The incidence of invasive aspergillosis (IA) [1] and its financial burden [2] have increased significantly in the last several decades with a parallel increase in the number of immunocompromised patients. The substantial increase in patients at risk for developing IA is due to the development of new intensive chemotherapy regimens, increased use of high-dose corticosteroids, worldwide increase in solid organ and bone marrow transplantation, and increased use of immunosuppressive regimens for autoimmune diseases [3]. The mortality rate associated with untreated IA is nearly 100% in some patient groups, and the overall survival rate among patients treated with amphotericin B is ~34% [4, 5].

There has been a recent surge in the development of newer antifungals, including new formulations of older drugs (i.e., cyclodextrin-itraconazole, liposomal nystatin, and PEG-amphotericin B) and entirely new classes of drugs with novel targets. Newer strategies have also been explored with combination antifungal therapies, as well as cytokine therapy, with or without antifungals, to ameliorate the host response system. The therapeutic options for IA have so markedly increased in the last few years that clinicians need to be aware of both the proven therapies of today and the anticipated treatments of tomorrow.

We reviewed available reports on the experimental and clinical investigations of newer IA therapy. Although a discussion of every prospective antifungal tested in the last few years is beyond the scope of any single review, we present the agents with the most promise for clinical use against IA and those undergoing clinical trials. The discussion does not cover empirical therapy for continued fever, the numerous fungal prophylaxis strategies in use, or the early diagnostic techniques for IA still in their infancy. New combination therapies are analyzed in a devoted and more thorough discussion of combination therapy for IA elsewhere [6] in this issue of Clinical Infectious Diseases. Our focus is treatment of suspected or proven IA, without reiteration of the existing 2000 Infectious Diseases Society of America Aspergillus guidelines [7]; instead
we consider the many recent advances and future trends in the treatment of IA.

OVERVIEW OF ANTIFUNGAL CLASSES

Because this recent increase in the development of antifungals has created new hope to combat disappointing mortality figures, clinicians need to be updated as to the agents now available in the expanding armamentarium and those in development (table 1). Until recently, there were only 2 agents with activity against *Aspergillus*, amphotericin B deoxycholate and itraconazole, and those compounds were often ineffective and frequently toxic or unpredictable [4]. A recent survey of clinicians experienced in the treatment of IA found that most used amphotericin B monotherapy for their most immunosuppressed patients, whereas itraconazole alone or amphotericin B followed by itraconazole was used for less-immunosuppressed patients [8].

**Polyenes.** The oldest antifungal class is the polyene macrolides, amphotericin B and nystatin, which bind to ergosterol, the major sterol found in fungal cytoplasmic membranes. This binding creates channels in the cell membrane that increase permeability and cause cell death through leakage of essential nutrients. The fungicidal activity is believed to be due to the lack of a barrier to essential nutrients and ions [9, 10]. These drugs also have oxidant activity and disrupt cellular metabolism in the fungal cell [11].

**Azoles.** Theazole antifungals are heterocyclic synthetic compounds that inhibit the fungal cytochrome P-450 14DM (also known as lanosterol 14α-demethylase), which catalyzes a late step in ergosterol biosynthesis. The drugs bind through a nitrogen group in their 5-membered azole ring to the heme group in the target protein and block demethylation of the 14C of lanosterol, leading to substitution of methylated sterols in the membrane and depletion of ergosterol. The result is an accumulation of precursors with abnormalities in fungal membrane permeability, membrane-bound enzyme activity, and coordination of chitin synthesis [12, 13].

The azoles are subdivided into imidazoles and triazoles on the basis of the number of nitrogens in theazole ring [9], with the structural differences resulting in different binding affinities of theazole pharmacophore for the cytochrome P-450 enzyme system. With the exception of ketoconazole, the imidazoles are limited to superficial mycoses, and none, including ketoconazole, have activity against *Aspergillus*. Of the older first-generation triazoles, fluconazole is ineffective against *Aspergillus* and itraconazole has unpredictable bioavailability in some patients. Newer second-generation triazoles (voriconazole, posaconazole, and ravuconazole) are modifications of itraconazole or fluconazole with activity against *Aspergillus*, and they generally have lower MICs than do the older compounds [14].

**Echinocandins.** For years, most development of new systemic antifungals focused on chemically modifying existing classes [15]. An entirely new class of antifungals, the echinocandins and the amino-containing pneumocandin analogues, are cyclic hexapeptide agents that interfere with cell wall biosynthesis by noncompetitive inhibition of 1,3-β-glucan synthase, an enzyme present in fungi but absent in mammalian cells [12, 14]. This 1,3-β-glucan, an essential cell wall polysaccharide, forms a fibril of 3 helically entwined linear polysac-

---

**Table 1. Selected antifungal agents with activity against *Aspergillus*.**

<table>
<thead>
<tr>
<th>Drug class, drug name (brand/investigational name)</th>
<th>Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Polyene</strong></td>
<td></td>
</tr>
<tr>
<td>Amphotericin B deoxycholate (Fungizone)a</td>
<td>Intravenous</td>
</tr>
<tr>
<td>Amphotericin B lipid complex (Abelcet)a</td>
<td>Intravenous</td>
</tr>
<tr>
<td>Amphotericin B colloidal dispersion (Amphocil; Amphotec)a</td>
<td>Intravenous</td>
</tr>
<tr>
<td>Liposomal amphotericin B (AmBisome)a</td>
<td>Intravenous</td>
</tr>
<tr>
<td>Liposomal nystatin (Nyotran)</td>
<td>Intravenous</td>
</tr>
<tr>
<td><strong>Triazole</strong></td>
<td></td>
</tr>
<tr>
<td>Itraconazole (Sporanox)a</td>
<td>Oral, intravenous</td>
</tr>
<tr>
<td>Voriconazole (VFend)a</td>
<td>Oral, intravenous</td>
</tr>
<tr>
<td>Posaconazole (SCH 56592)</td>
<td>Oral</td>
</tr>
<tr>
<td>Ravuconazole (BMS-207147; ER-30346)</td>
<td>Oral</td>
</tr>
<tr>
<td><strong>Echinocandin</strong></td>
<td></td>
</tr>
<tr>
<td>Caspofungin (Cancidas)a</td>
<td>Intravenous</td>
</tr>
<tr>
<td>Anidulafungin (VER-002; LY303366)</td>
<td>Intravenous</td>
</tr>
<tr>
<td>Micafungin (FK463)</td>
<td>Intravenous</td>
</tr>
</tbody>
</table>

*a* Licensed for clinical use in United States.
charides and provides structural integrity for the fungal cell wall [16, 17].

Echinocandins inhibit hyphal tip and branch point growth, converting the mycelium to small clumps of cells, but the older septated cells with little glucan synthesis are not killed [15]. Therefore, the echinocandin in vitro activity end point is morphological change, not clearing of the medium. Echinocandins are generally fungicidal in vitro, although not as rapidly as amphotericin B [14, 18], but appear to be more fungistatic against Aspergillus [19]. As a class, these agents are not metabolized through the cytochrome P-450 enzyme system but through a presumed O-methyltransferase, lessening some of the drug interactions and side effects seen with the azole class.

Although echinocandin B was first described in 1974 as a natural product of Aspergillus nidulans [20], drug development in this class has only recently expanded. Cilofungin, the first echinocandin B, had activity in a murine model of IA [21], but clinical investigation was discontinued because of toxicity of the vehicle, polyethylene glycol [22]. Three new compounds in this class (caspofungin, micafungin, and anidulafungin) are in the final stages of clinical trials, including February 2001 US Food and Drug Administration (FDA) approval of caspofungin for refractory IA.

Allylamines. The allylamine class of antifungals [23], which includes terbinafine, inhibits the enzyme squalene epoxidase in the fungal biosynthesis of ergosterol [24] and is currently indicated for the treatment of superficial dermatophyte and yeast infections. In vitro studies have shown terbinafine to have fungicidal action [25] against Aspergillus similar to that of amphotericin B or itraconazole [26]. Limited reports show some efficacy for IA, and combination therapy remains a possibility.

Nikkomycins. The naturally occurring nikkomycins are nucleoside-peptide antibiotics that inhibit the synthesis of cell wall chitin, a polysaccharide found in most fungi but not in mammalian cells, as competitive analogues of the substrate UDP-N-acetylglucosamine for the enzyme chitin synthase [14, 27]. The effect is analogous to the action of the β-lactam antibiotics on bacteria cell walls, leading to osmotic lysis [28]. Nikkomycins were first described as a class of drugs in 1976, isolated as a result of a program to discover fungicides for agricultural use [29]. The antifungal activity of nikkomycin Z against opportunistic fungi has been reported to be low in many experimental fungal models, including ineffective growth inhibition against Aspergillus fumigatus [28, 30–32], leaving combination therapy as a potential option.

Other investigational antifungal classes. Protein synthesis is an attractive target for antibacterials, but that action is hampered in antifungals because of the eukaryotic nature of fungi and thus similarity between fungal and mammalian protein synthesis. Most previous targets used for antifungals exploit differences in the 2 cells, for example ergosterol, 1,3-β-glucan, or chitin [33]. The new sordarin class affects the translocation step of the elongation cycle of protein synthesis [34] by inhibiting elongation factor 2, an essential factor for fungal protein synthesis, and halting the addition of amino acid residues to the growing peptide chain [33].

The sordarins have minimal in vitro activity against Aspergillus [35] and in a disseminated murine model have shown an irregular response and poor survival efficacy [36]. With such a limited protective effect against IA, this class of antifungals was pursued for clinical use for other fungal infections, but IA combination therapy studies remain to be done. Further investigation of this class for antifungal use is presently suspended (F. Gomez de las Heras, personal communication).

NC1175 is an α,β-unsaturated ketone and is fungicidal, although only at concentrations much higher than those of amphotericin B or itraconazole. In vitro data show that overall, NC1175 is less effective than amphotericin B or itraconazole against susceptible A. fumigatus isolates. However, there was demonstrated activity against both amphotericin B–resistant isolates and itraconazole-resistant isolates. When tested with a murine model of IA, the survival rate of animals treated with NC1175 was not significantly better than that of control subjects; however, the fungal load was reduced significantly at a rate ∼15% less than that of amphotericin B [37]. Toxicity is a concern, because NC1175 can act as an alkylating agent, but it may serve as a prototypical molecule for further development to obtain greater potency and selectivity.

Partricins are semisynthetic polyenes with in vitro and in vivo activity against other fungi. SPA-S-753 (Societa Prodotti Antibiotici) is a derivative of partricin A and, unlike amphotericin B, is water soluble. There have been in vivo reports of lower animal toxicity when SPA-S-753 was administered intraperitoneally (ip) [38–40] and substantially less when given intravenously (iv) [41]. Additionally, in vivo studies have noted increased [39] or equivalent efficacy [41] of SPA-S-753 compared with that of amphotericin B in other fungal models. Another partricin, SPA-S-843, showed generally 2-fold–lower MICs compared with those of amphotericin B for 13 Aspergillus isolates but similar fungicidal activity [42]. Although no work has yet examined IA models, these in vitro observations warrant further development.

The pradimicins and structurally similar benanomycins disrupt the fungal cell membrane by binding to the terminal D-mannosides. They have shown some in vitro [43] and in vivo [44] activity against Aspergillus organisms, but further study is needed to investigate potential use as monotherapy or combination therapy for IA.
ANTIFUNGAL SUSCEPTIBILITY TESTING AND RESISTANCE

Preceding any discussion of the in vitro results with newer antifungals has to be analysis of antifungal testing. However, before reviewing antifungal testing, one must evaluate the methodology of the available data. Studies have used diverging and imprecise definitions, parameters for estimation of growth inhibition or measurement, and in vitro conditions. This nonstandardized methodology plagued early antifungal susceptibility testing, and these inconsistencies led to conflicting reports. In vivo testing is therefore preferred, because of a more predictive nature of human pharmacokinetic effects and toxicities.

The increasing frequency of fungal infections has prompted better testing in clinical practice and research as well as correlation between in vitro data and in vivo results [45, 46]. In vitro antifungal susceptibility testing itself has limited utility if it cannot predict patient outcome. However, clinical relevance might best be related to patient factors (e.g., recovery of neutropenia, cessation of glucocorticoid therapy) and not intrinsically related to the susceptibility of the fungus itself. Nevertheless, isolates with varying MICs have been found, and there has been a correlation with in vivo outcome, suggesting clinical significance.

Amphotericin B susceptibility testing can be of limited clinical value; one study was unable to correlate mouse model outcomes with MICs, despite testing 32 different formats with varying media, inoculum sizes, incubation temperatures, and incubation times [47]. However, another study retrospectively examined Aspergillus isolates from 29 patients, and all 6 infections with fungi susceptible to amphotericin B were treated successfully, whereas 22 of 23 patients with isolates resistant to amphotericin B (MIC >2 μg/mL) died despite standard treatment. The generally amphotericin B–resistant Aspergillus terreus isolates required considerably higher MICs, and all of those patients died; MICs for A. fumigatus and Aspergillus flavus were lower, but strains from patients who died required MICs of >2 μg/mL [48]. Another study, with 6 A. fumigatus patient isolates and itraconazole [49], was readily able to differentiate the susceptible and resistant strains by use of both in vitro conditions and in vivo models, highlighting a direct clinical correlation between laboratory results and patient outcome.

A number of factors contribute to clinical efficacy, including the complex interaction between fungal virulence, pharmacokinetics, and availability of antifungals at the site of infection; intrinsic or acquired fungal resistance; and the host immune condition. Resistance to antifungals is becoming more common among Aspergillus species, and the clinician needs to be alert to resistance as a possible reason for clinical failure. Several years ago, the first isolates of A. fumigatus resistant to itraconazole were reported from 2 patients; MICs correlated with poor clinical outcome and were consistent with those determined in a murine model. Two proposed possibilities for resistance included changes in the target enzyme, sterol 14α-demethylase, or alteration in a membrane transporter [50].

Additional studies found the frequency of itraconazole resistance to be low (<2%) in an in vitro analysis of 230 Aspergillus isolates [51]. In vitro susceptibility of respiratory Aspergillus samples revealed 3 patients with acquired itraconazole resistance after long-term therapy [52]. However, a recent review of 593 Aspergillus isolates from a major medical center found a steady increase in isolation of Aspergillus over a 5-year period but no increase in antifungal resistance [53]. Cross-resistance among the azole class, including the 3 newer triazoles, has emerged in vitro [54]. An in vitro study of 17 A. fumigatus isolates noted 11 itraconazole-resistant isolates, with a concurrent elevation of posaconazole MICs but no elevation of voriconazole or ravuconazole MICs. The susceptibility patterns to voriconazole and ravuconazole suggest similar mechanisms of action and resistance, and the susceptibility patterns to posaconazole and itraconazole do the same. Interestingly, the lowest voriconazole and ravuconazole MICs were seen for highly itraconazole-resistant isolates [55].

Standardization and testing of filamentous fungi such as Aspergillus are still in the preliminary stages [56, 57]. Only recently did the NCCLS publish accepted standard conditions for molds (M38-A), specifying conidial suspension and MIC end point criteria [58]. Previous studies used an extrapolation of the M27-A criteria for yeast testing [59]. Additional studies have compared visual versus spectrophotometric MIC determination with use of the broth microdilution standards [60] or E-test (AB Biodisk) as an alternative to broth microdilution [61].

Even standardized antifungal testing is not without its pitfalls. For instance, the NCCLS in vitro susceptibility testing method [57] for filamentous fungi cannot clearly identify amphotericin B–resistant A. fumigatus isolates but can identifyazole-resistant Aspergillus isolates [62]. Echinocandin activity on Aspergillus does not give classic MICs in vitro by use of broth dilution techniques, but echinocandins do demonstrate clear morphological inhibition in vitro [63].

One potentially important distinction between antifungals is whether their action is fungicidal or fungistatic. The difficulty lies in testing and interpretation; a drug may be fungicidal or fungistatic depending on laboratory variables, such as media used, duration of incubation, drug concentration, or inoculum size. Drugs determined to be fungistatic in vitro may be fungicidal in vivo in cooperation with host cells. Conversely, drugs fungicidal in vitro may be fungistatic in vivo because of tissue penetration, binding, or pH.

Because one of the primary host defenses, neutrophils, is absent in neutropenic patients, an antifungal with fungicidal activity may prove to be crucial for patient survival, analogous to the need for bactericidal agents in neutropenia [64].
gistatic activity is reversible and does not clear the organism; once the drug is removed, the organism recovers rapidly and functions normally [10]. With a fungistatic agent, a patient with an impaired immune system will be unable to clear non-growing organisms, so relapse can occur after cessation of treatment.

SPECIFIC ANTIFUNGALS

Polyenes

**Amphotericin B deoxycholate.** Amphotericin B is clearly not a newer antifungal—since its initial approval for use in 1958, it has remained the reference standard for treatment of IA [7] as well as the standard of comparison for all newer antifungal agents. However, the fact that amphotericin B remains at such a post is not by virtue of its effectiveness but rather because alternatives were lacking, until recently [65]. Amphotericins A and B are natural fermentation products of a soil actinomycete collected in Venezuela in 1953, but although each has antifungal properties, amphotericin A was not developed [66]. Amphotericin B is so named because it is amphoteric, forming soluble salts in both acidic and basic environments [67]. However, because of its insolubility in water, amphotericin B for clinical use is actually amphotericin B mixed with the detergent deoxycholate in a 3:7 mixture [67, 68]. The lipophilic amphotericin B acts by preferential binding to fungal membrane ergosterols, creating transmembrane channels that cause an increased permeability to monovalent cations. It also inhibits proton ATPase pumps, depletes cellular energy reserves, and promotes lipid peroxidation, to result in an increase in membrane fragility and ionized calcium leakage [68].

The drug is released from its carrier and distributes very efficiently (>90% of the drug is distributed) with lipoproteins, taken up preferentially by organs of the mononuclear phagocytic system. Amphotericin B has an apparent volume of distribution of 4 L/kg; distribution follows a 3-compartment model, with high concentrations reaching the liver, spleen, and lungs. CSF values are only 2%–4% of serum concentrations [69]. There is an initial 24- to 48-h half-life, reflecting uptake by host lipids, very slow release and excretion into urine and bile, and then metabolism and a subsequent terminal half-life of up to 15 days [70]. Experimental in vitro and in vivo studies support concentration-dependent killing with a prolonged post-antifungal effect, suggesting that large daily doses will be most effective and that achieving optimal peak concentrations is important [18]. Serum levels of amphotericin B are proportional to doses administered but reach a plateau at the third consecutive day of a constant dose [67]. Peak levels are achieved 1 h after the end of a 4-h infusion, and there is a relationship between total dose administered and tissue concentrations, suggesting a progressive accumulation with continued drug administration [71]. Other authors point out the scant support for higher doses of amphotericin B [72], emphasizing the varying experimental conditions in published studies and no evidence of a clinical dose effect [73] to support such higher doses.

The recommended dosage of amphotericin B for IA is 1.0–1.5 mg/kg/day, and optimal duration of therapy is unknown but largely dependent on underlying disease, extent of the patient’s IA, resolution of neutropenia, lessening immunosuppression, and the return of graft function following bone marrow or organ transplantation [7, 19]. There is no total dose of amphotericin B recommended, and the suggested key to success is to give a high dose in the first 2 weeks of therapy and to change to another treatment if amphotericin B therapy fails [3].

Tolerance of amphotericin B is limited by its acute and chronic toxicities. In addition to fungal ergosterol, the drug also interacts with cholesterol in human cell membranes, which likely accounts for its toxicity [74]. Up to 80% of patients receiving amphotericin B develop either infusion-related toxicity or nephrotoxicity [67], especially with concomitant therapy with nephrotoxic drugs such as aminoglycosides, vancomycin, cyclosporine, or tacrolimus [75–77]. Renal function usually returns to normal after cessation of amphotericin B, although permanent renal impairment is common after larger doses [65]. Risk factors associated with amphotericin B–related nephrotoxicity include a total dose of >4 g, sodium depletion, age >30 years, and concomitant therapy with nephrotoxic drugs [77]. Sodium supplementation has decreased nephrotoxicity, and it has been recommended to infuse 500 mL of 0.9% saline before administration of amphotericin B [65, 78].

**Amphotericin B lipid formulations.** In addition to conventional amphotericin B, 3 fundamentally different lipid-associated formulations have been developed that offer the advantage of an increased daily dose of the parent drug, better delivery to the primary reticuloendothelial organs (the lungs, liver, and spleen) [79, 80], and reduced toxicity. The US FDA approved amphotericin B lipid complex (Abelcet; Enzon) in December 1995, amphotericin B colloidal dispersion (Amphotec; AstraZeneca or Amphotech; Interune Pharmaceuticals) in December 1996, and liposomal amphotericin B (AmBisome; Fujisawa Healthcare) in August 1997 [81]. The different pharmacokinetics and toxicities of the lipid formulations are reflected in the dosing recommendations: amphotericin B lipid complex is recommended at 5 mg/kg/day, amphotericin B colloidal dispersion at 3–5 mg/kg/day, and liposomal amphotericin B at 1–5 mg/kg/day. Most clinical data have been obtained with the use of these preparations at 5 mg/kg/day. Animal studies clearly indicate that on a similar dosing schedule, the lipid products are almost always not as potent as amphotericin B, but the ability to safely administer higher daily doses of the parent drug improves their efficacy [69].

Amphotericin B lipid complex is a tightly packed ribbon-
like structure of a bilayered membrane formed by combining dimyristoyl phosphatidylcholine, dimyristoyl phosphatidylglycerol, and amphotericin B in a ratio of 7:3:3. Amphotericin B colloidal dispersion is composed of disk-like structures of cholesterol sulfate complexed with amphotericin B in an equimolar ratio. Liposomal amphotericin B, the only true liposomal product, consists of small uniformly sized unilamellar vesicles of a lipid bilayer of hydrogenated soy phosphatidylcholine–distearyl phosphatidylglycerol–cholesterol–amphotericin B in the ratio 2:0.8:1:0.4 [82, 83].

According to the US FDA, all 3 lipid formulations are currently indicated for patients with systemic mycoses, primarily IA, who are intolerant of or have infections refractory to conventional amphotericin B, which could be defined as follows: development of renal dysfunction (serum creatinine level ≥2.5 mg/dL) during antifungal therapy; severe or persistent infusion-related adverse events despite premedication or comedication regimens; and disantifungal therapy; severe or persistent infusion-related adverse events. There was a nonlinear plasma pharmacokinetic profile, with a maximal concentration at 10 mg/kg/day, found no demonstrable dose-limiting nephrotoxicity or infusion-related toxicity. There was a nonlinear plasma pharmacokinetic profile, with a maximal concentration at 10 mg/kg/day, but the study was not statistically powered to determine dose-dependent efficacy [87]. In another study, liposomal amphotericin B at 10 mg/kg for 4 days followed by 3 mg/kg/day significantly increased survival (76% vs. 45% survival) and reduced chitin content in rat lungs compared with results with amphotericin B at 1 mg/kg/day [88]. However, a randomized trial of 87 patients found no clinical difference between liposomal amphotericin B at 1 or 4 mg/kg/day [89].

Complexation with lipids appears to stabilize amphotericin B in a self-associated state, so that it is not available to interact with the cholesterol of human cellular membranes, the presumed major site of toxicity [83, 90]. Another theory for the decreased nephrotoxicity of lipid formulations is the preferential binding of its amphotericin B to serum high-density lipoproteins, unlike conventional amphotericin B’s binding to low-density lipoproteins [91]. The high-density lipoprotein–bound amphotericin B appears to be released to the kidney more slowly or to a lesser degree. Amphotericin B lipid complex was 10-fold less nephrotoxic in mice than was amphotericin B [92], and lipid formulations have resulted in renal improvement in patients with amphotericin B–induced or pre-existing renal insufficiency [77, 93, 94]. For infusion-related toxicity, there is a general agreement that liposomal amphotericin B has less toxicity than amphotericin B lipid complex, whereas amphotericin B colloidal dispersion appears closer in toxicity to conventional amphotericin B [95, 96].

There are no data or consensus opinions among authorities indicating improved efficacy of any new amphotericin B lipid formulation over conventional amphotericin B [77, 79, 81, 95, 97]. One exception is that liposomal amphotericin B has shown less frequent infusion-related adverse events than have the other lipid formulations and conventional amphotericin B [81, 98]. This leaves the clearest indication for a lipid formulation over conventional amphotericin B to be reducing glomerular toxicity. However, mortality was slightly lower in patients treated with liposomal amphotericin B than in those treated with conventional amphotericin B in a study of a small number of patients with documented IA [99]. Another retrospective study with amphotericin B colloidal dispersion showed response rates and survival rates higher than with conventional amphotericin B; however, baseline differences in neutropenia in the 2 groups could confound survival rate findings [100].

**Newer amphotericin B formulations.** A new formulation of liposomal amphotericin B is complexed to a hydrophilic phospholipid derivative of polyethylene glycol 1900 (PEG). This creates a hydrophilic coating on the surface of the liposomes, which reduces blood protein binding and uptake by the mononuclear phagocyte system, achieving a long blood residence time of the liposomes [101]. Superior efficacy of the PEG formulation of amphotericin B over liposomal amphotericin B was demonstrated in mice with IA [102]. A single dose of PEG-liposomal amphotericin B gave results similar to those with a 10-day course of amphotericin B, although it showed no sig-
significant improvement in mortality over amphotericin B, and increased doses of PEG-liposomal amphotericin B offered no increased efficacy [101].

Another new formulation of amphotericin B in cholesterol hemisuccinate vesicles uses cholesterol, linked to a stabilizing hydrophilic anchor such as succinate, to form bilayer vesicles similar to liposomes with phospholipid vesicles. One murine study saw higher therapeutic efficacy than that of amphotericin B and efficacy and toxicity similar to those of the lipid formulations [103]. Amphotericin B Hydrosomes (Access Pharmaceuticals) are a novel formulation of hydrophilic, heparin-surfaced nanoparticles containing amphotericin B designed to target infected sites by local adhesion. The beef-lung heparin coats the core amphotericin B and converts the amphotericin amphotericin B into a completely hydrophilic formulation [104]. Amphotericin B Hydrosomes are estimated to be 7-fold less toxic than amphotericin B in a murine model [105], but there are no published clinical reports.

Aerosolized amphotericin B. Aerosolized amphotericin B was first mentioned in 1959 [106] as a solution to amphotericin B’s known distribution to the liver, spleen, and kidneys [71], leading to both toxicity and potential clinical failure for pulmonary IA. Aerosolization of amphotericin B is possible with various nebulizers [107], and in animals, pharmacokinetics show much higher concentrations of the drug in the lungs compared with that after ip injection, with undetectable amounts in the kidneys, spleen, liver, and brain [108]. Conventional amphotericin B is less efficiently nebulized than liposomal amphotericin B, but similar measured aerosol concentrations reached the lungs, although the lipid remained associated with the drug after nebulization [109]. The physiological side effects of aerosolized amphotericin B have been bronchospasm, especially in asthmatic patients, and nausea and vomiting; however, in general the aerosol is well tolerated [110].

Most studies have focused on prophylaxis [111], but aerosolized amphotericin B has been shown to prolong survival and decrease fungal burden in the treatment of murine IA [112]. Aerosolized treatment with conventional or liposomal amphotericin B showed efficacy compared with control rats but did not reduce dissemination of disease, unlike reports with systemic amphotericin B. However, systemic amphotericin B with and without aerosolized therapy were not compared [113]. Aerosolized liposomal amphotericin B was well tolerated in 56 patients after lung transplantation, including 6 patients with IA, and infections resolved [114]. In a similar approach to aerosolized amphotericin B, CT–guided percutaneous instillation of amphotericin B into IA lung lesions was recently described with some success in patients for whom systemic therapy failed [115].

Liposomal nystatin. Nystatin, a tetraene diene macrolide, was the first polyene antifungal. It was discovered in 1949 in a soil sample from Virginia [116] and named after the state of New York [117]. Nystatin was licensed for use in 1951 against superficial Candida infections [117]; however, problems with solubility and toxicity with parenteral use limited nystatin to topical use [118]. Recent liposomal reformulation has reduced toxicity and preserved antifungal activity in vitro [119, 120]. The new liposomal nystatin (Nyotran; Antigenics) is a multi-lamellar liposomal formulation of dimyristoyl phosphatidyl choline, dimyristol phosphatidyl glycerol, and nystatin (7:3:1) [121]. Nystatin has plasma pharmacokinetics markedly different from those of all 4 amphotericin B formulations, achieving comparatively high peak plasma concentrations with dose-dependent antifungal efficacy [21], an elimination half-life of $\leq 6$ h [18], and markedly less toxicity to mammalian cells [122], with renal toxicity similar to that of conventional amphotericin B [123].

In vitro studies have been recently reviewed [118] and yield mixed results. In one in vitro study, liposomal nystatin was more active against 10 A. fumigatus isolates than were all 3 lipid formulations of amphotericin B but less active than conventional amphotericin B. Against 30 A. fumigatus isolates, liposomal nystatin was more active than amphotericin B colloidal dispersion and liposomal amphotericin B but was not as active as amphotericin B lipid complex or amphotericin B deoxycholate [124]. Another in vitro evaluation of 40 Aspergillus isolates demonstrated MICs equal to or slightly higher than those of amphotericin B, amphotericin B lipid complex, amphotericin B colloidal dispersion, liposomal amphotericin B, and itraconazole [125]. An additional in vitro study revealed liposomal nystatin MICs higher than those of all 4 amphotericin B preparations and itraconazole for A. fumigatus and Aspergillus niger but lower than those of amphotericin B lipid complex and liposomal amphotericin B and equivalent to those of amphotericin B colloidal dispersion and amphotericin B against A. fumus. However, liposomal nystatin was markedly better than any amphotericin B preparation against A. terreus, although still inferior to itraconazole [126]. MICs of liposomal nystatin have been considerably lower than those of nystatin [127, 128], suggesting a complex interaction with lipid-complexed polyenes.

Despite liposomal nystatin having in vitro activity better than that of amphotericin B against clinical isolates in some studies, a murine model of IA showed that survival rates for amphotericin B were significantly better than those for liposomal nystatin at 4 mg/kg/day (93% vs. 74% survival). This study also found the absence of Aspergillus in the liver in all but 1 mouse treated with liposomal nystatin and absence in all mice treated with amphotericin B [129]. Liposomal nystatin had activity in another mouse model with an isolate with reduced susceptibility to amphotericin B, implying that although the mechanisms of action of these 2 polyenes are similar, mechanisms of resistance are not identical. In this study, liposomal nystatin at
5 mg/kg was the most successful in terms of survival. Liposomal amphotericin B at 5 mg/kg was second best, followed by equal results with amphotericin B lipid complex at 5 mg/kg, amphotericin B at 1 or 5 mg/kg, and liposomal nystatin at 1 mg/kg. Liposomal nystatin at 5 mg/kg resulted in fewer positive results of culture of liver samples than did any other treatment but was inferior to liposomal amphotericin B at 5 mg/kg. Liposomal nystatin at 5 mg/kg was superior to all regimens with respect to positive renal culture results [130].

In a neutropenic rabbit model, the survival rate of animals treated with liposomal nystatin at 1 mg/kg/day did not differ from that of control subjects. Treatment with 2 mg/kg/day increased survival to 70% (7 of 10 animals), compared with 40% (4 of 10) survival in the group treated with 1 mg/kg/day amphotericin B. However, treatment with standard-dose amphotericin B was significantly more effective at reducing fungal burden than was treatment with liposomal nystatin, a decrease to a 7% fungal burden versus 31%–47% on the basis of various liposomal nystatin doses [123]. No significant difference in number of pulmonary hemorrhagic infarcts was seen in those animals treated with amphotericin B versus liposomal nystatin.

In 1 case report, 5 patients with IA were treated with liposomal nystatin after therapy with amphotericin B failed because of disease progression or toxicity. Liposomal nystatin cured 4 patients, and all 5 had previously elevated serum creatinine levels return to normal [131]. A phase II trial noted that 7 of 16 evaluable patients with amphotericin B–refractory IA were alive on day 30 after treatment with 4 mg/kg/day liposomal nystatin [132]. Two large trials for treatment of IA have been closed because of lack of enrollment, and the pharmaceutical company is not pursuing further research with liposomal nystatin at present (E. Hawkins, Antigenics, personal communication, 2002).

Azoles

Itraconazole. First publicly described in 1983 [133, 134] and available for treatment of Aspergillus infection in 1990, itraconazole (Sporanox; Ortho-Biotech) adopted a triazole nucleus with higher specificity for the fungal cytochrome P-450 enzyme system over the older imidazoles [12]. Itraconazole’s fungicidal activity is not as efficient as that of amphotericin B, because inhibition of sterol synthesis takes longer than directly creating channels in the cell membrane [10]. Historically there have been several constraints with itraconazole: no parenteral formulation, erratic oral absorption in high-risk patients, and significant drug interactions.

Itraconazole has a high volume of distribution and accumulates in tissues, and the tissue-bound levels are more clinically relevant than serum levels [13]. Elimination of itraconazole is primarily hepatic, so there is no need for dosage adjustment in renal failure [13]. Itraconazole is poorly water-soluble, is not reliably absorbed from the gastrointestinal tract in its capsule formulation, and has high protein binding [18]. It has a relatively long half-life of 25–50 h, which allows for once-daily dosing [135]. Dosing is dependent on patient population, with children, especially infants, requiring higher doses [136]. The metabolism of itraconazole is also varied in specific ethnic populations because of genetic polymorphisms in the cytochrome P-450 2C19 enzyme [137].

Dissolution and absorption of itraconazole are affected by gastric pH. Patients with achlorhydria or who are using histamine receptor antagonists may demonstrate impaired absorption, whereas coadministration of the capsule with acidic beverages, such as cola or cranberry juice, may enhance absorption [138]. Administering a dose with food significantly increases the absorption of the capsule formulation, but the new oral suspension is better absorbed on an empty stomach [69]. Side effects are relatively few and include nausea and vomiting (in 10% of patients) and elevated transaminase levels (5%) [139]. There has also been a report of 58 cases suggestive of congestive heart failure associated with itraconazole therapy [140].

As a potent inhibitor of the fungal cytochrome P-450 3A4 enzyme, itraconazole also has some affinity for the human enzyme and therefore has important drug interactions. Prior or concurrent use of rifampin, phenytoin, carbamazepine, and phenobarbital should be avoided. Any drug handled by this cytochrome pathway with normally low bioavailability, extensive first-pass metabolism, or a narrow therapeutic window may be especially vulnerable [24]. Patients with IA often also have hypochlorhydria and/or enteropathy as a result of a variety of conditions, so when oral itraconazole is used, serum concentrations must be assessed to ensure adequate absorption [7].

Patients receiving cyclosporine who are treated with a triazole should have an immediate dose reduction of cyclosporine followed by frequent monitoring of serum levels. Furthermore, the azoles may increase serum, and thus intracellular, levels of cytotoxic drugs such as vincristine and anthracyclines [74] or protease inhibitors [141]. Azole-drug interactions may lead to decreased plasma concentration of theazole, related to either decreased absorption or increased metabolism, or increased concentration of any coadministered drugs [79]. In an open-label comparative pharmacokinetic study of 10 patients, there was an increase in tacrolimus or cyclosporine levels 48 h after completion of an iv itraconazole loading dose, so a 50% tacrolimus or cyclosporine dose reduction is recommended after an iv itraconazole loading dose [142].

Itraconazole’s insolubility has led to hampered oral absorption when administered in capsules. To overcome problems with variable absorption, itraconazole has now been solubilized in cyclodextrin, with substantial improvement as an oral solution [13, 143]. Cyclodextrins are naturally occurring doughnut-shaped glucose oligomers produced by enzymatic degra-
dation of starch that have been chemically modified for medical use. The external face of the molecule is hydrophilic to facilitate solubilization of the complex in water and shield a lipophilic guest molecule [144]. Once itraconazole is released from the host cyclodextrin, it follows its standard pharmacokinetics, with steady-state concentration reached in the second week of daily dosing. However, a murine model showed a >100-fold increase in the area under the serum curve (AUC) by cyclodextrin complexation [145], and bioavailability increased from 30%–33% to 55% in healthy volunteers when given in oral solution compared with capsule formulation [143]. Because elimination of cyclodextrin is dependent on glomerular filtration, the vehicle may accumulate in patients with significantly impaired renal function, but clinical significance has yet to be fully determined [12, 146].

A new iv formulation of itraconazole was recently approved by the US FDA for treatment of pulmonary and extrapulmonary aspergillosis in patients who are intolerant of or have infections refractory to amphotericin B [147]. The iv formulation can rapidly achieve high and steady-state plasma concentrations [148, 149], as opposed to the 7- to 10-day period needed for the capsule or oral formulation [150]. Generally, with the oral formulations of itraconazole, dosages of 800 mg for 3 days or 600 mg for 4 days are required. Because of this new formulation, some centers are modifying their protocols to include use of iv itraconazole during periods of neutropenia and mucositis and then a switch to oral solution [151]. The iv formulation was recently shown to be effective in experimental IA [152, 153]. In a neutropenic rabbit model, excellent tolerance and dose-dependent survival were seen with dosages of 8 and 12 mg/kg/day, but seizures were seen at dosages of 16 mg/kg/day [153].

In a multicenter open-label study, 31 patients with pulmonary IA received iv itraconazole for 14 days, followed by capsules for 12 weeks. The iv form was well tolerated, and target therapeutic concentrations (>0.25 μg/mL) were obtained within 2 days in 91% of patients and in all patients within 1 week after iv treatment. These levels were also maintained after switching to oral therapy. A complete or partial response was seen in 48% of patients (15 of 31 patients) [149]. These 2 new itraconazole formulations will allow better pharmacokinetics, especially in high-risk patients who may have difficulty using capsules because of mucositis, vomiting, or graft-versus-host disease.

The recommended dosage of itraconazole is 400 mg/day, and recent guidelines dictate a logical approach to include itraconazole as oral therapy after disease progression is arrested with parenteral amphotericin B [7]. Although there is a theoretical concern of polyene-azole antagonism due to ergosterol mechanisms of action, this regimen appeared safe in a recent survey of clinical practice of 93 patients treated with this sequential therapy [8]. This reserves itraconazole for maintenance therapy once the patient’s condition improves or as initial therapy for less-immunosuppressed patients. In some studies, itraconazole is a useful alternative therapy to amphotericin B, with comparable response rates [154, 155]. The introduction of iv itraconazole now offers a new option for patients intolerant of amphotericin B.

**Voriconazole (UK-109,496).** Voriconazole (VFend; Pfizer Pharmaceuticals) is a new second-generation triazole synthetic derivative of fluconazole. First described in 1995, it was developed as part of a program to enhance the potency and spectrum of activity of fluconazole. The addition of a methyl group to fluconazole’s propyl backbone and the substitution of a triazole moiety with a fluoropyrimidinid group increased the affinity for the target enzyme in A. fumigatus by 1 order of magnitude [156]. Both fungicidal [10, 156, 157] and fungistatic activity [157, 158] against Aspergillus have been demonstrated. In addition to its actions on the 14-α-demethylase, like other azoles, voriconazole also inhibits 24-methylene dehydrolanoster demethylation, explaining why it is active against a mold such as Aspergillus when fluconazole is not [156]. Independent of its activity on the cytochrome P-450 enzymes, voriconazole is also the only antifungal under investigation that inhibits Aspergillus conidiation and pigmentation [159].

Voriconazole is extensively hepatically metabolized and shows ~90% bioavailability. It appears that cytochrome P-450 2C19 plays a major role in the metabolism of voriconazole, and this enzyme exhibits genetic polymorphism, dividing the population into poor and extensive metabolizers as a result of a point mutation in the gene encoding the protein of cytochrome P-450 2C19 [160]. Approximately 5%–7% of the white population has a deficiency in expressing this enzyme, so genotype plays a key role in the pharmacokinetics of voriconazole [161].

Voriconazole is 44%–67% plasma-bound, with nonlinear pharmacokinetics; has a variable half-life of ~6 h [162], with large interpatient variation in blood levels [163]; and has good CSF penetration [12, 14, 164–167]. Its main side effects include reversible visual disturbances (increased brightness, blurred vision) in as many as 33% of treated patients, occasional elevated hepatic transaminases with increasing doses [167], and occasional skin reactions likely due to photosensitization [12, 156]. The skin manifestations are probably consistent with an indirect retinoid effect of voriconazole associated with a facial phototoxic reaction, because some patients showed improvement with application of sunscreen [168]. A multicenter randomized dose-escalation study of oral voriconazole treatment for patients at risk for fungal infection showed a dose-dependent reporting of visual side effects (mild blurred vision and photophobia), which were all reversible and transient and required no intervention [169]. These visual effects can be associated
with changes in electroretinographic tracings, but no permanent retinal damage has been described [170].

Oral absorption was nonlinear and rapid, with an ~5-fold accumulation over the 14 days tested in 1 study of patients with hematologic malignancy, consistent with reports in healthy volunteers [167]. In a study of 41 healthy men, nonlinear pharmacokinetics were again proven, and most subjects achieved steady-state levels by day 4 after switching from iv to oral drug administration. Maximum plasma voriconazole levels occurred at the end of the 1-h iv infusion and between 1.4 and 1.8 h after oral administration. Mean voriconazole levels did fall after oral administration to 63%–90% of levels achieved after iv administration, but most subjects achieved steady state at day 4, and trough levels remained above voriconazole MICs for *Aspergillus* species [161]. A pharmacokinetic study of 6 hepatic cirrhosis patients and age-matched control subjects demonstrated that patients with hepatic impairment should receive the same oral loading dose but half of the maintenance dose [171]. In contrast to adults, children show linear elimination of voriconazole, and they have a higher elimination capacity and therefore require a larger maintenance dose than adults do (4 mg/kg twice daily vs. 3 mg/kg twice daily) [172].

As with some other azoles, the potential exists to modify the metabolism of other drugs, including cyclosporine and phenytoin [14]. Voriconazole does inhibit metabolism of the commonly used immunosuppressive drug tacrolimus. In 1 study, coadministration of voriconazole and tacrolimus elevated trough tacrolimus levels in 1 patient after liver transplantation by nearly 10-fold, which was substantiated in an in vitro liver microsome model that showed that clinically relevant voriconazole levels inhibited the metabolism of tacrolimus [173]. In a study of patients who underwent renal transplantation, concomitant administration of voriconazole with cyclosporine also resulted in a 1.7-fold increase in the geometric mean AUC for cyclosporine, so it is recommended that the cyclosporine dose be halved and levels monitored frequently [174].

One study compared in vitro antifungal activity against 62 clinical isolates of *A. fumigatus* and demonstrated slightly more in vitro activity of voriconazole than amphotericin B and itraconazole [175]. Another study of 29 clinical *Aspergillus* isolates reported statistically significantly better in vitro activity of voriconazole compared with that of amphotericin B and itraconazole [175]. Another study of 29 clinical *Aspergillus* isolates reported statistically significantly better in vitro activity of voriconazole compared with that of amphotericin B. The voriconazole MICs for all isolates were <4 μg/mL, suggesting that voriconazole is effective at clinically achievable concentrations, whereas only 55% of isolates (16 of 29 isolates) had amphotericin B MICs <1 μg/mL, suggesting a possible predisposition to clinical amphotericin B treatment failure [165]. Another in vitro report of 23 isolates of *A. fumigatus* and *A. flavus* showed voriconazole MICs less than those of itraconazole and amphotericin B for *A. fumigatus* but greater than that of itraconazole for *A. flavus* [176]. One large review of in vitro studies showed that voriconazole had activity superior to itraconazole and amphotericin B against *A. fumigatus*, although itraconazole was superior for *A. nidulans* and *A. terreus*, and all had equivalent activity against *A. niger* [177].

One study compared data for 205 *Aspergillus* isolates from patients in clinical trials and showed that voriconazole and itraconazole were more potent in vitro against *Aspergillus* than was amphotericin B, with itraconazole having the lowest MICs [178]. In another in vitro study of 216 clinical and laboratory isolates, voriconazole had excellent activity against *Aspergillus* species at significantly lower concentrations than required of amphotericin B, but itraconazole had significantly better activity compared with voriconazole against *A. fumigatus*. Against itraconazole-resistant isolates, a modest rise in voriconazole MIC was noted, but the drug remained active and indeed was more active against amphotericin B–resistant isolates than was itraconazole [179]. Another study of 3 *A. fumigatus* isolates that were in vitro and clinically resistant to itraconazole showed that voriconazole had good in vitro activity [180]. In vitro testing of 130 clinical and 20 environmental isolates of *A. fumigatus* revealed no confirmed voriconazole resistance and similar voriconazole and amphotericin B MICs, although both were about double that of itraconazole. Voriconazole was also active against an itraconazole-resistant control isolate [181].

*A. terreus* infection is known to be frequently refractory to amphotericin B [182], and in vitro testing of 101 clinical isolates with amphotericin B compared with voriconazole showed only 2% of isolates were susceptible to amphotericin B (MIC ≤1 μg/mL) and 100% were susceptible to voriconazole (mean 48-h MIC, 0.22 μg/mL) [183]. The minimum lethal concentrations (MLCs) were elevated to 13.4 μg/mL for amphotericin B and 17.4 μg/mL for voriconazole, suggesting a lack of fungicidal activity. Voriconazole, usually fungicidal, also demonstrated fungistatic properties for this *Aspergillus* species. In an analysis of 413 clinical isolates of *Aspergillus* from phase III clinical trials of voriconazole, in vitro voriconazole was generally equivalent to itraconazole and superior to amphotericin B; however, itraconazole had lower MICs than did voriconazole for 42 *A. terreus* isolates [184].

In vivo studies of IA have mirrored the excellent in vitro efficacy against *Aspergillus*. In a rat model, voriconazole was better absorbed than itraconazole after oral administration, with much higher peak levels. Survival rates were equal with 4 mg/kg ip amphotericin B compared with 30 mg/kg voriconazole, but voriconazole was less effective at lower dosages. In vivo comparison of voriconazole versus itraconazole (both at 30 mg/kg) demonstrated that survival was 100% in the voriconazole-treated group and 75% in the itraconazole-treated group [185]. However, these results were not statistically significant, and no report was made of efforts to maximize oral absorption or monitor for continued therapeutic levels of itra-
conazole. These survival differences may represent the variable absorption of itraconazole versus the superior bioavailability of voriconazole. Interestingly, there is a problem with sustaining measurable concentrations of voriconazole in rodents, attributed to rapid first-pass metabolism. Oral administration of grapefruit juice, a known cytochrome P-450 enzyme inhibitor, does allow measurable concentrations for at least 10 days for experimentation during in vivo experimentation [186, 187].

In a guinea pig model of disseminated aspergillosis [188], voriconazole approximated the ability of amphotericin B to reduce tissue burden and was more effective in reducing the number of positive results of culture of liver samples. The same study also showed increased survival rates with voriconazole at either 5 or 10 mg/kg/day orally or itraconazole cyclohextrin solution at 10 mg/kg/day orally, compared with itraconazole at 5 mg/kg/day orally or amphotericin B at 1.25 mg/kg/day. However, this is a low dose of ip amphotericin B and may therefore not be a fair comparison. In vitro fungicidal activity was documented for voriconazole and amphotericin B against all isolates and against most for itraconazole. According to NCCLS criteria, at 48 h amphotericin B lacked fungicidal activity against 4 of 24 isolates (MLC range, 1 to >16 μg/mL) and itraconazole lacked fungicidal activity against 8 of 28 isolates (MLC range, 2 to >8 μg/mL), but voriconazole maintained fungicidal activity against all 28 isolates. However, the authors used the upper-limit MLC values for fluconazole, because none existed for voriconazole [188]. In another guinea pig IA model, 80% of guinea pigs (12 of 15 animals) treated with voriconazole at 10 mg/kg/day orally survived, whereas only 20% (3 of 15) treated with amphotericin B at 1 mg/kg/day ip survived. Fungal burden was also less at autopsy in voriconazole-treated animals [189].

In a rabbit model, significant reduction in tissue burden in the liver only was seen with voriconazole at dosages of 30 or 45 mg/kg/day and in the kidney at 45 mg/kg/day. Amphotericin B at 1.5 mg/kg/day was significantly more effective at decreasing tissue burdens. The tissue burden in the lungs in the voriconazole group was equal to that in untreated, sublethally challenged control subjects. Voriconazole at both dosages and amphotericin B eliminated mortality in the lethally and sublethally challenged animals [190]. In a guinea pig model, voriconazole performed markedly better than itraconazole in cyclohextrin in prophylaxis and treatment of Aspergillus endocarditis. All of the animals given oral voriconazole at 10 mg/kg twice daily survived, compared with none of those treated with oral itraconazole at 10 mg/kg twice daily. In addition, the higher-dose voriconazole sterilized the heart valves, with a dose-dependent recovery of Aspergillus with the lower doses of voriconazole. The mitral valves of the itraconazole-treated animals all grew hyphae, but the mean serum itraconazole level was subtherapeutic (0.35 μg/mL) [191]. Voriconazole has been shown to penetrate the aqueous humor of healthy rabbits, but because of significant variability in levels, more study is required to determine actual ocular treatment levels [192].

Clinical reports of patients treated with voriconazole are now appearing. A child with relapsed leukemia who had failure of therapy with itraconazole, amphotericin B, and liposomal amphotericin B showed improvement with voriconazole therapy [193], and 1 patient with cerebral aspergillosis improved after voriconazole treatment after failure with amphotericin B [194]. Another patient with cerebral aspergillosis had therapy failure with amphotericin B, liposomal amphotericin B, and then itraconazole with intrathecal amphotericin B. Evidence of infection resolved with voriconazole, but the patient died of leukemia [195]. A patient with sinusitis worsened and developed osteitis of the skull base despite 2 courses of itraconazole, but after their course of therapy was changed to voriconazole, there was marked clinical improvement and no evidence of disease recurrence 5 years later [196]. There have also been successful responses in patients with chronic granulomatous disease (CGD) whose treatment included voriconazole [197, 198]. However, it is somewhat difficult to evaluate all reports of sequential failure with 1 agent followed by success with another, because the contribution of the initial therapy to the eventual good outcome can sometimes be questioned. A review of 42 children with IA treated with voriconazole showed that the drug was well tolerated and had an overall response rate of 43% [172].

The largest prospective clinical trial of voriconazole involved 392 patients at 92 centers in 19 countries over 3 years and compared initial randomized therapy with voriconazole (2 doses of 6 mg/kg on day 1, followed by 4 mg/kg twice daily for at least 7 days, followed by 200 mg orally twice daily) versus amphotericin B (1–1.5 mg/kg/day) followed by other licensed antifungal therapy. Patients who initially received voriconazole had statistically significantly better complete or partial response (53% of patients), compared with those initially receiving amphotericin B (32%). Survival rates also improved to 71% for voriconazole, versus 58% for those initially receiving amphotericin B [199]. Analysis in an open, noncomparative multicenter study of 116 patients treated with voriconazole as primary therapy (60 patients) or salvage therapy (56 patients) also yielded encouraging results: 14% of patients had a complete response, 34% had a partial response, and 21% had a stable response to voriconazole, whereas 31% failed to respond to therapy [163]. These data forged the way for US FDA approval of voriconazole for initial therapy for IA in May 2002.

Although the comparator in the pivotal trial was primary therapy with amphotericin B, extrapolation to the amphotericin B lipid formulations may be considered. As noted above, no comparative large clinical trial has documented a difference in outcome or survival comparing conventional amphotericin B with any of the lipid products, although there are clear and relevant differences in toxicity. Therefore, although not specif-
ically studied, it has been argued that the clear superiority of voriconazole over amphotericin B should extend to the lipid formulations. However, in that large voriconazole–amphotericin B study, the high dropout rate in the amphotericin B arm and consequent switch to other licensed antifungal therapy led to a discontinuation of the comparator and interruption of therapy in that arm. This raises the question whether better-tolerated therapy, given continuously, might have produced a better result. Perhaps it is more accurate to conclude that voriconazole was more successful than other licensed antifungal therapy for IA.

Although voriconazole therapy has not been compared with other treatment modalities, such as itraconazole or echinocandins or combination therapy, in a randomized trial, the superiority of voriconazole demonstrated over the reference standard, initial therapy with amphotericin B, makes it the current first choice for primary therapy for IA, with a few caveats. Although voriconazole has a broad spectrum of activity, it has no activity against the Zygomycetes [200], which often produce a clinically and radiologically indistinguishable picture and which may also require surgical intervention for improved outcome [201].

The additional role of voriconazole in combination with newer agents such as caspofungin remains hopeful yet unproven [202].

**Posaconazole (SCH 56592).** Posaconazole (Schering-Plough Research Institute) is a second-generation triazole and is closely related to itraconazole. It is fungicidal in vitro, and its activity is slightly superior at 48 h to that of itraconazole and voriconazole yet inferior to that of amphotericin B [203]. Posaconazole has a long half-life of at least 18–24 h in humans [12, 14] and likely time-dependent killing [18]. Presently only an oral formulation is available, but a new carrier system is in development.

In vitro testing showed that posaconazole is 4- to 8-fold more active than itraconazole and 4- to 16-fold more active than amphotericin B against 22 *Aspergillus* isolates [204] and overall more active than itraconazole against 39 other *Aspergillus* isolates [205]. Another in vitro study showed lower MICs for 4 *Aspergillus* species compared with itraconazole and amphotericin B [206]. In vitro comparison of the 3 second-generation triazoles and amphotericin B against 106 *A. fumigatus* isolates showed posaconazole with the best activity, followed by voriconazole, itraconazole, and then amphotericin B. Of note, the itraconazole-resistant strain did not show cross-resistance to posaconazole or voriconazole [207]. Other in vitro studies of *Aspergillus* isolates showed posaconazole with generally lower MICs than voriconazole, itraconazole, and amphotericin B for all *Aspergillus* species tested [208], as well as consistently significantly lower MICs than all 3 newer triazoles [209].

An in vitro study comparing posaconazole against 2 echinocandins, caspofungin and anidulafungin, demonstrated fungicidal activity for posaconazole and very little fungicidal activity for the other antifungals. The same study reviewed published MIC data demonstrating better in vitro activity of posaconazole against *Aspergillus* species than that of amphotericin B, itraconazole, voriconazole, caspofungin, and anidulafungin [210]. A review of 593 isolates showed susceptibility for all antifungals tested yet lower posaconazole MICs than amphotericin B, itraconazole, and voriconazole MICs [53].

In 1 study, 60 clinical isolates of *Aspergillus* were tested to compare in vitro activity of posaconazole with that of amphotericin B and itraconazole [64]. The study demonstrated that geometric mean posaconazole MICs were ∼3-fold lower than itraconazole MICs and 20-fold lower than amphotericin B MICs. In addition, *A. terreus*, frequently resistant to amphotericin B, was the *Aspergillus* species most susceptible to posaconazole among those tested, offering another treatment option for this historically lethal species. Posaconazole was also active against itraconazole-resistant isolates tested. In another study, posaconazole had activity similar to that of itraconazole against an amphotericin B–resistant isolate of *A. fumigatus* but superior activity against a voriconazole-resistant isolate [203]. Cross-resistance of posaconazole to itraconazole- and voriconazole-resistant isolates, as might be expected because of structural similarities [64], is low in several studies [203, 211]. One study evaluating 12 itraconazole-resistant *A. fumigatus* isolates found increased posaconazole MICs for 6 isolates, whereas voriconazole and ravuconazole MICs remained similar to those of susceptible isolates. These strains had a point mutation in the *cyp51A* gene. The other 6 isolates required increased MICs of all antifungals tested, thereby suggesting at least 2 mechanisms for itraconazole resistance in *A. fumigatus* [212]. Another study confirmed the point mutation leading to a single amino acid substitution and posaconazole resistance [213].

Another in vitro study showed superior activity of posaconazole compared with amphotericin B, itraconazole, and voriconazole [203]. For 284 clinical isolates of *A. fumigatus*, posaconazole had a lower geometric mean MIC than amphotericin B, itraconazole, or voriconazole. Additionally, in a laboratory-derived voriconazole-resistant strain of *A. fumigatus*, posaconazole maintained the best activity compared with the same antifungals [211]. A recent in vitro study of 198 *Aspergillus* isolates showed posaconazole with clearly better activity than either amphotericin B or itraconazole and activity equivalent to or superior to that of both voriconazole and ravuconazole [214]. An in vitro study of 15 *Aspergillus* isolates showed lower MICs and minimum fungicidal concentrations (MFCs) of posaconazole than of voriconazole, ravuconazole, and itraconazole by use of various testing media [215]. In vitro analysis of 353 *Aspergillus* isolates found posaconazole to be the most effective triazole, followed by voriconazole, ravuconazole, and itraconazole, and all 3 triazoles and caspofungin were...
superior to amphotericin B for *A. terreus* [216]. Another in vitro study showed that posaconazole and voriconazole had superior activity against 2 *A. fumigatus* strains compared with that of amphotericin B, itraconazole, and terbinafine [217].

A murine pulmonary 1A model showed in vivo efficacy of posaconazole against *A. fumigatus* and *A. flavus* strains [205]. In a rabbit model of pulmonary aspergillosis, survival rates and antifungal efficacy of posaconazole in clearing tissue burden were equal to those of amphotericin B at 1 mg/kg/day and superior to those of itraconazole, given in cyclodextrin, at equal dosages, with MICs and MFCs consistently 4 times lower [218]. In another rabbit model, posaconazole significantly prolonged survival compared with itraconazole or amphotericin B; however, fungal tissue burden was not reduced compared with amphotericin B treatment [219]. In a murine model, treatment with posaconazole, or itraconazole in cyclodextrin, for infection with an itraconazole-susceptible strain showed a significant reduction in mortality, compared with amphotericin B (5 mg/kg/day ip). Posaconazole treatment for infection with an itraconazole-resistant strain achieved 100% survival, compared with 50% survival in the amphotericin B treatment arm. Posaconazole was also significantly better than amphotericin B and itraconazole at reducing fungal burden in assays of residual fungal tissue burden [220]. In another murine model of IA, posaconazole achieved significantly prolonged survival, greater than that seen with high-dose amphotericin B (6 mg/kg/dose) [221].

Phase I clinical trials demonstrated good oral absorption in 31 patients with cancer and neutropenia [222]. Posaconazole should not be coadministered with phenytoin because of interactions causing a large variability in antiepileptic concentration [223]. In a multicenter study, which included 25 patients with IA, to evaluate salvage therapy for patients who have refractory invasive fungal infections, posaconazole was well tolerated and effective in 53% of patients (8 of 15 patients) at week 4 and in 85% (6 of 7) at week 8; however, there was no mention of the patients who did not have complete follow-up [224].

Ravuconazole (BMS-207147, ER-30346). Ravuconazole (Bristol-Myers Squibb) is structurally similar to fluconazole and voriconazole, containing a thiazole instead of a second triazole. It is often fungicidal [225, 226] and has 47%–74% bioavailability, with linear pharmacokinetics [12] and a long terminal half-life of ~100 h [227]. The drug is well tolerated, with headache a main side effect, and urine studies suggest no cytochrome P-450 isoenzyme induction [14]. The drug is well absorbed following oral administration, and its absorption is enhanced by food [226]. Ravuconazole was well tolerated in healthy human subjects in single [227] and multiple doses [228]. Ravuconazole alone or coadministered with simvastatin was well tolerated in 20 healthy subjects, and ravuconazole is a less potent inhibitor of the cytochrome P-450 3A4 enzyme than are other triazole antifungals [229]. Ravuconazole did not affect nelfinavir levels in 14 healthy volunteers [230].

One in vitro study of 16 *Aspergillus* isolates demonstrated comparable fungicidal activity and MICs of ravuconazole, itraconazole, and amphotericin B [231]. In vitro comparisons against the 2 other new triazoles and amphotericin B and itraconazole demonstrated similar MICs among the 4azole compounds and general superiority over amphotericin B against various *Aspergillus* species [232]. Against 177 clinical isolates of *Aspergillus*, all 3 new triazoles showed similar and excellent activity, and all were superior to itraconazole. Overall, posaconazole was the most active agent, inhibiting 94% of isolates at a MIC of ≤1 μg/mL, followed by ravuconazole (93%) and voriconazole (89%) [214]. Another study found ravuconazole MICs to be slightly higher than itraconazole or amphotericin B MICs, but no ravuconazole-resistant isolates were detected [225]. One study found good in vitro activity of ravuconazole against 141 *Aspergillus* isolates, including *A. terreus* [233].

One murine study revealed that both ravuconazole and itraconazole led to decreased lung fungal burden in a dose-dependent fashion [234]. Penetration of ravuconazole into healthy rat tissue showed that the concentration of drug in the lungs was 2–6 times higher than the corresponding blood concentration [235]. Another murine model revealed similar survival efficacy with itraconazole when both were given at 40 mg/kg, but ravuconazole was better when both were compared at 10 mg/kg [236]. In a guinea pig model of disseminated aspergillosis, ravuconazole as well as itraconazole (10 mg/kg/day) displayed 100% survival (8 of 8 animals), compared with 87% survival with amphotericin B at 1.25 mg/kg/day ip. Ravuconazole was also more effective in reducing positive results of culture of organ samples than were amphotericin B or itraconazole [237].

Another study showed survival efficacy of ravuconazole in a rabbit model of IA as well as a decrease in tissue fungal burden comparable to the effect of amphotericin B, and *Aspergillus* infection was cleared in 90% of rabbits challenged [238]. This group of researchers, with 15 years of experience with rabbit models of IA, report that in studies of the efficacy of amphotericin B, liposomal amphotericin B, fluconazole, saperconazole, itraconazole, voriconazole, and posaconazole, only amphotericin B and ravuconazole consistently eliminated *A. fumigatus* from organ tissues of challenged rabbits [238]. These findings were reproduced by another group, who showed dose-dependent reduction in fungal burden and increased survival with ravuconazole at 5 mg/kg compared with amphotericin B at 1 mg/kg (95% vs. 50% survival), with significantly less elevation in serum creatinine levels [239].

**Echinocandins**

*Caspofungin* (MK-0991, L-743,872). Caspofungin (Cancidas; Merck) is a fungicidal water-soluble semisynthetic deriv-
that dosing at 50 mg/m² appears to be more appropriate than and a reduced half-life. Pharmacokinetic projections suggest in children, with lower caspofungin levels in smaller children such patients [248]. Pharmacokinetics appear slightly different plasma concentrations in patients with mild hepatic insuffi-
cance and is indicated for patients with refractory aspergillosis or intolerance to other therapies.

At present there is no maximal tolerated dose and no toxicity-determined maximal duration of therapy. The usual course is to begin with a load followed by a lesser daily dose [15]. Pharmacokinetics in healthy men revealed linear pharmacokinetics and dose-proportional AUC concentration data, with modest nonlinearity following multiple doses [248]. Two pharmacokinetic studies of mild-to-moderate hepatic insuffi-
ciency showed clinically insignificant elevations of caspofungin plasma concentrations in patients with mild hepatic insuffi-
ciency, but a dose reduction from 50 mg to 35 mg daily follow-
ing the standard 70-mg loading dose was recommended for such patients [248]. Pharmacokinetics appear slightly different in children, with lower caspofungin levels in smaller children and a reduced half-life. Pharmacokinetic projections suggest that dosing at 50 mg/m² appears to be more appropriate than at 1 mg/kg/day [249]. Plasma concentrations of tacrolimus were modestly reduced by ~20% with coadministration of caspo-
fungin, necessitating the close monitoring of tacrolimus levels. Cyclosporine increased the AUC of caspofungin by ~35%, although plasma concentrations of cyclosporine were not altered by coadministration of caspofungin [250]. Mycophenolate and caspofungin have no relevant interactions [247].

Caspofungin is also nonhemolytic for human and mouse red blood cells, and because 1,3-β-glucan is a selective target present only in fungal cell walls and not in mammalian cells, this elim-

ates drug mechanism–based toxicity [16]. For caspofungin, there is an ~1-h lag period before antifungal action, indicating that fungal growth is required for killing to occur. The rate of killing for caspofungin is significantly longer than for ampho-

tericin B, which does not require cell growth for activity [16]. Caspofungin was approved by the US FDA in February 2001 and is indicated for patients with refractory aspergillosis or intolerance to other therapies.

In a neutropenic rabbit model, improved survival rates over those of control subjects and linear pharmacokinetics, with plasma drug levels above the MIC for the entire dosing interval, were shown. However, despite dose-dependent hyphal damage, there was no reduction in residual fungal burden or galactomannan antigenemia, unlike results with amphotericin B [256]. Another in vivo study demonstrated equivalent efficacy of caspofungin and amphotericin B for treatment of IA in both tran-

siently and chronically immunosuppressed murine models. In the transiently immunosuppressed model, treatment with caspofungin at 0.5 and 1.0 mg/kg/day resulted in 70% and 90% survival rates, respectively, compared with survival rates of 90% and 50%, respectively, in animals treated with amphotericin B at the same dosages. In the chronic-suppression model, survival at day 28 after challenge was nearly identical for caspofungin and amphotericin B (both 1 mg/kg/day ip) [257].

In a murine disseminated aspergillosis model, caspofungin showed 50% effective doses (ED₅₀), with daily dosing, compara-
tible to those of amphotericin B [244]. Another study demon-

strated equivalent efficacy of caspofungin and amphotericin
B in a 5-day course of therapy [258]; quantitative PCR was used to determine residual fungal burden instead of analysis of colony-forming units to alleviate the possibility of error in counting a filamentous hyphal mass.

In a pivotal clinical study leading to US FDA approval, 56 patients with acute IA underwent salvage therapy after experiencing failure of primary therapy (45 patients) for >1 week or developing significant nephrotoxicity. These patients were compared with 210 historical control patients evaluated after 1 week of primary therapy. Recipients generally tolerated caspofungin well and had better outcome than did the historical control patients; 41% of patients (22 of 54) had a favorable response with caspofungin [259]. A recent update on all 90 patients enrolled in that trial revealed that 45% had a complete or partial response, and the drug was generally well tolerated [260]. A Spanish study done before licensure revealed a 67% (8 of 12 patients) favorable response rate among patients with proven or probable IA [261].

**Micafungin (FK463).** Micafungin (Fujisawa Healthcare) is an echinocandin lipopeptide compound [12, 262, 263] with a half-life of ~12 h and, like all echinocandins, is fungistatic in vitro versus *Aspergillus* [264]. Compartmental pharmacokinetics in healthy rabbits in doses of 0.5 and 2 mg/kg revealed a rapid initial distributive phase followed by a slower elimination phase, with an estimated elimination half-life of ~3 h in single-dose studies [265]. There were dose-independent linear plasma pharmacokinetics and dose-proportional increases in AUC with increasing dosage after single and multiple dosing. Plasma drug concentrations fit best to a 2-compartment open pharmacokinetic model. The highest drug concentrations were detected in the lung, followed by the liver, spleen, and kidney at potentially therapeutic concentrations. Drug concentrations increased proportionally to dosage. There were no abnormal elevations in serum creatinine or hepatic transaminase levels after 8 days of treatment. In plasma, drug concentrations were maintained in a dose-dependent manner severalfold in excess of the MIC<sub>90</sub> for *Aspergillus* isolates. Micafungin was undetectable in CSF; however, low levels were detected in brain tissue and the choroidal layer and vitreous humor of the eye but not in the aqueous humor [241].

In vitro, micafungin was shown to be more efficacious than either amphotericin B or itraconazole in several studies [264, 266, 267]. In vitro evaluation against 17 clinical isolates of *A. fumigatus* showed MICs of micafungin to be lowest compared with those of itraconazole, amphotericin B, and 5-fluorocytosine [266]. However, MICs were determined by the Japanese Society of Medical Mycology method, which does not refer to absence of visible growth as the MIC [268]. Against 64 clinical isolates of 4 *Aspergillus* species, micafungin was more active than itraconazole and amphotericin B. The MFCs were much higher than the MICs, indicating fungistatic activity [264]. Another in vitro study of 44 clinical isolates of *A. fumigatus* and *A. niger* revealed micafungin MICs lower than those of both amphotericin B and itraconazole [267]. An in vitro study of 6 *Aspergillus* isolates showed activity of micafungin far superior to that of amphotericin B and itraconazole [269]. In vitro testing showed activity against *A. fumigatus*, *A. flavus*, and *A. niger* by MIC as well as disk diffusion methods [270]. In vitro testing of 45 *Aspergillus* isolates showed micafungin with lower minimum effective concentrations (MECs) than amphotericin B, including activity against amphotericin B–resistant isolates [271].

In a murine pulmonary aspergillosis model, mice were treated with micafungin at 10 mg/kg iv. Under scanning electron microscopy, the hyphal surfaces were rough and irregularly deposited, with a fibril-like structure and markedly flattened hyphae. Transmission electron microscopy revealed swollen cells, cells with thinned walls resulting in lysis, and a thick and short hyphal cell with cytoplasmic vacuolation [272]. In a neutropenic rabbit model of pulmonary aspergillosis, rabbits treated with micafungin dosages of 0.25–2 mg/kg/day had no quantitative reduction in concentration of *A. fumigatus* in tissue compared with that in untreated control subjects, whereas amphotericin B and liposomal amphotericin B reduced tissue burdens [273]. Additionally, there was a persistence of galactomannan antigenemia, contrasted with the significant reduction of galactomannan levels in amphotericin B– and liposomal amphotericin B–treated animals. Micafungin caused dose-dependent damage of hyphal structures in the lung tissues of animals but did not clear histologically detectable *Aspergillus* from lung tissues. However, there was a significant reduction in the level of organism-mediated pulmonary tissue injury and a significant improvement in animal survival rates comparable to those treated with liposomal amphotericin B (5 mg/kg/day). The authors postulated that the survival effect was due to favorable safety profile and ability to damage hyphae, thereby reducing levels of angioinvasion.

A murine model of disseminated aspergillosis showed complete survival at 22 days of all mice treated with micafungin at 1 mg/kg/day. However, the ED<sub>50</sub> calculated on the basis of survival rate at 15 days after infection showed that micafungin had an efficacy 1.7–2.3 times inferior to that of amphotericin B, although it did have a partial inhibitory effect on fungal growth [274]. A murine study of pulmonary aspergillosis confirmed 100% survival of mice treated with 1 mg/kg/day at 22 days and an ED<sub>50</sub> similar to that of amphotericin B [275]. Another murine systemic IA model demonstrated that micafungin at 10 mg/kg/dose was effective at prolonging survival and reducing fungal burden in the brain and kidney compared with control animals [276, 277].

In a study of 20 patients, micafungin was well tolerated, with no severe adverse events, and a maximum tolerated dose was
not reached at 4 mg/kg/day [278]. In an open-label, multicenter study of micafungin monotherapy that included 10 patients with IA, overall clinical response was an improvement in 60% of patients, with no safety-related issues [279]. A recent study of micafungin combined with an existing antifungal agent in pediatric and adult bone marrow transplant recipients with IA revealed an overall complete or partial response by 39% of patients, including improvement in 40% of allogeneic transplant recipients [280].

**Anidulafungin (VER-002, LY-303366).** Anidulafungin (Vicuron) is a semisynthetic terphenyl-substituted antifungal derived from echinocandin B, a lipopeptide fungal product [281]. It has linear pharmacokinetics [14], with the longest half-life of all of the echinocandins (~18 h) [164, 282] and has shown fungistatic or fungicidal activity in different settings [283]. In vitro data on 22 *Aspergillus* isolates show that it is 2- to 4-fold more active, measured by a 75% reduction in growth for the echinocandins, than caspofungin against *A. fumigatus* and *A. flavus*. Both drugs were considerably more active than amphotericin B or itraconazole, as determined by use of the same criteria for the echinocandins and itraconazole and a classic MIC for amphotericin B [284]. An in vitro study with 20 *Aspergillus* isolates demonstrated lower MECs, but higher MICs, of anidulafungin than of amphotericin B [281]. In vitro evaluation of an additional 26 *Aspergillus* isolates revealed anidulafungin MICs lower than caspofungin MICs; however, both were generally less active than posaconazole [210]. Favorable antifungal interactions with anidulafungin and neutrophils or monocytes have been demonstrated [285], similar to findings with caspofungin [253].

Pharmacokinetic analysis in healthy rabbits revealed linear pharmacokinetics, with dose-proportional increases in AUC [286]. Anidulafungin fit a 3-compartment open pharmacokinetic model, with a terminal elimination half-life of up to 30 h. Tissue concentrations after multiple dosing were potentially therapeutic and highest in lung and liver, followed by spleen and kidney, with measurable concentrations in the brain tissue at dosages of ≥0.5 mg/kg/day. No concentration-dependent effect was seen in IA. The pharmacokinetics showed ~6-fold lower mean peak concentrations in plasma and 2-fold lower AUCs compared with values with similar doses of caspofungin and micafungin. Anidulafungin had no effect on residual fungal burden of *A. fumigatus* in rabbits with IA, despite dose escalation up to 20 mg/kg, and led only to dose-dependent morphological damage of hyphal structures. However, there was a significant improvement in survival and reduction in pulmonary tissue injury.

Survival in a rabbit model of IA treated with anidulafungin was comparable to that seen with amphotericin B (1 mg/kg/day); however, tissue fungal burden was not reduced and was actually significantly higher than in untreated control subjects. The serum *Aspergillus* antigen level was lowered but not eliminated, and higher doses produced mottled livers and necrotic lungs in a rabbit model [238]. Another rabbit model study confirmed survival efficacy but noted an upper threshold of toxicity at 20 mg/kg/day. However, although amphotericin B yielded a significant reduction in *A. fumigatus* growth in lung tissues and bronchoalveolar lavage fluid, no difference in growth was observed in animals treated with either anidulafungin or placebo. Analysis of that lung tissue showed dose-dependent damage of hyphal elements, with a progressive reduction in length and increased swelling. By comparison, tissues from amphotericin B–treated rabbits seldom revealed any hyphal elements [283].

In another mouse model, amphotericin B was superior to anidulafungin in reducing fungal counts in renal tissue, but anidulafungin was effective against an amphotericin B–resistant isolate [287]. Of concern is a report of lethal toxicity in a mouse model, through an unclear mechanism, with anidulafungin and cortisone, hydrocortisone, or triamcinolone but not dexamethasone [288]. Recent studies show that this phenomenon also occurs with micafungin, so the action might be a property of the echinocandin class [277]. This animal model finding is of concern, because most patients with progressive IA are also taking glucocorticoid therapy for their underlying disease.

A phase I study has shown anidulafungin to be safe and well tolerated in 29 healthy volunteers, with the highest-dose cohort experiencing transient elevations in liver function test results that exceeded twice the upper limit of normal [289]. In a separate study, 12 subjects with mild or moderate hepatic impairment did not show clinically significant changes in the pharmacokinetic parameters of anidulafungin [290]. A phase III open-label, noncomparative multinational study of anidulafungin with a lipid formulation of amphotericin B for IA scheduled to enroll 60 patients was recently discontinued (Vicuron, press release, 2001).

**Allylamines**

**Terbinafine.** Since its introduction into clinical practice in 1991, oral terbinafine (Lamisil; Novartis Research Institute) has been used by clinicians mainly for dermatophyte infections of the skin and nails [25]. Terbinafine is well tolerated [291], has a bioavailability of 70%–80% after oral administration, and is highly lipophilic, with a terminal half-life of up to 3 weeks [292]. The metabolism of terbinafine is not dependent on the cytochrome P-450 system, but metabolism takes place extensively in the liver [69], with subsequent excretion in the urine [25]. Terbinafine is rapidly absorbed following oral administration in humans [293], with a maximal plasma concentration of ~0.8–1.5 g/mL after a single 250-mg oral dose [294]. There have been reports of side effects with terbinafine, including...
hepatitis [295, 296], pancytopenia [295, 297], hair loss [298], and drug interaction with tricyclic antidepressants [299, 300].

The lipophilic terbinafine concentrates highest in the sebum and hair, with quantifiable concentrations 56–90 days after a final oral dose and a slow redistribution from the peripheral sites back to the central plasma compartment. Pharmacokinetic modeling indicated that at steady state, almost all (94%) of the terbinafine in the human body resides in adipose and skin tissues, with only 0.4% in the lung [301], which might theoretically lead to difficulty in treating systemic infection [302]. Tissue distribution in rats, as determined by high-performance liquid chromatography after a 6 mg/kg iv dose, revealed a slow uptake and efflux of terbinafine in the skin, with an estimated redistribution half-life from the skin of 1.6 days. Approximately 60% and 28% of the apparent volume of distribution of terbinafine was to the skin and adipose tissue, respectively [303]. Further analysis of terbinafine distribution in human blood shows a higher affinity for plasma proteins than blood cells in a concentration-independent manner [304].

Ryder [305] reviewed several in vitro studies showing good terbinafine activity against 5 different Aspergillus species. One study showed that terbinafine was active against A. flavus and A. niger at one-fourth the concentration of amphotericin B; however, amphotericin B was fungicidal at one-fourth the concentration against A. fumigatus [306]. In vitro testing of 28 Aspergillus species, including 1 strain of A. terreus, showed low terbinafine MICs (0.3–1.0 μg/mL). Terbinafine serum levels in rabbits after a 100-mg/kg loading dose and 50 mg/kg twice-daily dosing have been shown to be considerably above these MICs for Aspergillus species [307]. In vitro data on 100 clinical isolates show superior activity, compared with amphotericin B and itraconazole, against A. flavus, A. niger, and A. terreus but inferior activity against A. fumigatus. This presents a potential clinical problem, because A. fumigatus accounts for ~90% of human infections in some studies [3]. Interestingly, the terbinafine MIC was slightly lower for the itraconazole-resistant A. fumigatus isolates than for the itraconazole-susceptible isolates. Terbinafine was also more fungicidal for non-A. fumigatus isolates (88% vs. 35% isolates) [308]. A case report of a patient who died of Aspergillus ustus infection showed that, compared with amphotericin B, itraconazole, and voriconazole, terbinafine had the best in vitro activity and was the most fungicidal antifungal against that isolate [309].

A rat pulmonary IA model revealed no improvement in survival over control subjects at doses of 20–80 mg/kg for 1 week, and the lungs of the few survivors were grossly enlarged and filled with multiple abscesses at necropsy. Terbinafine concentrations determined by bioassay were adequate, and the authors concluded that terbinafine was inactive in their model despite adequate lung concentrations. Later in vitro testing revealed that MICs were 6-fold higher in serum than in laboratory media [310]. Others speculate that this decreased activity in the presence of serum is due to a reduced bioavailability resulting from nonspecific binding of the drug to major serum components. This could account for the low efficacy in experimental animal models and the high efficacy in dermatophyte infections [311]. After administration of ¹³C-labeled terbinafine in a rat model, the highest concentrations were found in the liver and pancreas but declined rapidly, whereas the concentrations in fat tissues declined more slowly, representing the drug’s high lipophilicity [294].

There are 2 poorly defined case reports of successful treatment of IA with terbinafine, each published as a personal communication. A 15-year-old boy with acute lymphocytic leukemia, graft-versus-host disease, and meningocerebral aspergillosis was treated with amphotericin B, itraconazole, fluconazole, and terbinafine for 169 days. At that point only terbinafine treatment continued until day 400, when the patient’s leukemia was in remission, and he fully recovered from IA [25]. A second patient with pulmonary IA was treated successfully with amphotericin B plus terbinafine and then continued to receive terbinafine monotherapy after the patient became intolerant of amphotericin B [312].

IMMUNOMODULATORY THERAPY

Host defense is paramount, because IA generally develops only in certain subsets of severely immunocompromised patients [313]. Two lines of host defense exist against Aspergillus. The first is formed by alveolar macrophages, and in vitro mouse studies have suggested that resident pulmonary macrophages are responsible for clearing inhaled Aspergillus conidia from the lung [314]. Phagocytes appear to kill the inhaled conidia better once the conidia are metabolically activated and swell, which takes place in a suitable moist environment such as the terminal airways of the lung [315]. After phagocytosis, germination of conidia into the invasive hyphal form is inhibited inside the phagolysosome. After 24 h, 90% of engulfed conidia are killed, and, 6–12 h later, they are completely digested [316].

If conidia escape this defense system, then, after transformation into the invasive Aspergillus hyphae, they become susceptible to neutrophil killing through the release of toxic oxygen radicals [315]. It appears that both mechanisms of host defense are important for Aspergillus to cause invasive disease. The host may be overwhelmed in the presence of neutropenia, and macrophages may be overcome with high challenge doses, activated conidia, or corticosteroid suppression of conidiacidal activity [317]. The ability of polymorphonuclear neutrophils (PMNLs) to kill hyphae was up-regulated in mice that survived infection and severely depressed in mice that died of infection [318].

Few patients with persistent neutropenia and IA survive, and indeed resolution of IA has followed recovery of neutrophil
levels in most cases. In bone marrow transplant recipients, the risk for IA remains even after engraftment, highlighting the fact that although the number of phagocytes is important, their ability to kill must be adequate [319]. Immunotherapy offers many therapeutic advantages through the availability of a wide range of recombinant cytokines that exert their effects indirectly through leukocyte activation rather than directly on the fungus. Immunotherapy is designed to increase the number of phagocytic cells and shorten the duration of neutropenia, modulate the kinetics or actions of those cells at the site of infection, and/or activate the fungicidal activity of phagocytes to kill fungal cells more efficiently [68, 320].

Corticosteroids are a well-known major risk factor for the development of IA [321] and can suppress the ability of monocytes/macrophages to kill conidia through inhibition of non-oxidative processes and impairment of lysosomal activity. Corticosteroids also inhibit PMNLs in their chemotaxis, oxidative bursts, and activity against hyphae [322–325]. This may be important in IA, because A. fumigatus has an quickened doubling time of 48 min in the presence of hydrocortisone in vitro [326]. Generally, corticosteroids suppress macrophages, whereas cytotoxic chemotherapy decreases neutrophil number and function. For instance, granulocytopenic rabbits had more hyphae in tissue and greater mortality through angioinvasion than did cyclosporine- and corticosteroid-immunosuppressed animals, which had more conidia [327]. Dexamethasone has also been shown to decrease the amount of granulocyte-macrophage colony-stimulating factor (GM-CSF) secreted by monocytes [328].

Interleukins. Antigen-specific immune responses result in selective stimulation of two CD4+ T helper (Th) cell subsets, leading to unique patterns of cytokine secretion and a differential regulation in mice that are resistant or susceptible to IA. Generally, Th1 cells confer protection against fungal diseases, and Th2 responses are associated with disease progression [318, 329]. Protective immunity is associated with CD4+ Th1 cells producing IFN-γ, IL-2, and IL-15, macrophages producing IL-12, or those mice treated with IL-4 or IL-10 antagonists. Disease progression is seen in mice producing Th2 cytokines IL-4, IL-10, or IL-13 or mice treated with neutralizing antibody to IFN-γ or IL-12 [95, 329, 330].

Bronchoscopic findings in a murine model also indicate resistance to disease progression associated with elevated production of TNF-α, IL-6, IL-12, IFN-γ, and IL-2 [331]. IL-4 production is decreased in mice surviving IA infections [318]. IL-4–deficient mice were found to be more resistant than wild type mice to infections. When treated with cyclophosphamide, the wild-type mice died of infection, but the cyclophosphamide-treated IL-4–deficient mice were resistant to infection and survived. Lung T helper cells from IL-4–deficient mice produced 50%–60% elevated levels of IFN-γ and IL-12 but 50% reduced IL-5 and IL-10 levels, compared with those in non–IL-4–deficient mice. This indicates that IL-4 down-regulates IL-12 production and, consequently, Th1 cell development [331]. Th1 resistance was impaired on IL-12 neutralization and in IL-12–deficient mice [331]. However, administration of recombinant IL-12 failed to increase protective effects in mice [318]. IL-12 immunotherapy remains to be fully studied because of potential deleterious inflammatory responses [332]. A disseminated aspergillosis murine model demonstrated decreased fungal burden and increased survival of IL-10 knockout mice compared with wild type [333]. One in vitro study found that IL-10 suppressed oxygen radical production and hyphal damage of A. fumigatus. Anti–IL-10 antibody, IFN-γ, and GM-CSF administration counteracted the suppressive host defense effects of IL-10 on phagocyte hyphal damage and oxygen radical production [334].

Granulocyte colony-stimulating factor (G-CSF). Human recombinant G-CSF has been approved for clinical use since 1991 [335]. The potential of exogenously administered G-CSF therapy seems to be in maintaining the innate signal for longer production of PMNLs or initiating that signal earlier if endogenous production is decreased or insufficient during a specific time, such as during neutropenia after bone marrow transplantation [336]. One fear has been the unwanted side effect of increased inflammatory products, such as the untoward release of reactive oxygen species and lysosomal contents, with G-CSF use [337]. However, in vivo and human studies have also shown that G-CSF reduces production of inflammatory mediators such as IL-1, TNF-α, and IFN-γ [336].

In addition to increasing the number of mature circulating PMNLs, G-CSF enhances phagocytic activity and oxidative burst metabolism. Human G-CSF affects function of granulocytes only, not macrophages, and has been shown to have a protective effect in murine models of IA. Prophylaxis with human G-CSF and amphotericin B or itraconazole showed some additive effect in neutropenic animal models of IA but not in those immunosuppressed with cortisone, which has a greater effect against macrophages. In a neutropenic (cyclophosphamide-induced) murine model, human G-CSF alone was ineffective but with amphotericin B showed synergy in survival greater than with itraconazole and G-CSF [338].

Pretreatment of neutrophils with G-CSF and/or IFN-γ can attenuate the inhibitory effect of corticosteroids on PMNL-induced hyphal damage [339]. G-CSF administered to human volunteers increased the fungicidal activity through enhanced respiratory bursts of their PMNLs against Aspergillus conidia of their PMNLs by 4-fold [340]. However, there is no clear evidence that G-CSF benefits patients with aspergillosis. One review found no significant reduction in fungal infections in patients with acute myelocytic leukemia treated with G-CSF [341].


S174  •  CID 2003:37 (Suppl 3)  •  Steinbach and Stevens
exhibit enhanced conidial phagocytosis, oxygen radical production, and hyphal damage [342, 343]. GM-CSF promotes differentiation, proliferation, and activation of cells in the macrophage/monocyte system, prevents the defective in vitro antifungal activity of corticosteroid-treated monocytes [322], and also enhances phagocytic activity of PMNLs [319]. GM-CSF also doubles the life span of neutrophils, exerts a stimulatory effect, and enhances their attachment to endothelial cells [344].

GM-CSF has increased spleen cellularity in mice, indicating potent stimulation of hematopoietic cells, and increased IFN-α production by concanavalin A compared with control cells [345]. In another murine model, the antifungal activity of bronchoalveolar macrophages treated with dexamethasone was significantly less than that of macrophages from dexamethasone-plus GM-CSF–treated mice. This study also showed that GM-CSF administered before dexamethasone blocked the deleterious effects, but if given after dexamethasone, GM-CSF could not reverse the effect on macrophages [346]. This confirmed earlier in vitro work demonstrating that coincubation of bronchoalveolar macrophages with GM-CSF followed by dexamethasone significantly prevented the conidialidal suppression. Also, coincubation of bronchoalveolar macrophages with dexamethasone and subsequent GM-CSF removed the deleterious effect if the dexamethasone was discontinued, but if the dexamethasone pretreatment continued despite GM-CSF use, the anticonidial effects persisted [347]. In another study, both murine and human GM-CSF can counteract dexamethasone suppression of murine macrophage function [348].

GM-CSF can act synergistically with TNF-α [349], and there are case reports of success with GM-CSF as part of a treatment regimen [350, 351]. GM-CSF has been shown to offer some protection against IA in a clinical trial of patients with acute myelogenous leukemia, decreasing the fungal infection–related mortality from 19% to 2% [352]. A small pilot study of GM-CSF in combination with amphotericin B (1 mg/kg/day) for treatment of proven fungal infection included 2 patients with refractory aspergillosis. One patient with pulmonary IA who underwent bone marrow transplantation because of breast cancer showed a partial response, whereas another patient with acute myelogenous leukemia and sinopulmonary IA had treatment failure [353]. However, use of GM-CSF is not without its concerns. In 1 case, a child with IA received GM-CSF to overcome neutropenia and, after bone marrow recovery, developed pulmonary cavitation and fatal hemoptysis [354]. Bone marrow recovery may lead to liquefaction of pulmonary foci and to potential erosive bleeding resulting from an increased inflammatory response, especially in the first week following cavitation [355].

M-CSF modulates mononuclear phagocyte functions such as H2O2 production and phagocytosis and enhances production of IL-1, IFN-γ, and TNF-α [356, 357]. M-CSF was found to enhance the nonoxidative mechanism of macrophages for inhibiting germination, but, although it recruited more macrophages to ingest conidia, it did not significantly effect ingestion of more conidia [357]. A neutropenic rabbit model demonstrated that prophylactic administration 3 days before inoculation and then throughout neutropenia augmented pulmonary host defenses against IA. Rabbits receiving M-CSF had increased survival rates and greater numbers of activated pulmonary alveolar macrophages than did control animals [358]. A phase I trial of M-CSF suggested some benefit in patients with Aspergillus infections, but an insufficient number of patients were treated to show a statistical benefit [343, 359].

TNF-α. TNF-α is a proinflammatory cytokine secreted by various macrophage populations and is shown to be a critical initiator in innate immunity against respiratory pathogens [360], including A. fumigatus [361]. In vitro, TNF-α appears to enhance early host defense against Aspergillus invasion, with slight increases in oxygen radical production by macrophages, up-regulation and activation of alveolar macrophage phagocytosis, and augmented production of other cytokines such as GM-CSF. It also augments a late defense with increased PMNL hyphal damage by production of oxygen radicals [356, 362].

In vitro, GM-CSF and TNF-α administration have been shown to counteract dexamethasone-induced immunodeficiency [322]. Animal model depletion of TNF-α results in increased fungal burden and mortality [361], and resistance is further impaired in IFN-γ–deficient mice [331]. Treatment of mice with neutralizing antibodies to TNF-α and GM-CSF reduces the influx of PMNLs into the lungs and delays fungal clearance [363]. Intratracheal administration of a TNF-α agonist resulted in survival benefits when given 3 days before A. fumigatus inoculation but not when given concomitantly with conidia, suggesting that pretreatment may provide macrophage priming [361]. However, excessive toxicities in doses required to have a biologically useful effect preclude safe administration to humans [319, 362].

IFN-γ. IFN-γ promotes TNF-α production [329] and enhances PMNL- and mononuclear cell–induced damage by increasing the oxidative burst of PMNLs in response to stimuli such as nonopsonized hyphae of A. fumigatus [356]. IFN-γ and G-CSF can each enhance the oxidative bursts and fungicidal activity in vitro of human PMNLs against A. fumigatus hyphae, with the combination of the 2 cytokines showing an additive effect [364]. IFN-γ can also restore the corticosteroid-suppressed fungicidal activity of human PMNL and elutriated monocytes [322, 342, 365], and IFN-γ–treated human monocytes show enhanced oxygen radical production and damage to A. fumigatus hyphae [342].

Exogenous administration of IFN-γ and TNF-α has resulted in protective effects in a murine model of IA [366] by decreasing mortality and the number of organs affected by Aspergillus.
Conversely, IFN-γ and TNF-α neutralization resulted in increased disease and increased expression of IL-10. Although IFN-γ is better than G-CSF or GM-CSF at enhancing PMNL hyphal damage, and both IFN-γ and GM-CSF result in enhanced hyphal damage by PMNLs in vitro [367], combination treatment does not increase damage [342, 368]. In vitro, IFN-γ augments PMNLs of patients with CGD by an undetermined mechanism [369], although previous work demonstrated a myeloperoxidase-dependent oxidative process [370]. IFN-γ has been proven to help prevent IA in patients with CGD [371], and there are case reports of the successful use of antifungals and IFN-γ for treatment of patients with CGD [372, 373]. One recent case report details use of liposomal amphotericin B and both GM-CSF and IFN-γ in successful treatment of sino- cerebral aspergillosis, with the addition of the IFN-γ temporally related to clinical resolution [374].

Combination antifungal and immune therapy. An additive effect with monocytes and monocyte-derived macrophages, but not PMNLs, and caspofungin was seen on Aspergillus hyphal growth [253]. An additive antifungal effect of hyphae cocultured with neutrophils and anidulafungin was also seen, and monocytes cocultured with hyphae preexposed to anidulafungin were synergistic [285]. Theoretically, effector cell, antifungal, and cytokine mechanisms may interact synergistically, with possible antifungal cell wall damage increased by a cytokine or cytokine stimulation of effector cells enhancing penetration of antifungals into cells [375]. Amphotericin B interacting with host cells can also have a positive effect by activation of macrophages through an oxidation-dependent process [11]. Neutrophils and monocytes with voriconazole have been shown to act additively in inhibiting fungal hyphal growth. GM-CSF treatment of neutrophils with voriconazole increases activity against hyphae compared with that of control neutrophils and voriconazole, but no comparable effect was seen on monocytes [365, 376]. An in vivo additive effect was also found with G-CSF and posaconazole in 1 study [325], and no antagonism was seen in another in vivo study [377].

Granulocyte transfusions. Granulocyte transfusions were first used to treat bacterial infections in neutropenic patients in 1934 [378], but the discovery that functionally intact transfused PMNLs are found in bronchoalveolar lavage fluid [379] has increased interest in the therapy for IA. However there are concerns with granulocyte transfusion: alloimmunization [380], which could complicate subsequent transplantation, or amphotericin B and neutrophil aggregation leading to pulmonary leukostasis and respiratory failure [381]. A review of 125 granulocyte transfusions given with concurrent amphotericin B to 31 granulocytopenic patients revealed pulmonary deterioration temporally related to therapy with amphotericin B, granulocyte transfusions, or both. However, they failed to show a specific detrimental interaction between the granulo-
solubility, but resistance has been described for both amphotericin B and itraconazole. Most experts agree that the present new antifungal for primary therapy against IA is voriconazole, with continued mounting evidence for its success.

To date no clinical study has convincingly answered the question of combination therapy or sequential therapy. Studies with amphotericin B and itraconazole, for instance, have produced a range of effects from synergy to antagonism [390], but guidelines [7] and experience [8] dictate clinical safety with amphotericin B followed by itraconazole. Therapy has most recently been viewed as a sequential approach, in which a patient with IA first is treated with conventional amphotericin B as a first-line drug, which is replaced with a lipid formulation if renal dysfunction occurs. If the patient survives and is able to take oral therapy, has good intestinal function, and is not taking drugs that induce the metabolism of the cytochrome P-450 enymes, itraconazole can be used as a maintenance drug [68]. Combination antifungal therapy for IA is reviewed in detail elsewhere [202].

Presently we are able to use less-toxic lipid formulations of amphotericin B, but the future holds the promise of newer antifungals, such as second-generation triazoles and the echinocandins with novel targets, as well as modification of the host immune response. Attacking the host immune response instead of the fungus itself is attractive, yet, to date, only data derived from vitro animal model studies and isolated or anecdotal clinical reports suggest improvement with immunomodulators. Data are lacking because of the lack of statistical power of many clinical studies to demonstrate a conclusive difference with immunomodulatory therapy [95, 391]. Newer antifungals and the possible synergistic effect of antifungal combinations, possibly with cytokine therapy, are a welcome, optimistic light in the dark history of treatment of IA.

References

Antifungal and Immunomodulatory Therapy • CID 2003;37 (Suppl 3) • S177

33. Dominguez JM, Kelly VA, Kinsman OS, Marriott MS, Gomez de las Heras F, Martin JJ. Sordarins: a new class of antifungal with selective
Antifungal and Immunomodulatory Therapy • CID 2003:37 (Suppl 3) • S179


331. Cenci E, Mencacci A, Del Sero G, et al. Interleukin-4 causes suscep-


368. Rex JH, Bennett J, Gallin JI, Malech HL, DeCarlo ES, Melnick DA.


