

Combination Antifungal Therapy: When Is Two Better Than One?

By Linsey R. McPheeters, Pharm.D.

Invasive fungal infections, especially in immunocompromised patients, are related to high rates of mortality. Fungi responsible for these infections include *Candida* spp., *Cryptococcus neoformans*, *Aspergillus* spp., and others.¹ Recently, the introduction of new antifungals, such as extended-spectrum triazoles and echinocandins, have increased the therapeutic options available. However, monotherapy is often limited by toxicity, intolerability, or a narrow spectrum of activity, and is too often ineffective against invasive infections.² In order to treat patients with invasive fungal infections, many practitioners are turning to combination antifungal regimens. Conflicting *in vitro* and animal data, and a relative lack of controlled clinical studies with well-defined outcomes, make the selection of antifungal combinations largely empiric. This review will address the rationale behind using combination antifungal therapies; summarize the results of *in vitro*, animal, and clinical studies; identify regimens that show the most promise; and suggest a rational approach to the treatment of patients with invasive fungal infections.

Each of the four main classes of antifungals has a different mechanism of action (see Figure 1). The azoles, which consist of both imidazoles (miconazole, clotrimazole, ketoconazole) and triazoles (itraconazole, fluconazole, and voriconazole) act by inhibiting the fungal cytochrome P450-dependent ergosterol biosynthesis, specifically by blocking 14- α sterol demethylase. Triazoles tend to have more affinity than imidazoles for fungal cytochrome P450 enzymes over mammalian ones. The primary differences between the azoles involve their spectrum of activity, pharmacokinetics, and chemical formulations. Itraconazole has a broader spectrum of activity than the imidazoles or fluconazole. Fluconazole has a similar spectrum of activity to ketoconazole, but is more water soluble, less protein bound, has a longer half-life, is better tolerated, and crosses the blood-brain barrier. Voriconazole is a derivative of fluconazole with a fluoropyrimidine group instead of a triazole side-chain and has a broader spectrum of activity than fluconazole. Amphoterin B, a polyene, works by binding to ergosterol in the fungal cell membrane, making the membrane more permeable. Flucytosine, or 5-fluorocytosine, is converted to 5-fluorouracil inside the cell, which inhibits DNA synthesis. Because of the high likelihood of developing resistance to flucytosine, it should never be used as monotherapy. Echinocandins, such as caspofungin, inhibit β -(1,3)-D-glucan synthase, an essential enzyme for the synthesis of the fungal cell wall. Echinocandins have a broad spectrum of activity and tend not to affect human cells due to their targeted mechanism of action.^{2,3}

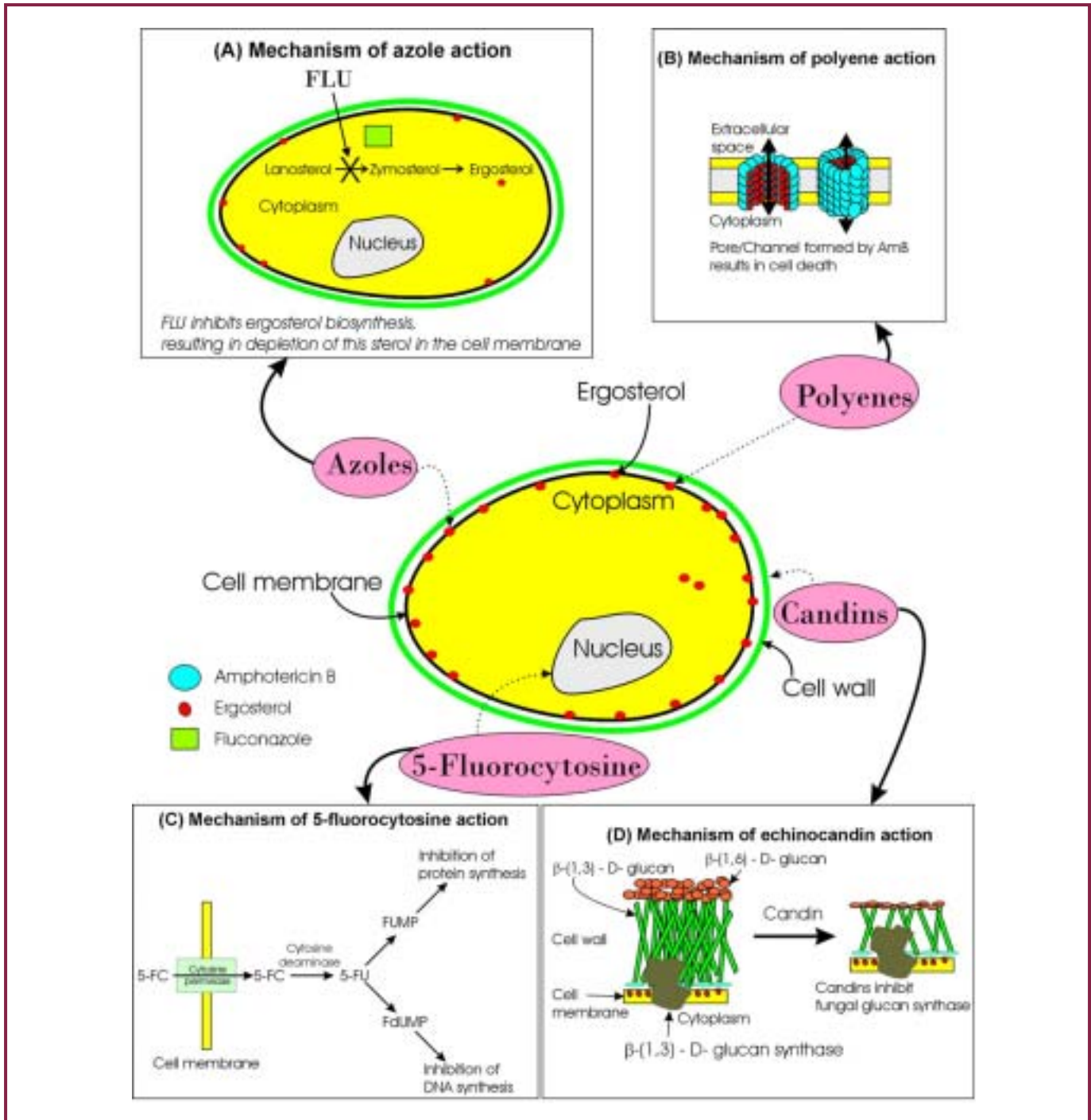
Combination antifungal therapy has potential advantages over monotherapy. By using two agents with different mechanisms of action, it may be possible to enhance the fungicidal activity of the two drugs and increase the distribution of the drugs into different tissues. Theoretically, drug combinations would broaden the spectrum of activity of the regimen and be useful for primary therapy when the fungus is unknown or if a patient has a mixed infection. A lower dosage of each agent could theoretically

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Figure 1: Sites of Action of Different Antifungal Agents²



²Reprinted with permission from Mukherjee PK, Sheehan DJ, Hitchcock CA, and Ghannoum MA. Combination treatment of invasive fungal infections. Clinical Microbiology Reviews Jan 2005;18(1):163-194.

Conflicting data make the selection of antifungal combinations largely empiric.

be used in a synergistic combination regimen to reduce the likelihood of dose-related toxicity; however, life-threatening invasive fungal infections require aggressive therapy. As demonstrated for flucytosine, combination therapies may also prevent the development of drug resistance.^{1,2,4}

Using more than one antifungal agent also carries theoretical disadvantages. Certain combinations have demonstrated antagonism, so that the activity of one or both of the drugs is less than when used alone. Combination therapy also increases the risk of drug-drug interactions and may result in additive toxicities.⁴ Finally, the cost of combination therapy is substantially greater than monotherapy, especially when combining the newer agents (see Table I).

Table I: UW Medicine Antifungal Acquisition Costs

Drug and Dose (based on 70kg)	Cost per dose
Amphotericin B 50mg IV (0.7mg/kg)	\$15.11
Caspofungin 70mg IV (loading dose)	\$399.72
Caspofungin 50mg IV	\$310.28
Fluconazole 400mg IV	\$35.35
Fluconazole 400mg PO	\$0.76
Flucytosine 1750mg PO (25mg/kg qm)	\$25.10
Itraconazole 200mg IV	\$177.84
Itraconazole 200mg PO	\$15.12
Voriconazole 420mg IV (6mg/kg loading dose)	\$262.68
Voriconazole 280mg IV (4mg/kg)	\$175.12
Voriconazole 200mg PO	\$25.75

Some experts point out that one of the limitations of *Aspergillus* clinical studies is a lack of well-defined surrogate markers of efficacy.

When an antifungal combination is indicated, it should involve two drugs with different mechanisms of action, be based on historical data with the combination, and take into account the adverse effects of the individual drugs and the clinical status of the patient.

The majority of combination antifungal studies that have been published to date are *in vitro* studies. *In vitro* studies are designed to show **synergy** (the combined effect of two separate drugs is greater than predicted when two agents are used in combination), **antagonism** (the combined effect of the drugs is less than predicted for the single most active agent alone), **additivity** (the combined effect is equal to the sum of the separate effects), or **indifference** (no additional effects observed over that of the most active drug).^{5,6} The results of *in vitro* studies are dependent on the fungal strain studied, the testing conditions, and the test method used. Since there is no standardized method of performing antifungal combination testing, the detection of conflicting results is not surprising.

The most common ways to test for synergy are the checkerboard method, the time-kill method, and the epsilometer strip test (E-test).² The checkerboard method determines the minimum inhibitory concentrations (MIC) for the organism by using a set of wells with serial microdilutions of the two drugs in question. The resulting MICs from the checkerboard test are used to calculate the fractional inhibitory concentration index (FICI). It has been proposed that a FICI <0.5 represents synergy, a FICI >4 represents antagonism, and a FICI 0.5–4 represents no interaction (additivity or indifference);² however, these divisions are not universally accepted and many authors choose different limits when interpreting FICI test results. The checkerboard method is easy to set up and interpret, but doesn't take into account the pharmacodynamics of the different drugs² or the differences in visual MIC endpoints for different agents.⁵ For the time-kill method, a standardized cell suspension is added to different drug combination concentrations and looks at the changes in antifungal activity over time. For each dilution, the cells are plated, incubated, and allowed to grow. The colony forming units (CFU) per milliliter of each dilution and incubation time are plotted to make a time-kill curve. Synergy and antagonism are determined by comparing the curve of the combination to those of the individual drugs. Time-kill studies take more time to perform and the calculations of CFU may be compromised by the filamentous growth of fungus. They are, however, better able to account for pharmacodynamic interactions of the combinations. Finally, the epsilometer test (E-Test) uses a calibrated strip containing different concentrations of antifungals. The MICs of the fungi are determined by where the zone of inhibition crosses the strip. The MIC of the combination is compared to those of single agents. The E-test is easy to perform, but also has limitations, including interpretation difficulties due to the inconsistent growth patterns of fungi.² Despite the limitations, the checkerboard method is the most widely used method of *in vitro* synergy testing.

As mentioned above, *in vitro* results vary greatly depending on the method, species, and strain tested. Table II consolidates the results from studies using checkerboard, microdilution, or macrodilution methods. Combinations that were shown to be predominately synergistic or additive by *in vitro* methods other than checkerboard or dilution, include amphotericin B with caspofungin (*Cryptococcus* spp.),⁷ amphotericin B with flucytosine (*Candida* and *Cryptococcus* spp.),^{8,9} and caspofungin with fluconazole (*Cryptococcus* spp.).⁷ Due to the variability between *in vitro* studies, however, it is important to note that *in vitro* antifungal combination data may not always correlate with *in vivo* results.

Animal and human studies are used to validate the usefulness of antifungal combinations. Combinations that are additive or synergistic *in vivo* are found in Table III. Many of the animal studies focus on tissue fungal burden, tissue sterilization, and survival.² Animal models have drawbacks, including failure to mimic the disease pathogenesis as seen in humans, being underpowered to detect efficacy, inter-study discrepancies in methods and models, and a lack of assurance of comparable pharmacokinetic parameters.⁵ While well-

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Table II. Consolidated Results of Combination Antifungal Regimens Studied *In Vitro* Via Checkerboard and Comparable Dilution Methods (Based on MICs)¹⁹⁻³¹

CANDIDA	Caspofungin	Fluconazole	Flucytosine	Itraconazole	Voriconazole
Amphotericin B	++	±	~50% synergistic, 50% indifferent ²³	0	±
Caspofungin		0	0	0	0
Fluconazole			±	0	0
Flucytosine				0	±
Itraconazole					0

CRYPTOCOCCUS	Caspofungin	Fluconazole	Flucytosine	Itraconazole	Voriconazole
Amphotericin B	0	+	++	+	0
Caspofungin		0	++	0	0
Fluconazole			++	0	0
Flucytosine				++	++
Itraconazole					0

ASPERGILLUS	Caspofungin	Fluconazole	Flucytosine	Itraconazole	Voriconazole
Amphotericin B	+	±	±	One study showed 40% synergy, 40% indifference; ²² another showed 34% indifference, 14% synergy. ²⁶	0
Caspofungin		0	++	++	One study showed 100% additivity; ³¹ another showed 87.5% synergy. ²⁶
Fluconazole			0	±	0
Flucytosine				0	-
Itraconazole					0

Key to Table II:

- ++ Synergy was shown for the majority of isolates
- + Additivity was shown for the majority of isolates
- ± Indifference was shown for the majority of isolates
- Antagonism was shown for the majority of isolates
- 0 Not reported

Amphotericin B with flucytosine against cryptococcal meningitis is the only antifungal combination that is standard of care.

compared fluconazole to amphotericin B in combination with fluconazole.¹⁰ This study showed that although the time to failure between the two groups was not statistically different, the combination group trended towards more successful therapy and faster clearance of the infection from the blood. Both groups had similar mortality rates; however, adverse events were higher with the combination therapy. Case reports of the successful treatment of candidiasis using fluconazole combined with flucytosine have also been published.¹¹

Most of the clinical data for *Cryptococcus neoformans* have assessed the efficacy of amphotericin B combined with flucytosine. Due to the trend toward better efficacy when compared to monotherapy, this combination has become the standard of care for cryptococcal meningitis.¹² One of the studies involving non-HIV infected patients found that the combination of amphotericin B (0.3mg/kg/day) with flucytosine (150mg/kg/day) produced a higher cure rate and faster rate of cerebrospinal fluid sterilization compared to amphotericin B monotherapy.¹³ Nephrotoxicity was less with the combination, however mortality rates were similar between both groups. A trial of AIDS patients with cryptococcal meningitis evaluated the combination of amphotericin B (0.7mg/kg/day) and flucytosine (100mg/kg/day) compared to amphotericin B monotherapy.¹⁴ At the end of two weeks, more patients in the combination group had negative cerebrospinal fluid cultures which correlated with lower intracranial pressures. Of the 21 patients who died during the trial, 14 had intracranial pressures measured and 13 had high intracranial pressures; however, overall mortality was not significantly different between the monotherapy and combination regimen groups. Another study also did not

designed human trials are needed to definitively establish the best antifungal combination therapies to use clinically, few such studies exist. Current human data mainly exists in the form of case reports and small, retrospective, single-institution studies. Clinical studies may be difficult to complete due to low rates of enrollment, the long follow-up necessary to confirm clinical cure, and the comorbidities and other confounding variables associated with human invasive fungal infections.⁵ Many of these clinical studies are underpowered to be able to demonstrate a difference in survival, and several of the *Candida* and *Cryptococcus* clinical studies used tissue sterilization or other measures as surrogate markers for clinical outcomes. Although many antifungal combinations have demonstrated good clinical responses, randomized, prospective trials, especially with high-risk patients and with newer agents, need to be performed to better establish the comparative efficacy of combination antifungals for invasive fungal infections.

One large, randomized study in non-neutropenic candidemia patients com-

Table III: Consolidated Results of Combination Antifungal Regimens Studied *In Vivo*^{2,10-18,31-38}

Combination Regimen		CANDIDA		CRYPTOCOCCUS		ASPERGILLUS	
		Animal	Human	Animal	Human	Animal	Human
Amphotericin B	Caspofungin	++					++ / +
	Fluconazole	+	++ / +	++			
	Flucytosine	++ / +		+	++	±	
	Itraconazole	±		+ / ±		±	++
	Voriconazole			±			
Caspofungin	Voriconazole			+		++	++
Fluconazole	Flucytosine	++ / ± (dose dependent)	++ / +	One study showed 59% antagonism; another showed 66% synergy	++	±	
	Itraconazole	++		±		±	
	Voriconazole			±			
Flucytosine	Itraconazole			+			
	Voriconazole			+			

Key: (++) Synergistic; (+) Additive; (±) Indifferent; Blank cell = No data

Based on clinical data, antifungal combinations associated with successful responses in humans include fluconazole with amphotericin B or flucytosine against candidiasis; amphotericin B with flucytosine against cryptococcosis; and caspofungin with voriconazole or amphotericin B, and amphotericin B with itraconazole in aspergillosis patients.

Primary antifungal therapy should be monotherapy; however, combination therapy may be warranted if the infection progresses.

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associated with monotherapy.¹⁶ Another study evaluating amphotericin B in combination with itraconazole showed that patients receiving the combination had a higher cure rate than those on monotherapy.² When the combination of voriconazole with caspofungin was compared to liposomal amphotericin B with caspofungin in six leukemia patients, all patients showed improvement and no toxicities were observed in either group.² A retrospective chart review in patients with *Aspergillus* that was clinically refractory to amphotericin B showed that the combination of caspofungin and amphotericin B led to increased survival.¹⁷ In yet another study, patients with presumed invasive aspergillosis who failed liposomal amphotericin B monotherapy were given the combination of liposomal amphotericin B and caspofungin.¹⁸ While the overall response rate for the combination was 42%, many of the patients lacked documentation of *Aspergillus* infection. In patients with documented aspergillosis, the response rate was only 18%.

For a practitioner faced with the challenge of treating a patient with an invasive fungal infection, deciphering all the data on antifungal therapy and deciding on a regimen can be an overwhelming task. Except for cryptococcal meningitis where the standard of care is a combination regimen of amphotericin B with flucytosine, initial therapy of invasive fungal infections should be monotherapy. For patients with serious disseminated infections that continue to progress on monotherapy, combining two antifungal agents that have demonstrated synergy or additivity should be considered. Based on the preponderance of data, reasonable antifungal combinations against *Candida* spp. include amphotericin B with caspofungin, fluconazole, or flucytosine, and fluconazole with flucytosine. Besides the standard combination of amphotericin B with flucytosine, other sensible combinations against *Cryptococcus neoformans* include amphotericin B with fluconazole or itraconazole, and fluconazole with flucytosine. Promising combinations against *Aspergillus* spp. include amphotericin B with caspofungin or itraconazole, and caspofungin with voriconazole. When an antifungal combination is indicated, it should involve two drugs with different mechanisms of action, be based on historical data with the combination, and take into account the adverse effects of the individual drugs and the clinical status of the patient.

show any improvement in survival when flucytosine was added to amphotericin B.¹⁵ The combination of fluconazole with flucytosine has also been used in the treatment of cryptococcal meningitis in AIDS patients.¹² Due to the increased toxicity of this regimen, however, it should only be an alternative to standard therapy.

Several clinical studies involving combination antifungals in *Aspergillus*-infected patients have been published recently. A retrospective review compared voriconazole combined with caspofungin to voriconazole alone in patients who had failed amphotericin B therapy and reported a 3-month overall survival rate in patients on the combination that was significantly greater than that asso-

References available on request or at <http://uw.pnrx.org/therapyTopics.asp>

Pharmacy & Therapeutics Committee Actions

Formulary Additions	Dosage Form & Strength	Therapeutic Classification	Use	Usual Adult Starting Dose*
Fenofibrate (Liofibra)	Tablet, micronized: 67mg, 134mg, 200mg	Antihyperlipidemic	Hypercholesterolemia; hypertriglyceridemia	Condition dependent.
	<p>Note: Added to Formulary with automatic substitution of the micronized capsule formulation (M) for the nonformulary nanoparticle tablet (N) or discontinued tablet (T) formulations of fenofibrate allowed as follows: 67mg (M) = 54mg (T) = 48mg (N); 134mg (M) = 108mg (T) = 96mg (N); 200mg (M) = 201mg (M) = 160mg (T) = 145mg (N).</p>			
Calcium carbonate and calcium citrate tablets	Calcium carbonate: 1500mg (600mg <i>elemental</i> calcium)	Mineral	Calcium Supplement	See note below.
	Calcium citrate: 950mg (200mg <i>elemental</i> calcium)			
<p>Note: Calcium supplementation is recommended in doses of 1200-1500mg/day of <i>elemental</i> calcium for men >65 years old and for postmenopausal women not taking HRT. Since most calcium supplements contain relatively small amounts of <i>elemental</i> calcium (see <i>UW Medicine Drug Formulary</i>), patients typically must take multiple tablets each day. Calcium carbonate requires an acidic environment for absorption, and thus should be taken with food. In contrast, calcium citrate is best taken on an empty stomach.</p>				
Formulary Deletions	Dosage Form(s), Strength(s)	Therapeutic Classification	Use	Comment
Calcium carbonate and calcium gluconate tablets	Calcium carbonate: 650mg	Mineral	Calcium Supplement	See note below.
	Calcium gluconate: 500mg			
<p>Note: Replaced by 1500mg calcium carbonate with automatic substitution by pharmacy at the nearest equivalent elemental calcium dose.</p>				
Calcium citrate + cholecalciferol (Citracal + D)	All dosage forms/strengths	Mineral + Vitamin D	Dietary Supplement	See note below.
	<p>Note: Replaced by calcium citrate without vitamin D with automatic substitution by pharmacy at the equivalent elemental calcium dose; however, the maximum dose of cholecalciferol will be 400 units/day</p>			

* Refer to product labeling for full prescribing information.

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