require a collaborative international effort and substantial support from both pharmaceutical companies and governmental agencies.

Presently, there are plans to conduct a major international trial of combination therapy with an extended-spectrum triazole and an echinocandin for treatment of invasive aspergillosis. This study

would be conducted by the National Institutes of Health-sponsored Bacteriology and Mycology Study Group (BAMSG), but details of this study are unavailable at this time. Another combination study that is being conducted by the BAMSG involves the combination of amphotericin B and higher-dose fluconazole for the initial therapy of cryptococcal meningitis among patients with AIDS. This study is important in that flucytosine is unavailable in most of the world, it is difficult to administer and monitor, and fluconazole is almost universally available throughout most of the developing world. The study is being conducted in the US and Thailand. Because of the availability of the echinocandins, there is much less interest in developing combination therapy for invasive candidiasis.

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