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MINIREVIEW

Combination Antifungal Therapy

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RATIONALE

The availability of new antifungal agents with novel mechanisms of action has stimulated renewed interest in combination antifungal therapies. In particular, and despite the limited clinical data, the high mortality of mold infections and the relatively limited efficacy of current agents have produced significant interest in polyene-, extended-spectrum azole-, and echinocandin-based combinations for these difficult-to-treat infections. With the recent publication of the first large randomized trial of antifungal combination therapy to be conducted in two decades (166) and the rapid proliferation of new in vitro and in vivo data on antifungal combinations, we have sought to review the recent work and future challenges in this area.

The focus of this review is on the efficacy of antifungal drugs in combination with respect to the extent or rate of killing of the fungal pathogen, although other potential interactions (such as pharmacokinetic drug interactions) can impact efficacy when these agents are used together. The value of giving two drugs because each is separately effective against a group of organisms exhibiting a variety of types of resistance is not specifically discussed, but this also is an obvious and straightforward reason to use a combination of agents.

It cannot be simply assumed that the use of two or more effective drugs with different mechanisms of action will produce an improved outcome compared to the results seen with a single agent. Combination antifungal therapy could reduce antifungal killing and clinical efficacy, increase potential for drug interactions and drug toxicities, and carry a much higher cost for antifungal drug expenditures without proven clinical benefit (106). Thus, it is important to critically evaluate the role of combination therapy as new data become available.

Conceptual models and terminology. Methods for studying antifungal combinations in vitro and in vivo have differed considerably over time and among investigators. These tools do not differ with respect to their application to combination antibacterial or antiviral therapies and have been discussed extensively and elegantly in the landmark 1995 review by Greco (79). In brief, all approaches to evaluating combinations can be reduced to two elements: (i) a conceptual model for predicting the expected result for a combination and (ii) a set of phrases used to categorize results that are better than expected, worse than expected, or as expected. Although many subtle variations are possible, the underlying mathematical model is based on either the assumption of additive interactions or the assumption of probabilistic (multiplicative) interactions. On the basis of the terminology employed by the author who first carefully described each of these models, the two models can be usefully referred to as the Loewe additivity model and the Bliss independence model (79).

The terminology used to place results into interpretive categories is often the subject of debate and confusion. Greco et al. (79) have proposed a set of consensus phrases that are instructive (Table 1). In this proposal, synergism and antagonism have clear and intuitive meanings. The phrases used to describe results that are neither synergistic nor antagonistic are, however, somewhat tricky. Mathematically, the term "additive" is indeed logical for the Loewe additivity model just as the term "independent" is logical for the probabilistic Bliss model. Unfortunately, the term "additive" often conveys an imprecise message and may be misinterpreted as referring to a positive interaction. Coined terms such as "subadditive" only reinforce this erroneous conception.

This situation has no perfect resolution. Possible alternatives to the term "additive" include the terms "summation" (44), "no interaction" (141), and "indifferent." The term "summation" unfortunately still carries a hidden positive message. Although somewhat imprecise, the terms "indifferent" and "no interaction" have an inherently conservative emotive nature and can be used to describe Loewe additivity and Bliss independence and also to describe results in cases in which the underlying model is not clearly specified. The use of these terms provides the reader with a constant reminder of the neutral nature of the result—although there can be value in an indifferent (additive) interaction, the biological relevance of such an interaction is not always obvious. For reasons related mostly to ease of expression (it is simpler to speak of indifference and indifferent interactions than to speak of noninteraction and noninteractive interactions), this review uses the phrases synergistic, indifferent, and antagonistic when interpretive categories are required.

With respect to the underlying mathematical model, Loewe

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TABLE 1. Consensus terminology for describing results of combination testing[a]

Category	Terminology for indicated conditions			
	Both agents are active alone; additive effects model is presumed	Both agents are active alone; multiplicative effects model is presumed	One agent is active alone; the other is not	Neither agent is active alone
Combination result is better than expected	Loewe synergism	Bliss synergism	Synergism	Coalism
Combination result is as expected	Loewe additivity	Bliss independence	Inertism	Inertism
Combination result is worse than expected	Loewe antagonism	Bliss antagonism	Antagonism	

[a] The terminology shown is that proposed by Greco et al. (79) following a consensus conference occurring in Sarriselka, Finland. Models based on the additive interaction concept first proposed by Loewe and Muischnek (111) follow the intuitive result in which a drug combined with itself produces a linear sum of effects. That is, 1 μg/ml plus 1 μg/ml gives the effect of 2 μg/ml and this result is neither synergistic nor antagonistic. Models based on the multiplicative interaction concept first proposed by Bliss (26) follow a probabilistic model in which the two agents truly act independently as determined on the basis of their separate probabilities of effect. If 1 μg/ml permits 40% of the target organism to survive, then 1 + 1 = 2 μg/ml should permit only 40% × 40% = 16% survival. As reviewed in detail by Greco et al. (79), both models have strengths and weaknesses but Loewe additivity-based models more often seem appropriate for combinations of antimicrobial agents.

additivity most often seems appropriate for combinations of antimicrobial agents. This result follows from the detailed comparison of the strengths and weaknesses of the additive and multiplicative models found in the review by Greco et al. (79). Although many other arguments can be put forward and the interested reader is strongly encouraged to study closely the review by Greco et al., the key argument for us is that Loewe additivity supports the thought experiment in which an agent combined with itself is neither synergistic nor antagonistic.

Quantitative analyses: the fractional inhibitory concentration index. Calculation of the fractional inhibitory concentration (FIC) index (FICI) by the use of the checkerboard method has long been the most commonly used way to characterize the activity of antimicrobial combinations in the laboratory (66). The FICI represents the sum of the FICs of each drug tested, where the FIC is determined for each drug by dividing the MIC of each drug when used in combination by the MIC of each drug when used alone. Stated in terms of the Loewe additivity model, the FICI model assumes that indifference is seen when this equation is true: $1 = (MIC_{\text{drug A in combination}}/MIC_{\text{drug A alone}}) + (MIC_{\text{drug B in combination}}/MIC_{\text{drug B alone}})$. To make this concrete, imagine an organism for which the fluconazole MIC is 2 μg/ml. If we perform a checkerboard study of fluconazole versus fluconazole, we should find that the well which receives the combination of 1 μg/ml + 1 μg/ml produces an effect identical to that of the wells containing 2 μg/ml [or FICI = (1/2) + (1/2) = 0.5 + 0.5 = 1, which indicates indifference or Loewe additivity].

Reproducible variations from an FICI of 1 represent nonindifference of at least some magnitude. However, the experimentalist must consider both the inherent inaccuracy of MIC methodologies and the question of biological relevance. Thus, it has been proposed that synergy be declared when the FICI is less than or equal to 0.5 and that antagonism be declared when the FICI is greater than 4 (66, 141; Instructions to authors, Antimicrob. Agents Chemother. **48:**i–xxi, American Society for Microbiology, 2004).

The logic behind these interpretive categories is worth discussing. They are based on the related assumptions that (i) testing employs concentrations separated by a factor of 2 (e.g., a sequence similar to 0.25, 0.5, 1, 2, and 4 μg/ml) and (ii) 1-dilution-step MIC changes are within experimental error ranges. For a result to be synergistic, these rules require that both drugs show a minimum drop in MIC of at least two dilution steps and thus, a fourfold drug concentration drop. As an example, consider drugs A and B, each of which has a MIC of 2 μg/ml for a given isolate. Synergy would only be declared when both drugs in combination showed a MIC of 0.5 μg/ml or less. Mathematically, this would be FICI = (0.5/2) + (0.5/2) = 0.25 + 0.25 = 0.5. Importantly, a FICI of 0.50000000001 does not meet the definition of synergy. For instance, if the MIC of one drug in our example were to drop to only 1 when used in combination, synergy could not occur under these rules no matter how low the other drug's MIC in combination were to become: FICI = (1/2) + (??/2) = 0.5 + some value greater than 0. This would result in a FICI value slightly greater than 0.5 and would thus be defined as indifference.

Conversely, antagonism is declared when at least one drug has at least a fourfold increase in MIC. To understand this rule, consider the boundary condition under which both drugs show a precisely twofold increase in MIC. Continuing with the same example, this would be the situation when the MICs of both agents increase to 4 and thus, FICI = (4/2) + (4/2) = 2 + 2 = 4. This result remains within experimental error limits; a FICI of precisely 4.00000 is defined as indifferent (or additive), whereas any value greater than 4 is defined as antagonistic. The rule that a FICI of >4 defines antagonism also handles the situation wherein a small amount of one drug dramatically increases the MIC for the other drug. For example, a small and ineffective concentration of beta-lactam A might induce the expression of a beta-lactamase active against beta-lactam B and, thus, cause beta-lactam B's MIC to increase fourfold or more. In this case, the FICI rule for antagonism in our example is again satisfied: FICI = (??/2) + (8/2) = some value greater than 0 + 4 = some value greater than 4.

More complex calculations and mixed interactions: beyond the FICI. While the FICI and its variants have long been employed to depict the characteristics of antimicrobial drug combinations, these approaches have had a number of limitations that have been well described by others (62, 106). The biggest challenge is that FICI-based approaches presume a smooth and linear interaction across all combinations of concentrations. In practice, this situation is often not seen; Eliopoulos and Moellering (66) provide some excellent examples of the curious results seen in practice. In an effort to develop approaches that resolve these difficulties, more sophisticated methods have been proposed (as discussed in great detail by

Greco et al.) (79). Many of these other methods have only occasionally been employed in studies reported in the antifungal literature. The method of Chou and Talalay (44) was reviewed in particular detail by Greco et al. (79) and is especially noteworthy for its frequent use in publications on antiviral and antineoplastic drug combinations. This method has more recently been applied to work with beta-lactamase inhibitors and appears to have broad applications (196). A few antifungal studies have employed other methods. For instance, a contour and surface plot methodology has been proposed and was found particularly useful in characterizing the nature of three antifungal agents in combinations using various concentrations of each agent (74). Similarly, newer and more sophisticated methods have been employed in recent years to depict the nature of these complex interactions in animal models of infection. These methods involve the use of response-surface plots which illustrate the Loess fit of the association for a particular outcome (such as weight change, fungal tissue burden, or survival) (60, 61, 101). These methods have been facilitated by improvements in the capabilities of technologies developed for the performance of more complex mathematical and statistical computation. In contrast to older isobologram approaches, the Loess method has the distinct advantage of allowing visualization of the relationship between agents that have different dose-response curves. The Loess method is flexible and fairly simple to perform using appropriate statistical software, but it requires a fairly high density of data (i.e., intense sampling) and is computationally complex. Unlike the results seen with other models (such as nonlinear regression), furthermore, the use of the Loess method does not result in a mathematical formula representing a regression function that can be easily shared with others. The Loess method has not been widely employed to date, but future investigations may use similar methods or even develop newer methods to depict these complex and often unpredictable dose-response interactions between multiple antifungal agents.

Ultimately, all of these methods attempt to reduce the interaction to one or more summary terms that capture the degree of deviation of the observed results from the expected results. The method of Chou and Talalay (44) has the advantage that its plot showing the fraction affected versus the combination index provides a simple and visual way to look at a series of summary terms across a range of possible drug combination concentrations. This approach is especially helpful when the study shows synergy under some conditions and antagonism under others (38, 39). When such a situation occurs, it is both logical and plausible to focus on the result achieved with the maximal tolerable systemic drug exposure (37).

No matter how it is defined, the analysis must ultimately apply some test of biological relevance to the result and propose a set of interpretive categories. While it is not possible to propose a fixed figure for use in defining interpretive categories, the historical model suggested by the ideas developed on the basis of the FICI (see above) seems logical: synergy begins when the activity of each drug appears to increase at least fourfold, antagonism begins when the activity of at least one drug decreases at least fourfold, and indifference lies in between. Although one can debate the point endlessly, these ideas have the virtue of suggesting changes in drug doses or effects that are potentially biologically relevant.

Approaches to analysis of in vivo and clinical studies. Applying these ideas to in vivo and clinical investigations of combination antifungal therapy is especially difficult, and no standards for interpretation of these data have been recommended to date. Analysis and comparison of results across in vivo and clinical studies requires careful consideration of the nature of pathogen, host, host immune status, study design, and study endpoints. Issues related to clinical trial design with antifungal agents have been extensively reviewed elsewhere (25, 167). Indeed, each type of study (in vitro, in vivo, and clinical) has its own set of strengths and weaknesses (Table 2).

A few issues particularly stand out with reference to the study of antifungal combinations in the clinical setting. First, the methods used to assess antifungal drug efficacy for humans are limiting. Whereas other disease states have surrogate markers, such as estimates of viral antigenemia (human immunodeficiency virus [HIV] infection) or sputum CFU (assessment of early bactericidal activity of mycobacterial drugs), that can yield rapid, early, and clinically relevant measures of drug efficacy, most antifungal clinical trials are limited to subjective efficacy assessments based on clinical outcome. Standardized criteria have been developed for the purposes of enrollment (8), and the use of blinded efficacy assessment expert panels can reduce bias (25, 85, 167); however, these tools still do not provide rapid or quantitative means for the evaluation of antifungal drug efficacy. Colony counts are routinely employed with animal models as a means of assessing efficacy, but with the exception of serial studies of cerebrospinal CFU levels in subjects with cryptococcal meningitis (R. A. Brouwer, A. Rajanuwong, W. Chierakul, G. E. Griffin, R. A. Larsen, N. J. White, and T. S. Harrison, Abstr. 15th Congr. Int. Soc. Hum. Anim. Mycol., abstr. 029, 2003), studies of humans are limited to less-invasive methods. Newer technologies can provide nontraditional methods for the estimation of fungal burden levels in human tissues or serum (29, 32, 45, 64, 65, 70, 72, 76, 83, 84, 86, 108, 109, 110, 112, 131, 132, 134, 177, 211, 220, 221), but these assays have generally been developed with the objective of diagnosing disease rather than determining drug efficacy. To further complicate matters, even traditional methods may fail, as illustrated by studies of caspofungin and its effects on colony counts of *Aspergillus* spp. (4, 82, 152). Development and validation of more-rapid and -reliable measures of antifungal drug efficacy that could be used in the clinical setting would facilitate clinical trials that use these agents.

Beyond this initial hurdle, host factors (such as changes in ongoing immunosuppression, altered physiologic state, and pharmacokinetic disposition of drugs) greatly impact the performance of an antifungal agent in the clinical setting and cannot always be simulated in studies employing in vitro or animal models. Although most of these issues are obvious, the possibility of a pharmacokinetic interaction between the study drugs (e.g., studies of the combination of rifampin with an azole would be limited by the fact that rifampin reduces azole blood levels) is often overlooked. The interplay between all of these factors and the pathogen in the human cannot necessarily be predicted on the basis of laboratory studies, and issues related to toxicities or lack of efficacy may only be apparent when studied in humans.

Several studies have suggested that different concentrations of each drug in combination can be associated with results that

TABLE 2. Technical and analytical issues associated with different model systems for studying combination antifungal therapy

Category	Characteristic		
	In vitro studies	Animal models	Clinical trials
Strengths	Easily repeated across a wide variety of drug concentrations	Studies can be done with homogeneous hosts	This is the answer that matters
	Easily subjected to statistical testing	Host factors are integrated	
	Easy to vary technical factors	Resistant isolates can sometimes be tested	
	Easy to test multiple isolates	Quantitative endpoints (tissue burden, rate of clearance) can be tested	
	Easy to test isolates with defined types of resistance	A range of doses and dose combinations can be tested	
Weaknesses	Relevance of in vitro methods not always clear	Infection models are often poor mimics of human disease	Subjects and infecting isolates are heterogeneous
	Host factors are ignored	Pharmacokinetic and toxicological behavior of test drugs (and their pharmacological effects on each other) may not mimic that seen in humans	No ability to control nature of infecting isolate
	Pharmacokinetic factors are ignored	Only limited numbers of isolates can be tested	Underlying disease cannot be controlled
		Only limited numbers of repetitions are possible	Lack of quantitative endpoints for many diseases
		Resistant isolates sometimes have reduced virulence	Limited ability to study a range of doses
			Very expensive
			Slow

range from synergy to antagonism (19, 157, 161; L. Ostrosky-Zeichner, M. Matar, V. L. Paetznick, J. R. Rodriguez, E. Chen, and J. H. Rex, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-1816, p. 415, 2002). Studying multiple-dose combinations in the clinical setting is especially difficult, considering the expense of clinical trials and the limited number of research candidates. However, dose selection of the agents under study can critically impact the trial results. Randomized clinical trials performed to date (24, 166, 213) have employed standard antifungal doses in combinations that are consistent with maximally tolerated doses used in monotherapy. This approach seems logical given limited resources. Although the possibility exists that greater or lesser benefit may occur with other dose combinations, such information is of limited practical use, as finely calibrated systemic exposure adjustments are usually not possible in the clinical setting. It is for this reason that we generally believe that the most important result is the result seen at maximal safe systemic exposure levels. Stated conversely, we do not see much relevance in the observation that (for example) two drugs are synergistic at 15% of their usual dosage but their effect in combination at maximal doses is no better than monotherapy with the more potent of the two compounds.

Perhaps the greatest limiting factor for conducting additional clinical studies of antifungal drug combinations is the combination of time and cost (167). Meaningfully powerful multicenter studies require several years to complete and require enormous financial commitments. Given that combinations are being used more frequently in the clinical setting at significant expense without evidence-based clinical support and that the potential for reduced efficacy or increased toxicity exists, it is critical to build upon the available in vitro an animal data and demonstrate efficacy and safety in the setting of clinical trials.

The approach taken in this review. Recognizing the challenges inherent in the study of combination antifungal therapy in the clinical setting, it is clear that in vitro and animal model investigations comprise the bulk of the literature on this topic. Therefore, this paper focuses on various methods of study and their applications and critically evaluates research findings from these investigations with the goal of providing a framework for future research as well as for translation into the clinical arena.

For the reasons mentioned previously, we will utilize the terms synergy, indifference, and antagonism to describe the required range of interpretive categories for in vitro investigations and to provide the basis for data aggregation and summarization. This often entails the remapping of the interpretive categories in the original manuscript, with the most common change being the conversion of a large number of phrases used to describe indifference into that single term. In analyzing in vitro data, we have followed as closely as possible the rules discussed above respecting the FICI. Analyses of in vivo and clinical data usually require a qualitative interpretation of a variety of results; we therefore focused on objective study endpoints such as time to sterilization of tissues or body fluid, survival, or reduction in log CFU in infected tissues or fluid, and we comment accordingly on relevant issues of study design and administration. At times, the terms positive and negative are used to describe interactions that are less rigorously characterized but that trend in one direction or another. When a combination displays a mixed interaction (synergy in some settings, antagonism in others), we note the contradiction but focus on the interaction most likely to be seen at maximal tolerated systemic exposures, since this is most clinically relevant interaction (as discussed previously in this paper).

MECHANISMS OF INTERACTIONS FOR THE ANTIFUNGAL AGENTS

Mechanisms of synergy. There are several mechanisms proposed for antifungal synergy. (i) Inhibition of different stages of the same biochemical pathway represents one type of interaction. An example is the combination of terbinafine and azoles (10, 11, 35, 148), in which both compounds inhibit ergosterol biosynthesis and, thus, impair the function of fungal cell membranes. (ii) Increased penetration of an antifungal agent as a result of cell wall or cell membrane antifungal activity from another agent is possible. This interaction has been proposed for combinations of amphotericin B or fluconazole with antibacterials such as rifampin (23, 125, 127) and quinolones (204, 205) and allows these agents to easily penetrate the fungal cell membrane to reach their target of fungal DNA synthesis. Such a mechanism may also explain potential synergism between azoles (3, 12, 14, 101, 124, 138, 223) or amphotericin B (126, 156) and flucytosine, in which case the azole or polyene damages the fungal cell membrane, enabling increased uptake of flucytosine. (iii) A transport interaction is proposed for amphotericin B-flucytosine (17, 18, 20), whereby amphotericin B acts on the fungal cell membrane and inhibits flucytosine transport across the cell membrane and out of the yeast cell. In this scheme, flucytosine exerts its lethal effects on any surviving fungus when amphotericin B degrades the cell membrane via an oxidative decay, allowing flucytosine to remain at the site of its action within the cell. (iv) Simultaneous inhibition of different fungal cell targets, such as cell wall and membrane targets, is also possible. This mechanism has been suggested for both the apparently synergistic interactions between echinocandins (cell wall active) and amphotericin B (5, 15, 71; S. Kohno, S. Maesaki, J. Iwakawa, Y. Miyazaki, K. Nakamura, H. Kakeya, K. Yanagihara, H. Ohno, Y. Higashiyama, and T. Tashiro, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1686, p. 388, 2000; and Ostrosky-Zeichner et al., Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-1816, 2002) or azoles (both cell membrane active) (147, 195; Kohno et al., Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1686, 2000; E. M. O'Shaughnessy, J. Peter, and T. J. Walsh, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-856, p. 385, 2002; and R. Petraitiene, V. Petraitis, A. Sarafandi, A. Kelaher, C. A. Lyman, T. Sein, A. H. Groll, D. Mickiene, J. Bacher, and T. J. Walsh, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-857, p. 385, 2002). (v) Potent initial activity of a rapidly fungicidal agent, such as amphotericin B, to reduce fungal burden, which then allows another agent to subsequently work well as consolidation or clearance therapy on a reduced number of fungal cells (107), is another possible mechanism. Some of these effects have been observed with certain fungal pathogens but not with others (156), so these interactions may depend on and differ according to certain target cell factors and even between different fungal species.

Mechanisms of antagonism. Antagonism among antifungal agents might occur in one of several ways. (i) Direct antifungal action at the same site results in decreased ability of other agents to exert their competitive effects on that site or at an altered target, as proposed for the azoles and amphotericin B (31, 49, 182, 183, 185, 186, 203). Azoles block the synthesis of ergosterol in the fungal cell membrane and may thus render amphotericin B inactive, since this agent exerts its activity by binding to ergosterol in the cell membrane. (ii) Adsorption to the cell surface by one agent inhibits binding of another antifungal agent to its target site of activity. This mechanism is proposed for lipophilic azoles (such as itraconazole and ketoconazole) which may adsorb to the fungal cell surface and inhibit binding of amphotericin B to fungal cell membrane sterols (183, 185). (iii) Modification of a target upon exposure to an antifungal agent occurs that renders the pathogen less susceptible to the effects of other antifungals. This mechanism has been proposed for sequential antagonism observed with azoles and amphotericin B, whereby preexposure to an azole compound causes replacement of membrane ergosterol with a methylated sterol derivative to which amphotericin B binds less well (51, 67, 75). (iv) Other unknown antagonistic mechanisms may exist such as that observed for polyenes and flucytosine. Some have suggested that antagonistic interactions between these agents might be related to changes in fungal cell membrane function due to the effects of amphotericin B (189); however, additional data are needed to explain this phenomenon, because these two drugs generally display an interaction which trends towards synergy.

FOCUSED REVIEW OF THE ANTIFUNGAL DRUG INTERACTION LITERATURE

The latter portions of this review focus on the available in vitro animal model and clinical data for the potential utility of antifungal combination therapy. Since published data are limited for uncommon pathogens such as *Histoplasma capsulatum*, *Coccidioides immitis*, and *Blastomyces dermatitidis* and less-common molds, our review focuses on studies of the three common fungal pathogens (*Cryptococcus neoformans*, *Candida* spp., and *Aspergillus* spp.). Analyses respecting these pathogens are more robust and less susceptible to the limitations imposed by the availability of only single cases and/or a few strains, as occurs with the less common fungi. However, the limitations of this review should not suggest that combination therapy does not have a place in treatment of less-common fungal infections but just that evidence-based data are too limited at present to support meaningful analysis.

***C. neoformans*. (i) In vitro data.** Numerous in vitro studies have attempted to characterize interactions among antifungal agents developed for the treatment of *C. neoformans* infections (Table 3), and various results among different investigations have been obtained. In general, antagonism has been an uncommon finding with most drug combinations and concentrations employed in laboratory investigations against *C. neoformans* isolates.

(a) Flucytosine-amphotericin B combinations. While many clinicians believe that the combination of amphotericin B and flucytosine is the preferred strategy for induction therapy treatment of cryptococcal meningitis in immunosuppressed patients, in vitro findings with this combination have not consistently demonstrated synergy (40, 74, 78, 80, 92, 126, 140, 189). Positive interactions between amphotericin B and flucytosine against *C. neoformans* strains have been reported (40, 126). Investigations that have not confirmed synergy (78, 80, 161)

TABLE 3. Summary of key findings reported in studies of *C. neoformans* with combinations of clinically relevant antifungal agents[a]

Combination	Settings studied	General findings	Comments
5FC + AmB	In vitro (40, 63, 74, 78, 80, 92, 126, 140, 156, 189)	Indifference (63, 74, 78, 80, 140, 156, 189) synergy (40, 126), antagonism with 5FC-resistant strain (80)	AmB reduces development of resistance to 5FC; 5FC-resistant strains have been used in numerous studies (80, 126, 189) and produce various results
	Mice (16, 27, 60, 80, 158) Rabbits (150)	Improved survival (16, 27, 158) Reduced tissue burden (16, 150)	Survival (27) or reduction in tissue burden (150) not necessarily better than results with AmB alone (60); combination more effective than AMB and 5FC alone against 5FC-resistant strains (158)
	Humans (24, 42, 43, 55, 103, 144, 213)	Similar (213) or improved (24, 171) clinical success overall, improved sterilization of CSF (24, 213)	Addition of 5FC leads to earlier sterilization of CSF (24); clinical success rates were similar between AmB + 5FC and AmB alone (73 vs. 83%) after 2 weeks of therapy (213); relapse has been associated with no use of 5FC during initial 2 weeks (171)
5FC + triazoles	In vitro FLC (3, 138, 140), KTC (31, 140), ITC (12), PSC (14)	FLC: synergy (138) KTC: indifference (31, 89) ITC, PSC: indifference or synergy (12, 14)	Synergy in 62% of 50 strains studied for FLU-5FC (138); various doses may be necessary to achieve greatest effect; addition of 5FC helped prevent emergence of 5FC-resistant mutants
	Animal studies FLC: mice (3, 60, 61, 101, 139, 157)[b] KTC: mice (50, 78, 158) and rabbits (150) ITC: mice (157), hamsters (89), and guinea pigs (212) PSC: mice (14)	Improved (3, 50, 61, 157), similar (14, 50, 60, 212), or worse (89) survival Reduced tissue burden (50, 78, 101, 212)	Combination associated with better survival than monotherapy and was consistent over a range of doses (3); effects more pronounced at lower doses (101), and single agents were very effective at higher doses alone; 5FC + KTC rarely cleared tissues better than either agent alone (150); hamsters with combination did worse than with ITC alone (89); ITC + 5FC performed similarly to ITC + AmB and better than ITC or 5FC monotherapy in guinea pigs (212); with 10 days of treatment of mice, combination prolonged survival more than either agent alone but not when treatment was limited to 5 days (157); PSC combination not better than monotherapy in terms of survival but better than monotherapy in reducing fungal counts in brain tissue (14)
	Humans FLC (48, 102, 124, 193, 223)	Good clinical success (48, 102, 193, 223) Increased survival (124)	63% success rate in cryptococcal meningitis (95% confidence interval, 48–82%) (102); improved survival (32%) versus FLC alone (12%) at 6 months in AIDS-associated cryptococcal meningitis (124)
AmB + triazoles	In vitro FLC (13, 74), KTC (78, 140, 150, 161), ITC (13), PSC (13)	Indifference (13, 78, 140)	FLC: indifference in 10/15 strains tested, indifference in 4/15, and synergy in 1/15 with NCCLS methods (13); indifference among 3 strains using an inoculum of 104 CFU/ml on yeast nitrogen base broth and response surface plots (74); KTC: no antagonism observed (78, 140, 150); synergy reported with one strain in two studies using nonstandard methodologies (140, 161); ITC: 14/15 strains indifferent; 1/15 synergistic (13); PSC: 8/15 strains indifferent; 5/15 synergistic; 2/15 indifferent in one study (13)
	Animal models FLC: mice (2, 13)[b] KTC: mice (50, 78, 158) and rabbits (150) ITC: mice (157) and guinea pigs (212)	FLC/KTC: improved survival compared to results with azole (2, 13, 78, 158)[b] and/or AmB (2, 158) ITC: did not improve or worsened survival (157) Reduced tissue burden (2, 13, 78, 212)	FLC: addition of AmB to FLC had dramatic impact on yeast burden in brain tissue[b], but survival with AmB was 100%; effects on survival were greatest at highest dosages of azole-AMB (2, 158); improved survival at lower doses of ITC + AmB, but survival was worse when higher doses were used (157); FLU preexposure did not reduce subsequent AmB activity (13)
	Humans—case report (47)		Case report of a woman with meningitis who responded to this combination after failing AmB
Caspofungin or anidulafungin + AmB	In vitro (71)	Synergy	Used higher levels of caspofungin than would be used for humans
Caspofungin or anidulafungin + FLC	In vitro (71, 169)	Indifference (71, 169) or synergy (71)	One study showed that echinocandins were no better than FLC monotherapy (169); no antagonism (71, 169)
ITR + FLC	Guinea pigs (212)	Reduced tissue burden	Survival was 100% in all treatment groups; improved sterilization of tissues compared to FLC but not ITC

[a] AmB, amphotericin B; CLT, clotrimazole; FLC, fluconazole; 5FC, flucytosine; KTC, ketoconazole; ITC, itraconazole; PSC, posaconazole; RVC, ravuconazole; SPC, saperconazole; TRB, terbinafine; VRC, voriconazole.

[b] Larsen et al., Abstr. 5th Int. Conf. *Cryptococcus* Cryptococcosis, abstr. P3.1, 2002.

used lower concentrations of flucytosine and amphotericin B only or used strains with reduced susceptibility to flucytosine, which may have influenced or biased their ability to characterize the full spectrum of antifungal interactions between these agents.

(b) Flucytosine-azole combinations. The results of early investigations of flucytosine in combination with older azoles such as econazole or miconazole (63) suggested that antagonism between these agents occurred. However, more recent reports of investigations of triazole-flucytosine combinations most commonly have indicated synergy or indifference (3, 12, 14, 74, 138, 140, 223). In similarity to the results seen with interactions of polyenes and flucytosine, it has been suggested that the addition of a triazole (such as itraconazole) suppresses emergence of flucytosine-resistant mutants (12). Perhaps not surprisingly, these combinations have been somewhat less impressive against strains with reduced azole susceptibility and variations among the different triazoles have been reported (12).

(c) Polyene-azole combinations. Polyene-azole interactions in the treatment of *C. neoformans* infections have been studied both with (74, 140) and without (13, 63, 74, 78, 150, 161) the use of flucytosine, but in vitro data are relatively scant. In contrast to the antagonism commonly observed with *Candida* spp. for this combination, polyene-azole interactions for *C. neoformans* have generally ranged from indifferent to synergistic. This may be related to the various sterol compositions seen with *C. neoformans* isolates (75). Indifferent effects have been reported most frequently (13, 78, 161), but synergy has also been found for combinations of ketoconazole (78, 161) or posaconazole (13) and amphotericin B. While positive interactions have been most commonly reported with concurrent combinations of polyenes and azoles, sequential exposure to an azole may reduce subsequent amphotericin B activity. In one study (13) preexposure to fluconazole appeared to inhibit subsequent killing activity of amphotericin B, especially against nonreplicating *C. neoformans* cells. Therefore, the timing of antifungal exposure may be critical to interaction results.

(d) Echinocandins with other agents. Echinocandins, which have negligible activity against *C. neoformans* alone (56, 69, 99, 169), have exhibited positive interactions in combination with amphotericin B (71) or azoles (56, 71, 169), with some reports indicating indifference (169) and others indicating possible synergy (71). It is important that the concentrations of caspofungin used have been much higher than could be clinically achieved at current doses with humans. Synergistic interactions have also been reported (56) for caspofungin and tacrolimus, a calcineurin inhibitor. Calcineurin is involved in signaling during the stress response and probably regulates 1,3-β-D-glucan synthase. Although promising conceptually, the effectiveness of tacrolimus as an antifungal in the clinical setting has been limited by its direct immunosuppressive effects.

(ii) Animal models of cryptococcal infection. Antifungal interactions have been studied extensively in animal models of *C. neoformans* infection (including studies of both meningitis and systemic disease) (Table 3). A common theme with combination treatments has been prolonged survival and/or reductions in tissue burden, but the effects were rarely greater than those seen with amphotericin B monotherapy.

(a) Flucytosine-amphotericin B combinations. Combinations of amphotericin B and flucytosine in studies of mice (16, 27, 60, 80, 158) and rabbits (150) with flucytosine-susceptible stains of *C. neoformans* indicate that survival is improved and that substantial reductions in levels of log CFU per milliliter occur with combination therapy (16, 150). Studies conducted with combination treatments whose results have included worse-than-anticipated effects on negative culture results (80) used substantially lower doses than those used by others.

(b) Flucytosine-azole combinations. In combination with triazoles, flucytosine (when combined with fluconazole) appears to have increased effects on survival, fungal tissue burden, and weight changes in infected animals (3, 60, 61, 101, 139). However, relatively high doses of each agent have been very effective as monotherapy (60, 61, 101); thus, it has been difficult to show superior or improved outcome. Deleterious effects of combinations have been uncommon, but investigators conducting one study did observe additive toxicity after 5 days of treatment with the combination (157). Combinations of flucytosine and itraconazole, ketoconazole, or posaconazole have resulted in improvements in survival (50, 157) and tissue clearance (14, 212) in cases of cryptococcal meningitis, despite limited penetration of these azoles into cerebrospinal fluid (CSF). However, ketoconazole-flucytosine combinations rarely resulted in better outcomes with regard to tissue clearance than ketoconazole alone or even amphotericin B monotherapy (50, 78, 150, 157). One group studying hamsters with systemic cryptococcosis (89) reported worse survival and higher fungal burden in brain tissue after 30 or 60 days of therapy for itraconazole-flucytosine in combination compared with the results seen with itraconazole alone. Fungal burden in brain tissue of infected animals was also worse with the combination than with flucytosine alone, but the use of flucytosine alone was also associated with poor survival (89).

(c) Polyene-azole combinations. Experience with amphotericin B and triazole combinations indicates that the azole compound is largely the beneficiary. It is difficult to improve on the rapid and impressive fungicidal activity of amphotericin B in animal models, and tolerability rather than direct antifungal activity is the main issue. Improvements in survival (R. A. Larsen, M. Bauer, A. M. Thomas, and J. R. Graybill, Abstr. 5th Int. Conf. *Cryptococcus* Cryptococcosis, abstr. P3.1, p. 121, 2002) and/or reductions in tissue burden have been reported, but these effects have usually not been superior to the results seen with amphotericin B alone. The results seen in early studies with ketoconazole-amphotericin B combinations indicated that the greatest impact of dual therapy might be on fungal burden in the tissues rather than in the form of dramatic improvements in survival (50, 78, 150, 158). The addition of an azole could result in better overall efficacy or possibly enable effectiveness with lower dose requirements for amphotericin B. In one model of murine cryptococcal meningitis (157), worse survival was observed when higher dosages of amphotericin B-itraconazole were used in combination; when lower doses were employed, survival was dramatically better among those animals receiving dual therapy. Among neutropenic and nonneutropenic guinea pigs (212), increases in rates of tissue sterilization with increases in dosages of concomitant amphotericin B (0.63 to 2.5 mg/kg of body weight/day) and itraconazole were observed relative to the results seen with itraconazole

alone. This combination also reduced colony counts in brain and meningeal tissue more than amphotericin B alone.

(d) Azole-azole combinations. Azole-azole combinations have also been employed on a limited basis. The combination of fluconazole and itraconazole in a guinea pig model of systemic cryptococcosis and meningitis (212) resulted in improvements in tissue sterilization compared to the results seen with fluconazole alone but did not add much to itraconazole monotherapy at similar doses. Survival in this model for all treatment groups was 100%.

(iii) Clinical data. *(a) Flucytosine-amphotericin B combinations*. As mentioned previously, the classic use of combination antifungal therapy in the clinical setting involves the use of flucytosine with amphotericin B for the treatment of cryptococcal meningitis. Several clinical trials have been used to compare this combination to amphotericin B monotherapy and have resulted in faster clearance of yeasts from the CSF and fewer relapses with the addition of flucytosine compared to the results seen with amphotericin B treatment alone (24, 103, 171, 173, 213), but overall mortality or clinical cure rates with the combination were not consistently better in any of these trials. In the first of the studies cited, the addition of flucytosine reduced dosage requirements for amphotericin B and thus reduced polyene toxicity (24). Clinical cure or improvement rates in this prospective, randomized multicenter comparative trial conducted with subjects with cryptococcal meningitis (24) were 68% (23 of 34 subjects) for subjects receiving amphotericin B (0.3 mg/kg/day) plus flucytosine for 6 weeks compared to 47% (15 of 32) for those receiving 10 weeks of amphotericin B treatment alone (0.4 mg/kg/day). There were significantly fewer deaths (24 versus 47% [$P < 0.05$]) and more-rapid conversion of CSF to negative culture results ($P < 0.001$) in the combination therapy arm. In a later trial, subjects were randomized to receive amphotericin B (0.7 mg/kg daily) plus flucytosine (100 mg/kg daily divided in four doses) or placebo for the initial 2 weeks of therapy followed by randomization to fluconazole or itraconazole consolidation therapy for 8 weeks. After the initial 2 weeks of therapy, 60% of subjects receiving the combination of amphotericin B plus flucytosine versus 50% of those randomized to amphotericin B alone achieved sterilization of the CSF ($P = 0.06$). No differences were reported between treatment groups with respect to clinical and microbiologic responses after this 2-week induction period (213), but the addition of flucytosine to the regimen was associated with fewer relapses (171).

(b) Flucytosine-azole combinations. In contrast to experience with amphotericin B, the addition of flucytosine to fluconazole in clinical studies has resulted in less-clear-cut benefits. In an observational study of HIV-infected individuals with cryptococcal meningitis, the addition of flucytosine to fluconazole for the treatment of subjects with a range of levels of illness consistently reduced the failure rate (222). In a small, randomized trial performed in Uganda, the addition of flucytosine to fluconazole for the first 2 weeks of induction therapy for the treatment of HIV-infected subjects with cryptococcal meningitis was associated with increased 6-month survival rates (32 versus 12% [$P = 0.022$]) without a high frequency of serious toxicities (124). In another study (102), the clinical success rate after 10 weeks of daily treatment with fluconazole (400 mg) plus flucytosine (150 mg/kg) was 63% (95% confidence interval, 48 to 82%), with a median time to CSF culture negativity of 23 days (which is longer than that observed, in general, with combinations of amphotericin B and flucytosine) (102, 173). However, these rates were substantially better than those reported from other studies conducted with fluconazole (103, 173) or amphotericin B (24, 173) alone. Dose-limiting adverse effects have been problematic with the combination of flucytosine and fluconazole and necessitated discontinuation of flucytosine treatment in 28% of the study subjects in one trial (102). This combination was also associated with toxicities in a study of a series of non-HIV-infected subjects with cryptococcal meningitis (P. G. Pappas, J. R. Perfect, and R. A. Larsen, Abstr. 36th Ann. Meet. Infect. Dis. Soc. Am., abstr. 101, 1998). Outcomes with itraconazole plus flucytosine in case series (218, 219) are comparable or better than those for itraconazole alone, which has erratic oral absorption characteristics and great interpatient pharmacokinetic variability (91). A total of 22 subjects with disseminated cryptococcosis received itraconazole (200 to 400 mg) daily with or without flucytosine (150 to 200 mg/kg/day) for 6 weeks in an open-label study (219) and experienced similar results with respect to treatment success. A total of 9 of 12 subjects receiving itraconazole alone experienced marked improvement, while 8 of 10 receiving the combination experienced a similar outcome. There was one treatment failure in each group. Significant toxicity was not observed during this induction period, but long-term followup data from the maintenance period were not well described.

(c) Triple combinations. Triple combinations of amphotericin B, flucytosine, and triazoles in treatment of cryptococcal meningitis have also been employed, with apparent success (42, 43; Brouwer et al., Abstr. 15th Congr. Int. Soc. Hum. Anim. Mycol., 2003). Recently, treatment of HIV-infected patients with the combination of amphotericin B and flucytosine for cryptococcal meningitis resulted in reduction of yeast counts in CSF that was faster than that seen with a triple combination with amphotericin B, single-agent therapy with amphotericin B, or the combination of flucytosine and fluconazole (Brouwer et al., Abstr. 15th Congr. Int. Soc. Hum. Anim. Mycol., 2003).

Published results from a large clinical trial (43) indicated that overall treatment success and time to resolution of fever were better among subjects receiving triple therapy with amphotericin B, flucytosine, and itraconazole (50 of 50 successfully treated; fever resolved in 5.9 ± 3.7 days) than the results seen with amphotericin B-flucytosine alone (45 of 50 successfully treated [$P = 0.03$]; fever resolved in 8.8 ± 5.1 days [$P = 0.02$]). In this study, 100 subjects with AIDS-associated cryptococcal meningitis were randomized to receive amphotericin B (0.3 mg/kg of body weight/day) plus flucytosine (150 mg/kg divided into doses administered four times daily) for a total of 6 weeks or amphotericin B and flucytosine in the same doses plus itraconazole (400 mg in 200-mg capsules administered twice daily) until negative culture results (mean time, 2.4 ± 0.6 weeks) were obtained; this regimen was followed by treatment with itraconazole alone in the same doses for a total of 6 weeks. Secondary prophylaxis for both treatment groups consisted of the administration of itraconazole capsules (200 mg daily). Fewer subjects receiving triple therapy experienced significant laboratory adverse events (21 of 50 versus 32 of 50 [$P = 0.045$]), which consisted mostly of decreased hematocrit and

metabolic acidosis in the subjects receiving amphotericin B and flucytosine. In addition, after 2 weeks of therapy, CSF sterilization rates were better for the triple therapy group than the results seen with controls. However, there were more relapses among subjects receiving the triple combination after they had been switched to itraconazole alone. The results of other studies (162, 171) have suggested that relapse rates among subjects with HIV-associated cryptococcal meningitis are higher with itraconazole as a secondary prophylaxis than with fluconazole. It should also be noted that the dose of amphotericin B used in this study was less than that recommended in Infectious Diseases Society of America guidelines (172).

(d) Polyene-azole sequential therapy. Sequential therapy with polyene with or without flucytosine followed by an azole (fluconazole or itraconazole) has been well studied in the clinical setting (171, 213), and it appears that pretreatment with amphotericin B with or without flucytosine during the induction phase might aid the positive impact of subsequent azole activity during the consolidation, clearance, or maintenance phase. This strategy is currently used clinically (171, 172). However, relapse rates have been higher with the use of itraconazole as maintenance therapy than the results seen with fluconazole (171, 213). This probably relates to the better CSF penetration of fluconazole and its more reliable pharmacokinetic profile.

(iv) Interpretation and recommendations. Judging on the basis of these data, flucytosine-amphotericin B combinations have not been particularly impressive with *C. neoformans* isolates in in vitro investigations; however, the results seen with animal models of cryptococcosis suggest significant positive effects of this combination with respect to survival and tissue clearance of organisms. As determined on the basis of clinical research experience, it appears that treatment with amphotericin B and flucytosine is the best combination available for cryptococcal meningitis at the present time; perhaps the differential performance of this combination in vivo relates to host factors that cannot be readily simulated in the test tube. Amphotericin B and flucytosine treatment represents the only combination antifungal therapy regimen recommended as an initial therapy for the treatment of cryptococcal meningitis in the guidelines published by the Infectious Diseases Society of America (172), and we agree with these recommendations.

Flucytosine may be a useful addition to azoles and in this setting could result in improved activity and reduced emergence of flucytosine-resistant yeasts. Factors other than in vitro potentiation, including differential pharmacokinetics, reduction of selective pressure, and the capability of being used in lower dosages and therefore of reducing associated drug toxicity, may be of more importance for these combinations in vivo. Judging on the basis of the results of investigations using animal models, fluconazole-flucytosine combinations appear beneficial. Data with other triazoles in combination with flucytosine have been less consistent, and these combinations are less likely to be investigated in the clinical setting. Infectious Diseases Society of America guidelines (172) have proposed fluconazole plus flucytosine as an alternative induction therapy for cryptococcal meningitis. Since there have been only poorly developed comparative outcome studies associated with this combination in cases of cryptococcosis and toxicity levels have been problematic, it will continue to be used as a secondary regimen until further supported by solid comparative clinical data demonstrating efficacy as well as safety.

Unlike the results seen with respect to the antagonism observed between polyenes and azoles in other fungal organisms, the combination of amphotericin B and triazoles (when used concurrently) appears to have positive effects in vitro and in animal models of cryptococcosis; however, the effects are not necessarily more positive than the effects of high dosages of amphotericin B alone. The addition of amphotericin B to a triazole could possibly enable reduced dosages of either or both agents and thus potentially reduce drug-associated toxicities, which are frequently observed at the high dosages of a polyene required for superior efficacy with humans. As sequential therapy, preexposure with a triazole may reduce subsequent activity of amphotericin B in vitro; however, polyene preexposure does not appear to reduce subsequent azole activity, and a sequential approach is supported by large-scale clinical trial results (213).

Combinations of amphotericin B and echinocandins are an interesting area of therapeutic study, but until further data or new echinocandins are available these combinations should be employed only in the experimental setting.

Three-drug combinations are a novel approach that appears to result in excellent clinical cure rates and reduced toxicities for subjects with cryptococcosis. The results of a few in vitro (74, 140) and animal model (60) studies suggest that this approach might be promising, and the results of small clinical trials (42, 43; Brouwer et al., Abstr. 15th Congr. Int. Soc. Hum. Anim. Mycol., 2003) have affirmed this potential. However, relapse rates have been unacceptably high (43), suggesting that the duration of induction therapy might need to be prolonged when lower doses of these agents are used in combination or that maintenance therapy after induction with three agents needs to be carefully selected to avoid subsequent clinical failures. Furthermore, it is not proven that these combinations have superior fungicidal activity compared to the combination of amphotericin B and flucytosine (Brouwer et al., Abstr. 15th Congr. Int. Soc. Hum. Anim. Mycol., 2003).

***Candida* spp. (i) In vitro evidence.** The relationships among numerous combinations of antifungal agents have been characterized in in vitro studies of *Candida* species (Table 4).

(a) Flucytosine-amphotericin B combinations. Flucytosine has been studied in combination with amphotericin B (21, 74, 92, 105, 122, 140, 157, 178, 191) with mixed results, depending on the isolate and test conditions. In concert with amphotericin B, the predominant finding has been that of synergy (40, 105, 133, 156, 191); however, indifference (21, 74, 92, 122) has also been reported. In similarity to the results seen with this combination in studies of *C. neoformans*, it has been suggested that the addition of amphotericin B helps prevent the emergence of flucytosine-resistant mutants (156). The addition of flucytosine in time-kill studies (92), however, did not appreciably affect the activity against *C. albicans* of both low and high concentrations of amphotericin B. In this study, no antagonism was observed even with preexposure to flucytosine but other study results have indicated apparent antagonism at higher concentrations (161) or when yeast cells were first exposed to flucytosine and then amphotericin B (122). Dramatic synergy when amphotericin B and flucytosine were combined in the setting of flucy-

TABLE 4. Summary of key findings reported in studies of *Candida* spp. with combinations of clinically relevant antifungal agents[a]

Combination	Settings studied	General findings	Comments
5FC + AmB	In vitro (21, 40, 63, 74, 92, 105, 122, 133, 140, 156, 161, 178, 191)	Synergy (40, 105, 133, 178, 191) or indifference (21, 74, 92, 122)	Addition of AmB helps prevent emergence of 5FC resistance
	Mice (157, 164, 191, 209) and rabbits (208)	Improved survival (164) Reduced tissue burden (164, 208, 209)	Most effective combination in one study when compared with results for AmB-rifampin, 5FC-KTC, and these agents alone (208); reduced dosages of the agents were possible in combinati on while maintaining efficacy (209)
	Humans with invasive disease (1, 36, 100, 155)	Good clinical success	AmB + 5FC cleared cultures faster than fluconazole in humans with peritonitis (100)
5FC + azoles	In vitro Econazole (63), miconazole (63, 191) CLT (22), KTC (19, 20, 140), FLC (74, 105, 129)	No consensus Synergy (129), indifference (19, 105), antagonism (74, 140)	Extended duration of postantifungal effect was reported in one study with fluconazole-flucytosine (129), low concentrations of 5FC-KTC appeared antagonistic for *C. parapsilosis* (19) contour surface plot methodology suggested negative interaction between fluconazole and flucytosine over a range of concentrations (74)
	KTC: mice (158) and rabbits (208) ITC: mice (157) FLC: mice (180) and rabbits (115)	Improved survival (157, 158) Reduced tissue burden (115, 208)	FLC doses in rabbits were equivalent to 1,600 mg/day in humans (115); 5FC-KTC appeared to prolong survival against some *C. albicans* strains in a murine model more than either agent alone (even in higher concentrations) but against other strains had no survival benefit over a single agent (158); effects most apparent with 5FC-resistant *C. albicans* strains; in rabbits (115) FLC-AmB combination sterilized cardiac vegetations faster than FLC but performed similarly to FLC in kidney
	Humans (181)		Case report of sepsis due to *C. albicans* that was treated successfully with 5FC plus FLC (181)
AmB + azoles	In vitro FLC (67, 74, 105, 107, 122, 154, 161, 175, 185, 186, 214, 216, 217),[b] sequential (67, 105, 107, 175) Miconazole (31, 49, 63, 154, 191)[c] CLT (22, 49) KTC (31, 140, 154, 161, 183, 198) ITC (154, 161, 184, 185)	Antagonism	One study suggested indifferent effects for AmB-FLC against *C. albicans* over a wide range of concentrations (74) Slight synergy with higher concentrations of KTC and AmB (161); short-term exposure with miconazole resulted in antagonism, long-term exposure resulted in positive effects (31)
	FLC: mice (113, 176, 199, 202) and rabbits (115, 176) ITC: mice (157, 203) KTC: mice (158) and rabbits (208) PSC: mice[d] SPC: mice (206) Sequential: mice (202, 203, 216)	Improved (FLC, PSC, SPC) (113, 157, 176, 202) or similar to worse (ITC, KTC) (157, 203) survival Reduced tissue burden (FLC, KTC) (115, 208) but ITC-AmB had poorer clearance of tissues (kidney) (203) with combination	AmB-FLC effects not as profound in a less-acute model of infection (202); in rabbits, combination was not better than AMB alone in sterilizing cardiac vegetations and kidneys (115). Rabbit model used FLC doses equivalent to 1,600 mg/day in humans (115). In mice, the combination resulted in worse survival and kidney fungal burden compared to AmB alone (113) against FLC-susceptible and low-level resistance (MIC, 64 to 125 μg/ml) strains AmB-FLC gave better survival than AmB but not FLC (199) and in another study gave better survival than FLC but not AmB (176). IT C-AmB resulted in 100% mortality in mice, while 90% of amB-treated mice survived; in neutropenic rabbits AmB-KTC improved sterilization rates in kidneys (208) relative to either agent alone but not as much as AmB-5FC combination; AmB-KTC prolonged survival against one *C. albicans* strain but not 2 others (158); combinations of AmB-KTC against 2 *C. albicans* strains were generally not better than AmB alone in prolonging survival in infected mice (158)
	Humans with candidemia (166)	Good clinical success	Comparable clinical cure rates to FLC alone, faster bloodstream sterilization with the combination regimen
AmB + nystatin	In vitro (31)	Indifference	

Continued on following page

TABLE 4—*Continued*

Combination	Settings studied	General findings	Comments
Caspofungin or anidulafungin + FLC	In vitro (169)[b]	Indifference	FLC reduced caspofungin activity against *C. albicans* biofilms[b]; in mice no additional benefit of combination therapy was observed with low doses of FLC and caspofungin on clearance of yeasts from kidneys[e]
	Mice (77)	Improved or similar tissue burden	Caspofungin + FLC over 4 dosing schemes did not improve tissue clearance of *C. albicans* from kidney tissue compared to FLC alone but not caspofungin alone
TRB + FLC or ITC	In vitro (10, 11)	Indifference (10, 11) or synergy (10, 11)	No antagonism observed (10, 11)
	Humans (73)		Case report of successful therapy of oropharyngeal candidiasis due to azole- and terbinafine-resistant *C. albicans* with TRB + FLC therapy
TRB + AmB	In vitro (10)	Indifference or synergy	No antagonism observed
AmB + rifampin	In vitro (23)	Synergy	Synergy in 6 of 8 strains tested; used method of Jawetz (90) to define synergy
	Neutropenic rabbits (208)	Similar or worse tissue burden	Worse clearance of yeasts from splenic tissue than with AmB alone but similar clearance in kidney, liver, and lung
TRB + cyclosporine A or tacrolimus	In vitro (142)	Synergy	Synergistic against *C. albicans* as well as *C. glabrata* and *C. krusei*; dependent on calcineurin
FLC + cyclosporine	In vitro (120)	Synergy or indifference	Results varied with endpoint used
	Rats (119)	Reduced tissue burden	FLC approximated high doses used in humans, but cyclosporine concentrations were higher than that used in humans; combination was the most effective regimen in clearing cardiac vegetations and kidneys even compared to AmB

[a] See Table 3 for drug name abbreviations.
[b] Also Bachman et al., Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-1813, 2002.
[c] Also Schacter et al., letter.
[d] Cacciapuoti et al., Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-1814, 2002.
[e] Bocunegra, L.K. Navjar, S. Hernandez, R.A. Larsen, and J.R. Graybill, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-864, p. 387, 2002.

tosine-resistant strains of *Candida* spp. was evident, with synergy observed with 18 of 20 isolates (133).

(b) Flucytosine-azole combinations. Antagonism was reported for flucytosine with the sterol biosynthesis inhibitor miconazole in an in vitro study of strains of *C. glabrata* (192), but others reported synergy of this combination in activity against the majority of *C. albicans* isolates tested (63). The effects seen with combinations of flucytosine and newer azoles (19, 20, 22, 63, 74, 105, 129, 140, 191) have also been inconsistent. For example, in a study using checkerboard methods Lewis et al. (105) reported indifference (FIC ≥ 1 and < 4) for all of three *C. albicans* and one *C. krusei* isolate and synergy (FIC $< = 0.5$) for one *C. glabrata* and one *C. tropicalis* isolate. A negative interaction has been suggested for fluconazole-flucytosine combinations against *C. albicans*, based on a study using contour surface plots depicting a wide range of concentrations (74). Studies of other azoles (such as ketoconazole) in combination with flucytosine have also given conflicting results, with synergy or indifference reported for *C. albicans* and non-*albicans Candida* spp. (including *C. tropicalis* and *C. glabrata*) (19). In contrast, antagonism was reported when low concentrations of each agent were used against *C. parapsilosis* and indifference was observed when higher concentrations of both ketoconazole and flucytosine were employed (19).

(c) Polyene-azole combinations. Most studies of in vitro antifungal interaction with *Candida* spp. have focused on interactions between polyenes and azoles, and all types of interactions have been reported (Table 4). Antagonism has been the most common finding among studies with older azoles such as clotrimazole, miconazole, or econazole across most *Candida* spp. studied (31, 49, 63, 140, 191; L. P. Schacter, R. J. Owellen, H. K. Rathbun, and B. Buchanan, Letter, Lancet **ii:**318, 1976). However, there are reports of positive interactions between some of these older azoles and amphotericin B (22, 31). Findings with combinations of fluconazole, itraconazole, or ketoconazole and amphotericin B have generally demonstrated antagonism (107, 122, 154, 185, 186, 198, 214). Most of these studies have been performed using *C. albicans* isolates, but results with the non-*albicans Candida* spp. that have been tested have not been dramatically different. When studied sequentially, the order of administration and duration of exposure appear to be important factors affecting the activity of the polyene-azole combinations. Pretreatment with fluconazole has generally resulted in the reduction of subsequent amphotericin B activity (34, 67, 105, 107, 114, 153, 175, 216), but in some series high azole concentrations, long duration of pretreatment, or high levels of inocula were required to produce these effects (67, 107, 185). Other azoles have also been reported to exert antagonistic effects on amphotericin B activity (31, 185, 216, 217), and preexposure with more-lipophilic

azoles such as itraconazole has produced more profoundly negative effects in some experimental systems (184, 185, 217) but not in others (216). One conflicting study report has also indicated that short preexposure to amphotericin B reduced subsequent azole activity but that similar preexposure to an azole did not inhibit subsequent polyene effects (153).

(d) Other combinations. Other combinations have been studied for activity against *Candida* spp. in vitro, including combinations of terbinafine and azoles (10, 11), terbinafine and amphotericin B (10), azoles and echinocandins (169, 195), and polyenes with echinocandins (33, 195, 201) Of these combinations, terbinafine and azoles seem most promising, with synergy or indifference observed most often (10, 11) and with no apparent antagonism. Data from echinocandin combination studies have been somewhat unimpressive; while these studies have not demonstrated antagonism, synergy has also been observed infrequently (169, 195, 201). Perhaps this is because echinocandins are typically fungicidal and have potent activity against most *Candida* species when administered alone. Furthermore, fluconazole may inhibit caspofungin's activity against biofilm-producing *C. albicans* strains (S. P. Bachman, G. Ramage, A. W. Fothergill, M. G. Rinaldi, B. L. Wickes, T. F. Patterson, and J. L. Lopez-Ribot, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., 2002, abstr. M-1813, p. 415, 2002) whereas caspofungin and amphotericin B appeared to have indifferent effects.

Other agents with limited antifungal activity on their own, including metronidazole (52), fluvastatin and pravastatin (41, 136), nonsteroidal antiinflammatory agents (155, 188, 224), and quinolones (135), have been studied in combination with amphotericin B or triazoles, with a suggestion of positive effects occurring in all cases. The results of a recent report also suggested that when combined with fluconazole, terbinafine, or caspofungin, combinations of calcineurin inhibitors such as cyclosporine A and tacrolimus are highly synergistic for fungicidal effects (142). These calcineurin inhibitors can make fluconazole fungicidal. Additional data are needed regarding these novel approaches for direct therapeutic strategies.

(ii) Animal models of invasive candidiasis. *(a) Flucytosine-amphotericin B combinations.* In murine and rabbit models of invasive candidiasis, combinations of flucytosine and amphotericin B have resulted in improved survival (158, 164, 208) or tissue sterilization (164, 208, 209). Effective doses were much lower in combination than those required to produce similar effects as monotherapy (209). These models have predominantly used *C. albicans*, but one study also used a model of *C. tropicalis* infection (208).

(b) Flucytosine-azole combinations. Experience suggests that flucytosine might be a useful addition to triazoles and can result in improved rates of survival (157, 158) or clearance of yeasts from infected tissues (115, 208). However, responses have been strain specific (158); thus, caution is warranted when making generalized statements. There has also been some variation in response depending upon the doses of the agents used in the combination, and no clear dose-response relationship has been observed (157, 158).

(c) Polyene-azole combinations. Polyene-azole combinations have been carefully studied in several animal models of invasive candidiasis, including models using mice (9, 113, 157, 158, 176, 199, 202, 203, 206; A. Cacciapuoti, M. Gurnani, J. Halpern, F. Gheyas, R. Hare, and D. Loenberg, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-1814, p. 415, 2002) and rabbits (115, 176, 208). Some of the findings with these animal models are contrary to those of in vitro reports of antagonism, and although positive effects have been observed, it is difficult for an azole-polyene combination to improve on the activity of amphotericin B alone. Combinations of fluconazole and amphotericin B have been associated with prolonged survival and/or tissue clearance in a number of studies (115, 176, 199, 202). In most cases, the combination had improved activity relative to fluconazole alone (115, 176, 202) but was not superior to amphotericin B. Only one study of acute invasive candidal infection (199) indicated improved survival with the combination compared to the results seen with amphotericin B alone. In contrast, other studies indicated trends toward (202, 203) or significantly (113, 115) worse survival results among mice receiving a combination of fluconazole and amphotericin B compared to amphotericin B alone. The combination was also associated with higher levels of yeast burden in kidneys (113, 115) and more cardiac vegetations (115) than amphotericin B alone. In contrast to the sometimes positive effects observed with fluconazole-amphotericin B combinations in the treatment of other fungal infections, animal studies of candidiasis treated with itraconazole, ketoconazole, or posaconazole in combination with amphotericin B have generally not indicated any advantages of the combination with regard to tissue clearance (203) or survival (157, 158, 203). Sequential therapy with azole and amphotericin B has resulted in the attenuation of amphotericin B activity after azole preexposure (115, 203); in contrast, however, preexposure to the polyene did not significantly reduce subsequent azole activity (115). In one study, both sequences of combination therapy had negative effects (203). For example, survival was worse among mice exposed sequentially to itraconazole-amphotericin B in either order (amphotericin B for 5 days and then itraconazole for 5 days and vice versa), compared to the results seen with amphotericin B alone (203).

(d) Echinocandin combinations. Only limited data have been published with respect to combinations of echinocandins and amphotericin B in animal model studies, but early experience with cilofungin and amphotericin B in mice with disseminated candidiasis indicated improved survival and reduced tissue burden relative to the results seen with either agent alone (201). These effects were particularly apparent when higher doses of the agents were employed in combination. A recent study (77) demonstrated that combinations of caspofungin and fluconazole were more effective in reducing yeast burden in kidneys of mice infected with *C. albicans* compared to fluconazole alone but were not better than caspofungin alone (77). Results were consistent over a range of dosages of both agents, but the study design was limited to one clinical isolate of *C. albicans* and the model did not focus on effects in the immunosuppressed host.

(e) Other combinations. Other combinations (including rifampin in combination with ketoconazole, flucytosine, or amphotericin B) were not superior with respect to results in guinea pig (68) or rabbit (208) model studies. However, combinations of fluconazole and the calcineurin inhibitor cyclosporine A in a study using a rat model of *C. albicans* endocarditis were more promising (119). The combination of relatively

high concentrations of cyclosporine and fluconazole was fungicidal and significantly decreased yeast burden in cardiac vegetations and kidneys compared to the results seen with either agent alone and amphotericin B.

(iii) Clinical data. *(a) Polyene-azole combinations*. Recent data from studies conducted with humans (166) have generated some excitement with respect to azole-polyene interactions since the first large-scale trial in the treatment of candidemia was completed. In this multicenter randomized study (166), nonneutropenic subjects with non-*C. krusei* candidemia received fluconazole (12 mg/kg of body weight) daily for 14 days after symptoms resolved plus either placebo or amphotericin B (0.7 mg/kg/day) for the first 3 to 8 days. Overall success rates were somewhat better for the combination regimen, with 69% (77 of 112 subjects) success versus 56% (60 of 107) success for fluconazole monotherapy ($P = 0.043$). In addition, more subjects receiving monotherapy with fluconazole had persistent fungemia than those receiving fluconazole plus amphotericin B (6 versus 17% [$P = 0.02$]). However, there were no differences between the two treatment groups in overall mortality or drug toxicity. Rates of study discontinuation due to nephrotoxicity were not significantly different between groups, with 3% of combination therapy subjects and 5% of fluconazole monotherapy subjects withdrawing. Thus, this study demonstrated no antagonism and the results tended towards a better outcome with the combination fluconazole-amphotericin B therapy (166). The results of small studies have suggested positive outcomes among neonates (88) and adults (130) with hematologic malignancies who received the combination of amphotericin B and fluconazole for candidemia. Others (145) have employed oral amphotericin B simultaneously with ketoconazole or fluconazole as prophylaxis in neutropenic patients, with the hope of preventing selection of resistant *Candida* species. Fluconazole-amphotericin B combinations in particular were associated with colonization rates in the gastrointestinal tract that were lower than the colonization rates observed during later years during which itraconazole alone was used as a prophylaxis. However, rates of breakthrough candidemia were similarly low with all treatments (145). Sequential azole-polyene therapy has been reported in studies of humans in the context of breakthrough infections while patients were receiving azole prophylaxis including fluconazole (174) or a combination of fluconazole and itraconazole (123). Neither study was designed to specifically examine the effectiveness of amphotericin B after azole exposure, but success rates of lipid formulations of amphotericin B for candidemia (174) and fevers or pneumonia of unknown origin (123) were similar to those reported for other published studies.

(b) Flucytosine-amphotericin B combinations. Therapy with amphotericin B and flucytosine in combination has been successfully employed in a study of invasive candidiasis in nonneutropenic subjects in intensive care units (1), with success rates among subjects with sepsis similar to those seen with fluconazole. Combination therapy was better than fluconazole in sterilizing tissues and in successfully treating peritonitis cases among subjects in this randomized study (1). Other clinical reports indicate excellent efficacy of combinations of amphotericin B and flucytosine for the treatment of candidal prosthetic hip infection in combination with surgical revision (165) and of candidal meningitis among HIV- and non-HIV-infected patients (36, 194).

(c) Other combinations. Limited data have been published regarding combination therapy with other antifungal agents for the treatment of *Candida* spp. infections. Ghannoum and Elewski (73) reported a case of fluconazole-resistant oropharyngeal candidiasis that was cured with a combination of fluconazole (200 mg) and terbinafine (250 mg) administered daily for 2 weeks. Dual azole therapy with fluconazole and itraconazole capsules has been employed as an antifungal prophylaxis during induction chemotherapy and in a comparative study (123) performed similarly to therapy with liposomal amphotericin B (3 mg/kg three times weekly) with respect to the prevention of fever and infection.

(iv) Interpretation and recommendations. The frequency of non-*albicans Candida* spp. infections, increases in the frequency of azole-resistant isolates, toxicities associated with typical treatment doses of amphotericin B, and high levels of mortality associated with invasive candidiasis support the need for more-effective and less-toxic treatment strategies.

Flucytosine has been added to agents such as amphotericin B and azoles for activity against *Candida* spp. in the laboratory, with mixed effects. Conflicting results may be a result of a number of factors, including different experimental practice factors such as growth media, strains, inoculum sizes, pH, temperature, drug concentrations, individual drug characteristics, and other undefined factors. Generally, however, these findings have been positive. Experiences in animal models more consistently suggest that flucytosine in combination with amphotericin B or triazoles has positive effects on survival and tissue burden.

Amphotericin B-azole combinations (and, in particular, sequential exposure to an azole followed by a polyene) could be detrimental, as determined on the basis of in vitro data and animal models. Concurrent fluconazole-amphotericin B combinations have been promising in these animal models, since there are several reports of improved survival or tissue clearance with this combination. Importantly, most of these animal models have used *C. albicans*; the potential of combination therapy could be even greater with less-susceptible species (9). Amphotericin and fluconazole have been employed concurrently for management of candidemia in a large clinical trial (166) and produced favorable results.

Combinations of echinocandins with azoles or amphotericin B have not been particularly impressive in vitro. Since echinocandins are highly active against most *Candida* spp., their fungicidal activity for yeasts may be difficult to improve upon. Combinations of terbinafine and azoles, however, are more promising, especially for resistant oropharyngeal candidiasis, and are deserving of additional study.

Due in good part to the availability of three monotherapies that are now recognized as producing consistently good outcomes (caspofungin and amphotericin B in the most severely ill patients and fluconazole in less-severe disease and as followup therapy), recently published treatment guidelines for invasive candidiasis mention but do not strongly encourage the use of the combination of fluconazole and amphotericin B for therapy of candidemia (143). We concur that single-agent therapy in most cases of invasive candidiasis and candidemia will most likely be the treatment of choice. Combination therapy may be

TABLE 5. Summary of key findings reported in studies of *Aspergillus* spp. with combinations of clinically relevant antifungal agents[a]

Combination	Settings studied	Findings	Comments
AmB + 5FC	In vitro (58, 87, 95, 104, 140)[b]	No consensus Synergy[b]; synergy or indifference (95); indifference (87, 104); antagonism or synergy (58)	Results differ between studies and are variable amongst strains in same study (58, 95), different methodologies and doses employed
	Mice (6, 157) and rats (187)	Improved survival (6, 157)	Improved survival with 5FC + AmB in mouse model (6). No survival benefit with 5FC + AmB vs. AmB alone in steroid-suppressed rats (187).
AmB + rifampin	In vitro (95)	Synergy (95); indifference or synergy (46, 58, 87)	Antagonism not observed in any study
	Mice (6) and rats (187)	No consensus	No survival benefit with rifampin + AmB vs. AmB alone in steroid-suppressed rats (187). Improved survival with rifampin + AmB in mouse model (6).
AmB + azoles	In vitro (58, 87, 97, 118, 140, 207)[b,c,d,e]	No consensus	Pretreatment with KTC (118) or ITC (97, 118) strongly attenuates effect of AmB; simultaneous treatment less antagonistic to indifferent; AmB then KTC weakly synergistic (118); no antagonism for AmB then ITC; indifferent effects with simultaneous ITC-AmB[e]. Studies using colorimetric analysis and response surface modeling demonstrated ITC-AmB antagonism with simultaneous use (207).[b]
		Synergy (58, 140), antagonism, (118, 140, 207)[c,b] or indifference (58, 87)[d]	
	ITC: Mice (179)[f] KTC: mice (157, 180) and rats (187) PSC: mice[g]	Concurrent: no survival benefit (ITC)[f] or worse survival (KTC) (157, 187)	Neutropenic mice had significantly worse survival when pretreated with KTC before AmB or AmB + KTC (180). Steroid-suppressed rats given simultaneous KTC and AmB had worse survival than with AmB alone (187). Mice pretreated with ITC before AmB or AmB + ITC had lower survival than without pretreatment (179). Neutropenic mice with CNS infection had equal survival with either agent or combination vs. no treatment[f] but nonneutropenic mice challenged intravenously had reduced survival times with combination therapy (157). In mice, no sequential antagonism of PSC by pretreatment with AmB[g].
		Sequential: no survival benefit with or worse survival (179) compared to AmB results alone	
AmB + echinocandins	In vitro Caspofungin (5, 15)[e] Anidulafungin or micafungin[h]	Synergy (5),[e] indifference or synergy (15)[h]	No antagonism seen. Eagle-like effect (antagonism at high doses) seen in one study[h].
	Mice Caspofungin[i] or micafungin[j,k]	Improved survival[j,k] Reduced[i,k] or similar[i] tissue burden	Neutropenic mice, fungal burden in kidneys at 4 days reduced (10/16 groups) or equivalent (6/16) with combination therapy vs. either agent alone. Increased survival, reduced fungal lung burden, reduced serum galactomannan titer with combination vs. monotherapy[j]. Steroid-immunosuppressed mice had 100% survival with combination therapy vs. 61% with micafungin and 53% with AmB.
Triazoles + caspofungin or micafungin	In vitro ITC-caspofungin[e,l,m] or micafungin[l] PSC-caspofungin[m] RVC-caspofungin[m] VRC-caspofungin or micafungin[d,m,n]	Indifference or synergy[d,l,l,m,n] or synergy[e,m]	No antagonism seen in most studies; increased susceptibility with preexposure to either agent[l]. VRC-caspofungin: indifference against caspofungin- or micafungin-resistant-strains,[d] ITC and PSC demonstrated synergy with caspofungin[m]; RVC and VRC demonstrated indifference with caspofungin[m].
	ITC-caspofungin: guinea pigs[o] ITC-micafungin: mice (117) KTC-micafungin: mice (117) RVC-micafungin: rabbits[p] VRC-guinea pigs (94)	Improved (117)[p] (94) survival or similar Reduced tissue burden (94)[o].	Fungal burden in kidneys at day 4 undetectable in 9/9 animals receiving ITC-caspofungin therapy[o]. RVC-micafungin increased survival with combination (9/12) vs. revuconazole alone (2/8) or micafungin alone (0/8)[p].

[a] See Table 3 for drug name abbreviations.
[b] Also see Te Dorsthorst et al., Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-850, 2002.
[c] Also see Gavalda et al., Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-1817, 2002.
[d] Also see M. A. Ghannoum, N. Isham, and D. Sheehan, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-855, p. 385, 2002.

Continued on following page

considered in difficult individual settings, such as those of hepatosplenic candidiasis, endocarditis, meningitis, and relapsing infections.

***Aspergillus* spp. (i) In vitro data.** *(a) Flucytosine- or rifampin-amphotericin B combinations*. The effects of amphotericin B and the azoles on the cell membrane of *Aspergillus* spp. may allow for enhanced penetration and improved activity of other antifungal agents, such as rifampin (46, 125) and flucytosine (127). The results of in vitro studies of amphotericin B combined with rifampin (46, 87, 95, 127) or flucytosine (D. T. Te Dorsthorst, J. W. Mouton, H. A. L. van der Lee, and P. E. Verweij, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-850, p. 383, 2002) have commonly demonstrated synergy (Table 5). Combinations of flucytosine and amphotericin B have demonstrated various effects, with reports of indifference (58, 87, 104), synergy (58, 95, 140), and antagonism (210). These effects did not differ significantly according to the species of *Aspergillus* tested or according to levels of baseline resistance to flucytosine (58), but in some cases results differed according to the testing methodology employed by investigators.

(b) Flucytosine-azole combinations. Experience with the combination of an azole with flucytosine is limited, with older reports indicating effects ranging from indifferent to synergistic (58, 140, 157). However, a recent report indicated that antagonism resulted when flucytosine and itraconazole were used together (Te Dorsthorst et al., Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-850, 2002).

(c) Polyene-azole combinations. A number of in vitro studies have attempted to substantiate the theoretical antagonism between the azoles and amphotericin B. Studies conducted with simultaneous exposure have shown results ranging from indifferent interactions (58, 87; T. M. Chiller, J. Capilla Luque, K. V. Clemons, R. A. Sobel, and D. A. Stevens, Abstr. 41st Intersci. Conf. Antimicrob. Agents Chemother., abstr. J-1614, p. 391, 2001; J. Gavalda, P. Lopez, M. Martin, M. Cuenca-Estrella, X. Gomis, J. L. Ramirez, J. Ruiz, J. L. Rodriguez-Tudela; A. Pahissa, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-1817, p. 416, 2002; and E. K. Manavathu, S. Krishnan, J. L. Cutright, and P. H. Chandrasekar, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 931, p. 368, 2000) to synergistic effects (140). Simultaneous use of ketoconazole, fluconazole, or itraconazole with amphotericin B has yielded variable results (118; Te Dorsthorst et al., Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-850, 2002), with antagonism or indifference most commonly observed. Preexposure to amphotericin B has been associated with subsequent synergy or indifferent effects for these triazoles (118). When an azole was first applied to the culture, however, strong antagonism upon subsequent exposure to amphotericin B was observed (97, 118).

(d) Echinocandin combinations. In contrast to the negative effects observed with polyene-azole combinations, in vitro experience with echinocandins in combination with azoles and amphotericin B has generally been neutral or positive, with indifferent to synergistic effects for most combinations. Caspofungin, micafungin, and anidulafungin in combination with amphotericin B in vitro have all demonstrated synergy or indifference (5, 15; Kohno et al., Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1686, 2000; and Ostrosky-Zeichner et al., Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-1816, 2002). However, the results of one study also indicated an unexplained antagonism (an Eagle-like effect) at high doses of the echinocandin (Ostrosky-Zeichner et al., Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-1816, 2002) and so the full spectrum of antifungal interactions with these agents has been observed. Triazole (itraconazole, voriconazole, ravuconazole, or posaconazole) combinations with echinocandins (caspofungin and micafungin) have shown encouraging results, with some investigators reporting synergistic activity (147, 190; E. K. Manavathu, G. J. Alangaden, and P. H. Chandrasekar, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-854, p. 384, 2002; Manavathu et al., Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 931, 2000; and O'Shaughnessy et al., Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-856, 2002) and none reporting antagonism when the agents were used either simultaneously or sequentially (5, 15; C. M. Douglas, J. C. Bowman, K. F. Bartizal, G. K. Abruzzo, J. W. Anderson, A. M. Flattery, C. J. Gill, B. Michael, T. Felcetto, G. Mickle, W. Shoop, P. A. Liberator, and K. F. Bartizal, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-1819, p. 416, 2002; Kohno et al., Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1686, 2000; D. P. Kontoyiannis, R. E. Lewis, G. S. May, N. D. Albert, and I. I. Raad, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-851, p. 384, 2002; E. K. Manavathu, G. J. Alangaden, and P. H. Chandrasekar, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-854, p. 384, 2002; Manavathu et al., Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 931, 2000; M. Nakajima, S. Tamada, Y. Yoshida, Y. Wakai, T. Nakai, F. Ikeda, T. Goto, Y. Niki, and T. Matsushima, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1685, p. 387, 2000; Ostrosky-Zeichner et al.,

[e] Also see Manavathu et al., Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 931, 2000.
[f] Also see Chiller et al., Abstr. 41st Intersci. Conf. Antimicrob. Agents Chemother., abstr. J-1614, 2001.
[g] Najvar et al., Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-1818, 2002.
[h] Ostrosky-Zeichner et al., Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-1816, 2002.
[i] Douglas et al., Abstr. 41st Intersci. Conf. Antimicrob. Agents Chemother., abstr. J-1836, 2001.
[j] Kohno et al., Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1686, 2000.
[k] Nakajima et al., Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1685, 2000.
[l] Kontoyiannis et al., Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-851, 2002.
[m] Manavathu et al., Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-854, 2002.
[n] O'Shaughnessy et al., Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-856, 2002.
[o] Douglas et al., Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-1819, 2002.
[p] Petraitiene et al., Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-857, 2002.

Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-1816, 2002; and Petraitiene et al., Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-857, 2002). Synergy was reported among 87.5% of voriconazole-caspofungin interactions in 48 clinical isolates of *Aspergillus* spp. (147). Other agents may also be a useful addition to echinocandins. For instance, calcineurin inhibitors or rapamycin in concert with caspofungin has demonstrated synergistic effects (98). Most results of in vitro combinations have been similar for both *Aspergillus fumigatus* and the small number of *A. terreus* and *A. flavus* isolates tested.

One of the most synergistic combinations in vitro is a combined block of two cell wall enzymes, glucan and chitin synthases. This synergistic activity was first observed for *Aspergillus* with the use of cilofungin (glucan synthase inhibitor) and nikkomycin Z (chitin synthase inhibitor) (149, 151).

(ii) Animal models of aspergillosis. Possible in vivo benefits from combination antifungal therapy have been investigated in a number of animal models.

(a) Flucytosine- or rifampin-amphotericin B combinations. Mice challenged intravenously with *A. fumigatus* and treated simultaneously with amphotericin B and either flucytosine or rifampin had significantly improved survival rates compared with the results seen with any monotherapy (6). Flucytosine also augmented the activity of both amphotericin B and itraconazole when administered in mice intravenously challenged with *A. fumigatus* (157). However, another study showed no benefit from the combination of amphotericin B plus rifampin or flucytosine for immunosuppressed rats (187).

(b) Polyene-azole combinations. Suggestions of azole-polyene antagonism arising from in vitro studies have been confirmed in some animal models. Importantly, pharmacokinetics of azoles in murine models make them difficult to study due to rapid clearance; therefore, guinea pig models may be the preferred system for studying these interactions in *Aspergillus* spp. infections. However, most studies to date have been performed using mice. Studies of simultaneous treatment with amphotericin B and ketoconazole combinations have indicated worse survival in the combination arms (157, 187). Similarly, mice treated with itraconazole in combination with amphotericin B had shorter survival times compared to the results seen with amphotericin B alone in another study (157). On the other hand, combinations of itraconazole or posaconazole and amphotericin B have resulted in survival times similar to the results seen with treatment with each agent alone (Chiller et al., Abstr. 41st Intersci. Conf. Antimicrob. Agents Chemother., abstr. J-1614, 2001, and L. K. Najvar, S. Hernandez, R. Bocanegra, J. Halpern, M. Gurnani, F. Menzel, A. Cacciapuoti, D. Loebenberg, and J. Graybill, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-1818, p. 416, 2002). Preexposure to azole agents has generally reduced the subsequent efficacy of amphotericin B treatment. For example, mice receiving ketoconazole or itraconazole followed by amphotericin B alone exhibited worse survival rates than mice given amphotericin B without initial azole exposure (179, 180). In contrast, sequential therapy with posaconazole followed by amphotericin B did not produce appreciable differences in the clearance of organisms from lung tissue or in survival rates compared to treatment with either agent alone (Najvar et al., Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-1818, 2002). Thus, the timing of administration may be important with regard to some of these triazole-polyene interactions; also, these combined effects may differ among the different azole compounds.

(c) Echinocandin combinations. Few studies have described the use of echinocandins in combination antifungal therapy in animal models. In a guinea pig model of *A. fumigatus* infection, treatment with caspofungin plus voriconazole produced survival rates similar to that of treatment with voriconazole alone. The combination was superior in terms of the sterilization of all organs studied (94). Prolonged survival with combined micafungin and ravuconazole treatment was observed in a rabbit model of pulmonary aspergillosis (Petraitiene et al., Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-857, 2002). Treatment with combinations of either caspofungin (Douglas et al., Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-1819, 2002) or micafungin (117) and itraconazole has resulted in reduced fungal burden (Douglas et al., Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-1819, 2002) or prolonged survival (117). In one study (117), micafungin-itraconazole was a more effective combination regimen compared to micafungin-amphotericin B in clearing cerebral aspergillosis but was similar to itraconazole alone. In another study, however, caspofungin combined with amphotericin B produced a trend towards decreased fungal burden in the kidneys (C. M. Douglas, J. C. Bowman, K. F. Bartizal, G. K. Abruzzo, J. W. Anderson, A. M. Flattery, C. J. Gill, V. B. Pikounis, P. A. Liberator, and D. M. Schwartz, Abstr. 41st Intersci. Conf. Antimicrob. Agents Chemother., abstr. J-1836, p. 398, 2001). Suboptimal doses of micafungin with amphotericin B also prolonged survival (117) but did not sterilize tissues completely. Finally, combinations of micafungin and nikkomycin Z at relatively low dosages significantly increased survival compared to treatment with either agent alone (117). This study helped validate the principle that two blocks in cell wall synthesis can improve anti-*Aspergillus* activity.

(iii) Clinical data. *(a) Flucytosine- or rifampin-amphotericin B combinations*. The excellent central nervous system penetration of rifampin and flucytosine has prompted their use as adjuncts to amphotericin B in the treatment of central nervous system aspergillosis, with occasional successes reported in the near-uniformly-fatal disease (54, 93, 137, 159, 163). However, the potential hematologic toxicity of flucytosine, the lack of a widely available intravenous formulation, and the drug interaction profile of rifampin have limited the widespread use of these agents in combination therapy of *Aspergillus* infections (57). Furthermore, there has been no study with a sufficient number of treated patients to allow an appreciation of the impact of these combinations.

(b) Polyene-azole combinations. With the introduction of itraconazole, combination therapy with azoles-polyenes became feasible for clinical cases of aspergillosis. Simultaneous use of these agents has been reported infrequently, and this is perhaps due to concern over the antagonism demonstrated in vitro and in animal models as well as to the early lack of an intravenous or a highly bioavailable oral formulation of itraconazole (200). However, successful outcomes in individual cases have been reported (53, 54, 96, 128). Investigators conducting one case series reported that 9 of 11 patients receiving

amphotericin B and itraconazole were cured or improved versus 5 of 10 receiving amphotericin B alone (160).

More frequently, patients who are exposed to both amphotericin B and an azole will have received the two drugs sequentially rather than simultaneously. Azoles have been studied as a prophylaxis against fungal infections in neutropenic and transplant patients as well as for the empirical treatment of febrile neutropenia (7, 28, 30, 81, 121). These patients may develop breakthrough *Aspergillus* infections and be switched to another agent, most commonly amphotericin B. Azoles may also be used for long-term consolidation or clearance therapy in patients who have received induction therapy with amphotericin B for invasive aspergillosis (146). Also, azoles may be added to a failing amphotericin B regimen or vice versa. Thus, azole treatment may be initiated either prior or subsequent to treatment with polyenes. Despite the large number of trials utilizing azoles in prophylaxis or treatment, few have specifically examined the outcomes of patients who are crossed over from an azole to a polyene or vice versa. One case report describes a kidney transplant patient whose previously controlled *Aspergillus* infection became disseminated when the patient was switched from itraconazole to amphotericin B treatment (179). Other cases have been reported that showed success with the use of itraconazole following treatment with intravenous amphotericin B in different *Aspergillus* infections (168, 215). Voriconazole has been used with good results (an approximately 50% response rate) as salvage therapy in invasive aspergillosis, with most patients being initially treated with amphotericin B (59).

(c) Echinocandin combinations. The arrival of caspofungin for the treatment of refractory aspergillosis has generated renewed excitement over the potential use of this new antifungal class in combination therapy. This enthusiasm is due to the unique target, low toxicity of the class, and its lack of fungicidal activity when used alone. Already, several case reports have appeared which indicate successful treatment of invasive aspergillosis with the combination of caspofungin and either lipid formulations of amphotericin B (116, 170; T. Gentina, S. de Botton, S. Alfandari, J. Delomez, S. Jaillard, O. Leroy, C. Marquette, G. Beaucaire, F. Bauters, and P. Fenaux, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-860, p. 386, 2002; and D. P. Kontoyiannis, R. Hachem, R. E. Lewis, G. Rivero, H. Kantarjian, and I. I. Raad, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-1820, p. 416, 2002), itraconazole (170), or voriconazole (Gentina et al., Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-860, 2002). The largest case series reported thus far examined 48 subjects with hematologic malignancy and documented or probable invasive aspergillosis, most of whom had progressive disease on liposomal amphotericin B (Kontoyiannis et al., Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-1820, 2002). The subjects received combination therapy with caspofungin and liposomal amphotericin B. The response rate was 22% in those with documented invasive aspergillosis and 60% in those with possible invasive aspergillosis. No significant toxicities were reported with this regimen. In addition, the results of a multicenter study using micafungin in combination with other licensed antifungal therapy (predominantly lipid formulations of amphotericin B) in bone marrow transplant recipients with invasive aspergillosis were recently presented (R. Ratanatharathorn, P. Flynn, J. van Burik, P. McSweeney, D. Niederwieser, and D. Kontoyiannis, Abstr. 44th Am. Soc. Hemat. Annu. Meet., abstr. 2472, 2002). Participants had either failed to respond to or progressed while receiving 72 h of effective antifungal therapy. The level of clinical, radiographic, and microbiologic success (as evaluated by an expert panel) in this group of 85 evaluable subjects, who had significant graft-versus-host disease and mostly proven, progressive invasive aspergillosis at baseline, was approximately 28%. Interestingly, 13 subjects received triple combination therapy with micafungin, an amphotericin B formulation, and an azole. Outcomes for these subjects were not presented separately, but this is an example of trends in current clinical practice that are continuing despite limited objective data for treatment outcomes with double and triple antifungal drug regimens for humans.

(iv) Interpretation and recommendations. Although numerous in vitro and animal model investigations have been performed, there are currently no prospective trials published that evaluate the use of antifungals in combination for the treatment of human disease due to *Aspergillus* spp. Current therapy guidelines (197) do not include the use of combination therapy (other than the use of oral itraconazole for consolidation after a course of intravenous amphotericin B treatment), but these recommendations will need to be updated in light of recent data on voriconazole as primary therapy (85). In this study, approximately 53% of subjects had a successful response to voriconazole treatment. This demonstrates that there continues to be a need to improve outcomes among patients with invasive aspergillosis.

Flucytosine-amphotericin B combinations have produced inconsistent results in vitro, whereas rifampin-amphotericin B combinations have exhibited synergy. Both of these combinations appeared to prolong survival in animals, but the improvements were not necessarily better than that observed with amphotericin B alone. Data for azole-rifampin regimens are more conflicting, with one positive result (157) and one negative result (187) in studies of infected animals. Due to their toxicity and drug interaction profiles, respectively (as well as to the lack of any clinical trials with these combinations), treatment with flucytosine and rifampin in combination with polyenes or azoles should be reserved for those situations (brain abscess, ocular infections, etc.) in which the excellent tissue penetration of these agents can be leveraged.

As judged on the basis of in vitro and animal data suggesting antagonism, simultaneous therapy with amphotericin B and the azoles should be employed with caution and study. However, it appears that the sequential use of azoles to complete a course of therapy after treatment with polyene is probably safe. A more complicated issue is that of the use of amphotericin B for patients previously treated with an azole. Such cases often occur when patients have received azoles for antifungal prophylaxis and may occur more frequently as voriconazole becomes the standard as primary therapy for invasive aspergillosis. Theoretically, these patients could be at higher risk for treatment failure with a polyene due to sequence-specific antagonism with the azoles. To date, no clinical studies have implicated previous azole prophylaxis as a cause of treatment failures in *Aspergillus* infection with a polyene, but patients in this situation should be carefully monitored.

Combinations of echinocandins with azoles or amphotericin B products in both in vitro and in animal models of aspergillosis have produced positive results. Their relative lack of toxicity also makes them an attractive option as add-on therapy. Clinical studies to explore the effectiveness of echinocandin-based combinations seem worth pursuing. Particularly since there have been some positive results when caspofungin has been used as salvage therapy, it seems logical that it might perform better when used earlier in the treatment course.

Finally, triple-antifungal-drug combinations may be attractive to clinicians who are striving to improve the dismal outcomes among patients with invasive aspergillosis. These strategies have not been widely studied in any system to date, so caution is warranted when employing these expensive and potentially detrimental combinations in clinical practice. There is such a strong desire to improve the outcome of patients with aspergillosis with newer agents possessing different mechanisms of action that we must be careful to analyze the true impact of combination therapy with evidence-based observations and studies.

At the present time, successes with combination therapy for the treatment of invasive aspergillosis remain more hope than fact; clinicians should use combination therapy with caution until more clinical studies are available.

CONCLUSIONS AND FUTURE DIRECTIONS

Numerous in vitro susceptibility testing and animal studies have explored the interactions between antifungal agents for many different fungal pathogens. Effects observed have differed for different agents within a class (for example, azoles) as well as among different fungal pathogens and under different study conditions. Despite their importance for the framing of new hypotheses, the limits of in vitro and animal testing (Table 2) should be emphasized (68, 121). For example, it is clearly difficult to extrapolate these data to humans, for whom the host's "net state of immunosuppression" is both crucial to outcome and differs over time. The effects observed in these models will not precisely apply to all aspects of the clinical setting. However, they represent the best-controlled data we have and, upon review, they help us gain a better understanding of how these drugs might behave when used together.

Taken together, the results of these studies represent a comprehensive data set to support future investigations in this area and should improve outcomes among patients with these serious fungal infections. If anything, the range of in vitro and in vivo results shows that almost any result can be achieved for any combination. There are general trends, but it appears that the differences among strains, differences in relative drug dosages, and differences in the underlying models make data aggregation difficult. Ultimately, it appears that the only way to resolve some of these issues is to use the available in vitro and in vivo data to drive the design of carefully selected clinical studies of combination therapy in patients.

As we move toward more using of these systematic investigations, we must consider the potential risks and benefits of these approaches and design these studies with the utmost care. Particular attention should be paid to issues of dose response and dose selection for these trials. In addition, clear definitions of study endpoints, use of the appropriate patient population, and selection of appropriate comparator agents are critical to answering the research question. We have used published data to identify some of the most promising antifungal drug combinations for the three major fungal pathogens as well as some of the combinations that may be detrimental.

For cryptococcosis, combination therapies in the clinics are well established and based on significant evidence. For candidiasis, it is likely that single-agent therapy will be used primarily and that the use of combination therapies will be considered for unique settings. For aspergillosis, many questions remain, with few solid clinical answers. Future attention to this specific area is mandatory.

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