New Drugs and Novel Targets for Treatment of Invasive Fungal Infections in Patients with Cancer

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INTRODUCTION

The incidence of both mucosal and invasive fungal infections in immunocompromised patients has increased steadily over the past two decades [1]. Apart from organ transplant recipients, individuals with AIDS, and patients hospitalized with severe illnesses, major increases in invasive fungal infections have been observed in patients with hematologic malignancies who receive induction or consolidation chemotherapy and those who undergo bone marrow transplantation (BMT) [2-6].

Candida albicans and Aspergillus spp. account for most of the invasive infections in the neutropenic cancer patient. However, non-albicans Candida spp. and previously uncommon opportunistic fungal pathogens such as Fusarium spp., zygomycetes, and dematiaceous molds are observed with increasing frequency in this population [2, 7-9] (Table 1).

For many years, the treatment of most invasive fungal infections was essentially limited to amphotericin B (AmB) with or without 5-flucytosine (5-FC). Therapeutic options did not expand until the late 1980s, when fluconazole and itraconazole were introduced (Table 2). Triggered by the increasing number of neutropenic patients and patients with AIDS, the past decade, however, has seen a major expansion in antifungal drug research. These efforts have resulted in the development of novel lipid-based formulations of AmB and the discovery or design of several new antifungal compounds that are currently at various stages of clinical investigation (Table 3).

In order to overcome these limitations, new antifungal compounds are being developed, which may improve our therapeutic armamentarium for prevention and treatment of invasive mycoses in high-risk patients with neoplastic diseases. The Oncologist 2000;5:120-135

This article reviews the current developmental agents for systemic treatment of fungal infections: the advanced-generation antifungal triazoles; novel cell-wall inhibitors such as echinocandins and nikkomycin; pradimicins; sor- darins; and the unilamellar form of liposomal nystatin, the first antifungal polyene discovered in the early 1950s. The lipid formulations of AmB, which have been

### Table 1. Fungal pathogens in cancer patients

<table>
<thead>
<tr>
<th>Opportunistic yeasts</th>
<th>Examples</th>
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<tbody>
<tr>
<td>Cryptococcus</td>
<td>C. neoformans</td>
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<tr>
<td>Other Yeasts</td>
<td>Trichosporon species, Blastoshizomyces species</td>
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<tr>
<th>Opportunistic molds (hyalohyphomycetes)</th>
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<tr>
<td>Aspergillus spp.</td>
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<td>Fusarium spp.</td>
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<td>Zygomyces</td>
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<th>Dematiaceous molds (phaeohyphomycetes)</th>
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<tbody>
<tr>
<td>Pseudallescheria boydii</td>
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<tr>
<td>Bipolaris</td>
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<tr>
<td>Alternaria and other rare pathogens</td>
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<tr>
<th>Endemic dimorphic molds</th>
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<tbody>
<tr>
<td>Histoplasma capsulatum</td>
</tr>
<tr>
<td>Coccidioides immitis</td>
</tr>
<tr>
<td>Blastomyces dermatitidis</td>
</tr>
<tr>
<td>Penicillium marneffei</td>
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* Most common organisms

This article reviews the current developmental agents for systemic treatment of fungal infections: the advanced-generation antifungal triazoles; novel cell-wall inhibitors such as echinocandins and nikkomycin; pradimicins; sor- darins; and the unilamellar form of liposomal nystatin, the first antifungal polyene discovered in the early 1950s. The lipid formulations of AmB, which have been
comprehensively reviewed [10-12], will not be considered in this article.

**Antifungal Compounds Targeting Cell Membrane**

**Antifungal Triazoles**

The azole antifungal agents in clinical use for systemic treatment contain either two or three nitrogens in the azole ring and thereby are classified as imidazoles (ketoconazole and miconazole) or triazoles (itraconazole and fluconazole), respectively. Azole antifungal agents inhibit the synthesis of ergosterol, the major sterol component in the fungal plasma membrane, by interfering with the cytochrome p-450-dependent enzyme lanosterol demethylase.

Fluconazole has proved to be an effective agent for mucosal forms of candidiasis in neutropenic patients [13] and it is as effective as AmB in the treatment of candidemia in non-neutropenic individuals, but better tolerated [14, 15]. In the neutropenic hosts, fluconazole can be used for the treatment of invasive *Candida* infection caused by susceptible organisms in patients who are stable and who have not been pretreated with an azole agent [16-18]. Fluconazole also can be effective in patients with chronic disseminated candidiasis [19, 20]. Fluconazole is not clinically indicated for treatment of *Candida krusei* and some *Candida glabrata* infections and it is ineffective against opportunistic filamentous fungi [21]. Apart from inherited resistance, acquired resistance to fluconazole has been increasingly observed in patients with HIV infection and oropharyngeal candidiasis. In addition, breakthrough fungemia due to resistant *C. albicans* has been reported recently in BMT patients [22-24]. *C. glabrata* may also acquire resistance to fluconazole and may cause breakthrough infection.

Fluconazole is generally well tolerated at the usual dosage range of 100-400 mg/day and even at doses of up to 1,200 mg/day. Nausea, vomiting, and other gastrointestinal symptoms are reported in less than 5%, skin rash and headaches in less than 2%, and usually reversible, asymptomatic elevations of hepatic transaminases in 7% of adult patients.

In contrast to fluconazole, which is only active against yeasts, itraconazole has clinically useful activity against filamentous fungi [25]. In an open clinical trial investigating treatment for invasive aspergillosis performed by the National

| Table 2. Systemic antifungal agents in clinical use |
|-----------------|--------------------------------------------------|
| **Class and Compound** | **Mechanism of Action** |
| **Polene antibiotics** | | |
| Amphotericin B | Interaction with ergosterol, formation of aqueous channels, increased membrane permeability to univalent and divalent cations, cell death. |
| Lipid formulation of amphotericin B | | |
| **Nucleoside analogue** | | |
| 5-Flucytosine | Intracellular deamination to 5-fluorouracil; interference with both DNA and RNA synthesis/function. |
| **Antifungal azoles** | | |
| Ketoconazole | Interaction with cytochrome p-450; inhibition of C-14 demethylation of lanosterol, causing ergosterol depletion and accumulation of aberrant and toxic sterols in the cell membrane. |
| Fluconazole | | |
| Itraconazole | | |
| **Allylamines** | | |
| Terbinafine | Inhibition of squalene epoxidase, resulting in ergosterol depletion and accumulation of aberrant and toxic sterols in the cell membrane. |
| **Cell mitosis inhibitor** | | |
| Griseofulvin | Inhibition of fungal cell mitosis at metaphase by interaction with polymerized microtubules. |

| Table 3. Current investigational systemic antifungal agents |
|-----------------|--------------------------------------------------|
| **I. Antifungal compounds targeting fungal cell membrane** | | |
| **Antifungal triazoles** | | |
| Voriconazole | Interaction with cytochrome p-450; inhibition of C-14 demethylation of lanosterol, causing ergosterol depletion and accumulation of aberrant and toxic sterols in the cell membrane. |
| UK 109496 | | |
| Posaconazole | Liposomal nystatin | Interaction with ergosterol, formation of aqueous channels, increased membrane permeability to univalent cations, and, ultimately, cell death. |
| Sch 56592 | | |
| Ravuconazole | Liposomal nystatin | | |
| BMS-207147 | | |
| **Polyene antibiotics** | | |
| Liposomal | | |
| | | | |
| **Pradimicins** | Calcium-dependent complexing with cell-wall mannoproteins, leading to perturbation of the cell membrane and, ultimately, cell death. |
| Benamomycin A | | |
| | | | |
| **II. Antifungal compounds targeting fungal cell wall** | | |
| **Echinocandins and pneumocandins** | | |
| LY 303366 | Inhibition of fungal β-(1, 3) glucan synthase complex, leading to depletion of cell-wall glucan and osmotic instability. |
| MK 0991 (L743872) | | |
| FK 463 | | |
| **Nikkomycins** | Inhibition of fungal chitin synthase, leading to inhibition of growth and osmotic fragility. |
| Nikkomycin Z | | |
| | | | |
| **III. Antifungal compounds targeting fungal protein synthesis** | | |
| **Sordarins** | Interaction with fungal elongation factor 2. |
| | | |
Each of these new agents is active following oral administration. The structure of posaconazole is very similar to that of itraconazole. Voriconazole (BMS-207147). While voriconazole and ravuconazole (UK 109496), posaconazole (Sch 56592), and ravuconazole (BMS-207147) are antifungal triazoles for systemic use. Among these are two benzodiazepenes may achieve elevated and sustained plasma concentrations, resulting in respiratory depression. The former three compounds have been associated with venous thrombosis through cytochrome p450 3A4 pathway. These include terfenidine, astemizole, cisapride, midazolam, and triazolam. The frequency of side effects of itraconazole in the capsule formulation is relatively low and includes nausea and vomiting (<10%), elevated transaminases (5%), skin rash and/or pruritus (2%), and headache and dizziness (<2%). A definite impediment to itraconazole treatment is its erratic oral absorption. An intravenous formulation is in the advanced stage of clinical development. The cyclodextrin oral solution improves bioavailability but may cause gastrointestinal symptoms at higher dosages (>5 mg/kg/day). Itraconazole carries the potential for interfering with the cytochrome p450 enzyme system, which leads to a number of significant drug-drug interactions, including cyclosporin A, tacrolimus, and vincristine, drugs that are often used in cancer patients [31]. Several drugs are contraindicated in combination with itraconazole; such agents share metabolism through cytochrome p450 3A4 pathway. These include terfenidine, astemizole, cisapride, midazolam, and triazolam. The former three compounds have been associated with ventricular arrhythmias when combined with itraconazole. The two benzodiazepenes may achieve elevated and sustained plasma concentrations, resulting in respiratory depression.

The continuing demand for safe and effective broad-spectrum antifungal agents with favorable pharmacokinetic properties has spurred both the design and development of new antifungal triazoles for systemic use. Among these are voriconazole (UK 109496), posaconazole (Sch 56592), and ravuconazole (BMS-207147). While voriconazole and ravuconazole are structurally closely related to fluconazole, the structure of posaconazole is very similar to that of itraconazole. Each of these new agents is active following oral administration; voriconazole is also available in a parenteral formulation.

Voriconazole (formerly UK 109496)

Voriconazole is a new triazole antifungal agent that is derived from the structure of fluconazole, having one triazole moiety replaced by a fluropyrimidine grouping, and a methyl group added to the propanol backbone (Fig. 1). These structural changes result in increased activity at the target enzyme lanosterol demethylase and an enhanced antifungal spectrum that includes filamentous fungi. Voriconazole is orally and parenterally active.

In Vitro Activity

Voriconazole exhibits a wide spectrum of activity against clinically important fungal pathogens such as Candida spp. [32-34]; Aspergillus spp. [35, 36]; Cryptococcus neoformans [37]; dimorphic fungi such as Histoplasma capsulatum, Blastomyces dermatitidis, Coccidioides immitis, Penicillium marneffei; emerging pathogens such as Fusarium spp. and other hyalohyphomycetes; and some of the dematiaceous molds [38, 39].

Compared to reference agents, on a milligram-to-milligram basis, voriconazole was 4- to 16-fold more active than fluconazole and two- to eightfold more active than itraconazole against Candida spp., including C. krusei and C. glabrata [37, 40]. However, for isolates of Candida spp. with decreased susceptibility to fluconazole and itraconazole, MICs of voriconazole were also higher [33]. Of note, voriconazole was more active than AmB or 5-FC against all species of Candida except for C. glabrata [33]. The compound was active against both fluconazole-susceptible and -resistant strains of C. neoformans. However, higher MICs were observed in the few fluconazole-resistant strains, suggesting cross-resistance [41]. Noteworthy was the high activity of voriconazole in vitro against A. fumigatus (mean MIC = 0.23 µg/ml), exceeding that of itraconazole [42, 43].

Experimental Animal Models of Invasive Fungal Diseases

Voriconazole was as effective as fluconazole and itraconazole in experimental systemic candidiasis in both non-neutropenic and neutropenic guinea pigs and had clinical activity in animals infected by fluconazole-resistant strains of C. albicans, C. krusei, and C. glabrata where fluconazole was inactive [44]. This compound had activity superior to that of AmB and itraconazole in experimental disseminated aspergillosis in neutropenic guinea pigs, and was more efficacious than itraconazole in experimental pulmonary aspergillosis in immunocompromised guinea pigs [45]. Similar findings were noted in temporarily neutropenic rabbits and in steroid-treated rats [36, 46]. Thus voriconazole prolonged survival and reduced organism burden in infected tissue in several models [47]. In addition, voriconazole was highly efficacious in both the prophylaxis and treatment of experimental left-sided Aspergillus endocarditis in the guinea pig [48]. Voriconazole had efficacy comparable to that of fluconazole and itraconazole in experimental studies.
Pulmonary and meningocerebral cryptococcosis in the guinea pig [49].

**Pharmacokinetics and Tissue Distribution**

Voriconazole displays nonlinear plasma pharmacokinetics [50]. In human volunteers, peak plasma levels are attained within 2 h after oral dosing. Bioavailability is up to 90% depending on the dose. Plasma protein binding in humans is approximately 65%. The drug is extensively metabolized: 78%-88% of a single dose appears in the urine but less than 5% is unchanged compound. The elimination half-life is approximately 6 h. Multiple dosing leads to reduced systemic clearance and up to eightfold accumulation. The apparent volume of distribution at steady state of 2 l/kg suggests widespread distribution throughout the body [51, 52]. It is of interest that animal studies revealed good penetration into the central nervous system (CNS) and cerebrospinal fluid (CSF) [53]. In the guinea pig model, steady-state concentrations of voriconazole in CSF were approximately 50% of the concurrent concentrations in plasma, whereas those in CNS tissues were approximately twofold higher.

**Clinical Studies**

Data from phase II clinical trials indicate that voriconazole is a promising new agent for the treatment of oropharyngeal candidiasis, esophageal candidiasis, and acute and chronic invasive aspergillosis [40, 54-56]. Even in severe CD4 lymphocyte-depleted patients with end-stage AIDS who had fluconazole-refractory candidiasis, switching to voriconazole was effective in the majority of patients [57]. Voriconazole has been reported to successfully treat disseminated *Scedosporium* infection in a neutropenic patient and a case of cerebral aspergillosis, an infection in which the mortality is exceedingly high [58, 59]. The therapeutic activity of voriconazole in acute and chronic invasive aspergillosis was evaluated in open, noncomparative studies [54-56]. Immunocompromised patients with acute invasive aspergillosis, 72% of whom had not responded to prior therapy with AmB or itraconazole, received voriconazole in different dosages. An interim analysis of data for 53 of 71 evaluable patients showed that treatment with voriconazole resulted in a favorable clinical response in 29 patients (74%). Non-neutropenic patients with chronic invasive aspergillosis, approximately 50% of whom had failed to respond to prior treatment with either amphotericin or itraconazole, were treated with voriconazole 200 mg p.o. twice daily for 4 to 24 weeks. At interim analysis, voriconazole achieved a favorable clinical response in 9 (69%) of 13 evaluable patients.

Two phase III clinical trials comparing voriconazole with established agents are in progress: treatment of invasive aspergillosis (voriconazole versus AmB) and empirical antifungal therapy in persistently febrile neutropenic patients (voriconazole versus liposomal AmB).

Side effects of voriconazole include elevated hepatic transaminases in 10%-15% of patients, skin rash in 1%-5%, and transient visual disturbances in 10%-15%, which in animal models had no morphological correlates. This compound otherwise appears to be safe.

**Posaconazole (Sch 56592)**

Posaconazole is a hydroxylated analog of itraconazole (Fig. 1). Compared with itraconazole and similar to voriconazole, it has potent activity toward a variety of medically important fungi. Sch 56592 is available as an oral formulation.
In Vitro Activity

Posaconazole possesses potent broad-spectrum activity against opportunistic and endemic fungal pathogens. These include Candida spp. [34, 60, 61], C. neoformans [62, 63], Aspergillus spp. [64], Trichosporon beigeli [65], other less common, filamentous fungi, including zygomycetes, Fusarium spp., and some of the dematiaceous molds [65]. Compared with currently available triazoles, the activity of posaconazole is similar to that of itraconazole and at least eightfold greater than that of fluconazole against Candida spp. However, similar to that which has been observed in voriconazole, higher MICs were observed among these infections in humans. Posaconazole showed efficacy against pulmonary aspergillosis in corticosteroid-treated mice while treatment with AmB showed little effect [70]. In a neutropenic murine model of invasive aspergillosis, posaconazole reduced fungal burdens in both groups of animals infected with itraconazole-susceptible or -resistant organisms, and was superior to AmB [71]. No synergy or antagonism was noted when posaconazole was administered concomitantly with AmB to mice infected with C. albicans or Aspergillus flavus [47].

Studies in animal models of endemic mycoses indicate that posaconazole warrants further evaluation in the treatment of these infections in humans. Posaconazole showed efficacy in murine models of disseminated coccidiomycosis, blastomycosis, and histoplasmosis [72-74].

Experimental Animal Models of Invasive Fungal Diseases

Posaconazole has demonstrated therapeutic efficacy in a number of animal models of fungal infections, including vaginal, oropharyngeal, and systemic candidiasis, systemic and pulmonary aspergillosis, cryptococcal meningitis, disseminated histoplasmosis, and coccidiomycosis [47]. Posaconazole was superior to fluconazole or itraconazole or both in dermatophytoes, vaginal and gastrointestinal candidiasis, and systemic candidiasis [66-69]. Posaconazole prolonged survival and reduced lung tissue counts of A. fumigatus in a model of invasive aspergillosis in corticosteroid-treated mice while treatment with AmB showed little effect [70]. In a neutropenic murine model of invasive aspergillosis, posaconazole reduced fungal burdens in both groups of animals infected with itraconazole-resistant organisms. In this model, compared with fluconazole and itraconazole, only ravuconazole caused a significant reduction in fungal burden in lung tissue. Ravuconazole showed dose-dependent therapeutic efficacy against pulmonary C. neoformans infections in immunocompromised mice caused by fluconazole-resistant organisms. In this model, compared with fluconazole and itraconazole, only ravuconazole caused a significant reduction in fungal burden in lung tissue. Ravuconazole showed dose-dependent therapeutic efficacy against pulmonary C. neoformans infections in immunocompetent mice. In a murine model of intracranial cryptococcosis in healthy mice, ravuconazole reduced the fungal burden in brain tissue as compared with itraconazole and control treatment. Ravuconazole demonstrated activity comparable to that of AmB in a model of disseminated aspergillosis in neutropenic rabbits [80].

Pharmacokinetics and Tissue Distribution

No data have been published regarding the disposition in human volunteers or patients. A preliminary pharmacokinetic evaluation of posaconazole was conducted following single-dose i.v. (in hydroxypropyl-β-cyclodextrin vehicle) and p.o. (suspension in methylcellulose) administration to mice, rats, dogs, and cynomolgus monkeys and p.o. administration to rabbits. In dogs, absorption from the gastrointestinal tract was slow and oral bioavailability was 43%-48% [75]. Posaconazole revealed dose-dependent disposition with an elimination half-life of 18 h and 22 h in dogs and monkeys, respectively. Twenty-four hours after a single oral dose, concentrations of posaconazole in plasma were above the MIC and MFC (minimal fungicidal concentration) of most clinically relevant fungal pathogens [76].

Ravuconazole (BMS-207147, formerly ER30346)

Ravuconazole is a derivative of fluconazole with an expanded spectrum of activity in vitro (Fig. 1). It is available in oral form.

In Vitro Activity

Ravuconazole possesses broad-spectrum activity against important fungal pathogens, including Candida spp., A. fumigatus, and C. neoformans, as well as most hyaline hyphomycetes (except Fusarium spp.) and some of the dematiaceous fungi (except Pseudallescheria boydii) [40, 77, 78]. Similar to voriconazole and posaconazole, ravuconazole has expanded coverage against C. glabrata and C. krusei in comparison with fluconazole [34].

Experimental Animal Models of Invasive Fungal Diseases

The therapeutic efficacy of oral ravuconazole was compared with that of fluconazole and itraconazole in murine models of systemic and pulmonary infection due to Candida spp., Aspergillus spp., and C. neoformans [79]. BMS-207147 was active against pulmonary candidiasis in immunocompromised mice caused by fluconazole-resistant organisms. In this model, compared with fluconazole and itraconazole, only ravuconazole caused a significant reduction in fungal burden in lung tissue. Ravuconazole showed dose-dependent therapeutic efficacy against pulmonary C. neoformans infections in immunocompetent mice. In a murine model of intracranial cryptococcosis in healthy mice, ravuconazole reduced the fungal burden in brain tissue as compared with itraconazole and control treatment. Ravuconazole demonstrated activity comparable to that of AmB in a model of disseminated aspergillosis in neutropenic rabbits [80].

Pharmacokinetics and Tissue Distribution

Plasma pharmacokinetics of ravuconazole have been studied following p.o. and i.v. administration of single doses to mice, rats and dogs [78, 81]. Over a dose range of 2-40 mg/kg in mice, dose proportional increases in C_max and area under the curve (AUC) were observed. The presence of food enhanced absorption. In dogs, the mean elimination half-life after i.v. injection was 8.8 h.

Liposomal Nystatin

Discovered in the early 1950s, nystatin is the first compound of the large class, polyene antifungal antibiotic
It was never developed for systemic use. However, in the late 1980s, Metha and co-workers demonstrated that encapsulation of nystatin in liposomes had tolerable toxicity and promising antifungal efficacy in vivo [82, 83]. Several recent studies have assessed the efficacy of liposomal nystatin against disseminated candidiasis and invasive *A. fumigatus* infection in both immunocompetent and neutropenic hosts [84-87]. Whereas free nystatin was lethal and non-effective at its maximal tolerated dose in a mouse model of systemic candidiasis, the liposomal formulation was tolerated and improved survival at equivalent doses, and had markedly increased activity at higher dosages. Liposomal nystatin was well tolerated and was effective in a survival model of disseminated aspergillosis. It was also effective against disseminated and invasive pulmonary aspergillosis in neutropenic rabbits where it prolonged survival and reduced residual tissue fungal burden [87].

In humans, the compound appears to follow linear plasma pharmacokinetics with peak plasma levels above the MIC of most relevant fungi, and a terminal half-life in the range of 5-7 h at dosages of 1-7 mg/kg/day [87, 88].

Early phase I clinical studies in patients with hematological malignancies and refractory febrile neutropenia revealed no dose-limiting nephrotoxicity at dosages of up to 8 mg/kg/day and a relatively low frequency of infusion-related reactions. Ten of 27 evaluable patients (37%) responded to therapy [89]. Currently ongoing clinical trials target non-neutropenic patients with candidemia, patients with neutropenia and persistent fever, and patients with invasive fungal infection refractory to standard antifungal therapy.

**Pradimicin**

The pradimicins and the structurally similar benanomicins constitute a unique class of fungicidal antifungal antibiotics originally derived from culture broth filtrates of *Actinomycetes*. The chemical structure of these compounds is characterized by a benzonaphthacene quinolone skeleton substituted by a D-amino acid and a disaccharide side chain (Fig. 3). The pradimicins and benanomicins appeared to possess a novel mechanism of action consisting of specific binding recognition to terminal D-mannosides of the cell wall of fungi, resulting in the formation of a ternary complex consisting of D-mannoside, pradimicin, and calcium that leads to disruption of the integrity of the fungal cell membrane [90, 91]. Both pradimicins and benanomicins have broad-spectrum in vitro antifungal activity against *Candida* spp., *C. neoformans*, *Aspergillus* spp., dematiaceous molds, and zygomycetes [90, 92-94]. In vivo, in healthy rabbits, the lead pradamicin selected for development, BMS-181184, demonstrated nonlinear, dose-dependent kinetics with enhanced clearance, reciprocal shortening of elimination half-life, and an apparently expanding volume of distribution with increasing dosage [95]. BMS-181184 was active against disseminated candidiasis and showed efficacy equivalent to that of amphotericin when used against pulmonary aspergillosis in persistently neutropenic patients.
rabbits, without producing any nephrotoxicity [96, 97]. Unfortunately, due to the occurrence of hepatotoxicity in early clinical trials, which had been unpredictable from pre-clinical toxicity studies, the lead clinical compound was withdrawn from clinical investigation.

**Antifungal Compounds Targeting Cell-Wall Synthesis**

Ever since the discovery that penicillin inhibits bacterial cell-wall synthesis, pharmaceutical and academic research alike has been searching for equivalent agents to target fungi. The fungal cell wall is a structure that is essential for the fungus and absent from the mammalian host, and, consequently, presents an attractive target for new antifungals.

With considerable variation among different species, the gross macromolecular components of the cell wall of most fungi include chitin, α or β linked glucans and a variety of mannoproteins. The dynamics of the fungal cell wall are closely coordinated with cell growth and cell division, and its predominant function is to control the internal turgor pressure of the cell. Disruption of the cell-wall structure leads to osmotic instability, and, ultimately, lysis of the fungal cell [98].

Current investigational systemic antifungal agents directed against or involving the major constituents of the fungal cell wall include the echinocandin lipopeptides and the nucleoside-peptide antibiotic nikkomycin Z [99].

**Echinocandin Lipopeptides**

Perhaps the most interesting novel class of antifungal agents currently being evaluated in clinical trials is the echinocandins. The echinocandins are natural cyclic lipopeptide antifungal agents that interfere with cell-wall synthesis by noncompetitive inhibition of 1,3 β-D-glucan synthase, an enzyme that is absent in mammalian cells but present in most pathogenic fungi [100] (Fig. 4).

The first member of this class, cilofungin, was only active against *Candida* [98, 101]. Insights into the structure/activity relationship of this drug have led to the generation of
semisynthetic echinocandins with considerable increased potency and expanded spectrum and favorable pharmacokinetic properties. The compounds that are currently being developed for clinical use include LY 303366, MK 0991, and FK 463. Because of their distinct mechanism of action, the echinocandins have the potential for use in combination regimens with currently available standard antifungal agents.

**LY 303366**

**In Vitro Activity**

LY 303366 is water-soluble and only parenterally administrable. It has potent, rapid in vitro antifungal activity against most *Candida* spp., *Aspergillus* spp., and against both the trophic and cystic form of *Pneumocystis carinii* [65, 102-106]. As expected, LY 303366 is active against both fluconazole-sensitive and fluconazole-resistant strains of *Candida* spp. [102, 104, 107, 108]. A notable omission in the spectrum of activity of these agents is *C. neoformans*, a fungus that has a different composition of glucan polymer with predominant β 1,6-linkage. LY 303366 was also not active against *B. dermatitidis* because of greater cellular reliance upon α-1,3 D-glucan because of β linkage [104].

**Experimental Animal Models of Invasive Fungal Diseases**

LY 303366 has activity in murine models of superficial and disseminated candidiasis, disseminated aspergillosis and *P. carinii* pneumonia in both normal and immunocompromised animals [109-114]. The compound was effective against esophageal candidiasis caused by fluconazole-resistant strains of *C. albicans* in immunocompromised rabbits [115]. LY 303366 prolonged survival in experimental invasive pulmonary aspergillosis in persistently neutropenic rabbits and in a neutropenic murine model of disseminated aspergillosis [116]. This echinocandin reduced the residual fungal load and prolonged survival against both AmB-susceptible and AmB-resistant invasive *A. fumigatus* infection in a neutropenic mouse model [117].

**Pharmacokinetics and Tissue Distribution**

In rabbits, LY 303366 demonstrated dose-proportional pharmacokinetics, potentially peak plasma concentrations in excess of MIC values reported for most susceptible fungal pathogens, and a large volume of distribution suggestive of extensive distribution into tissues [118]. Indeed, tissue levels after multiple dosing are above the MIC in major organs, including the brain. In human volunteers, the compound exhibits linear pharmacokinetics after single oral doses of 100-1,000 mg. Peak plasma levels in that study occurred 6-7 h after ingestion, and the elimination half-life was approximately 30 h. The drug was well tolerated at doses of up to 700 mg with adverse gastrointestinal effects defining the maximal tolerated dose [119]. With the long plasma half-life, once-daily dosing is anticipated.

**MK 0991**

**In Vitro Activity**

MK is water-soluble and is available only in parenteral form. It has potent antifungal activity in vitro against most *Candida* spp., *Aspergillus* spp., and some dimorphic molds, such as *H. capsulatum*, *C. immitis*, and *B. dermatitidis* [107, 120, 121]. As in the case of LY 303366, MK 0991 showed no evidence of cross-resistance to antifungal triazoles [122]. The compound had no in vitro activity against *C. neoformans*, *T. beigelii*, dematiaceous molds, *Rhizopus*, or *Fusarium* spp. In vitro synergy of MK 0991 with AmB, 5-FC, and azoles was demonstrated against *C. neoformans* [123].

**Experimental Animal Models of Invasive Fungal Diseases**

In both immunocompetent and either neutropenic or corticosteroids immunocompromised murine models of disseminated candidiasis, MK 0991 prolonged survival and led to a significant reduction in the residual burden of fluconazole-sensitive and fluconazole-resistant *C. albicans* in kidney tissue at dosages as low as 0.0125 and 0.05 mg/kg/day [124-127]. MK 0991 significantly prolonged survival in murine models of disseminated aspergillosis in a manner comparable to AmB deoxycholate, both in immunocompetent and neutropenic animals [125-127]. MK was effective in both the prevention and therapy of experimental murine pulmonary pneumocystosis and in the treatment of murine histoplasmosis [128, 129].

**Pharmacokinetics and Tissue Distribution**

In mice, rats, monkeys, and chimpanzees, the drug demonstrated dose-proportional pharmacokinetics [130]. Oral bioavailability was <1% in these species. Protein binding was 96% in mouse and human serum. MK 0991 is excreted by the hepatic and renal route with terminal half-life in the range of 5-7.5 h. The single and multiple dose plasma pharmacokinetics of MK 0991 following 1-h intravenous infusions have been investigated in healthy male volunteers [131]. The data are supportive of the evaluation of once-daily dosing regimens in clinical efficacy studies.

**Clinical Studies**

MK 0991 is currently undergoing clinical investigation. Preliminary results [132] from phase II trials comparing intravenous two-week therapy with MK 0991 50 mg or 70 mg/day and AmB 0.5 mg/kg/day for endoscopically documented *Candida* esophagitis have been reported. Clinical responses (defined as reduction in symptoms and endoscopic
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lesions) were documented in 85.1% of MK 0991-treated patients versus 66.7% in the AmB group. Adverse effects during treatment with MK 0991 were uncommon. Additional human clinical trials and pharmacodynamic studies will help to define the role of this novel antifungal agent.

FK 463

In Vitro Activity

FK 463 is a new parenteral echinocandin antifungal drug undergoing clinical development. FK 463 is a lipopeptide compound synthesized by chemical modification of a product from the environmental mold Coleophoma empedri. FK 463 exhibits broad-spectrum activity against clinically important pathogens including Candida spp. and Aspergillus spp. FK 463 was also active against azole-resistant Candida spp. FK 463 had no in vitro activity against C. neoformans, T. beigellii, and Fusarium solani [133].

Experimental Animal Models of Invasive Fungal Diseases

In a murine model of disseminated candidiasis, FK 463 significantly prolonged the survival of infected mice and reduced the residual fungal load in kidney. The efficacy against pulmonary aspergillosis in neutropenic mice was comparable to that of AmB [133, 134]. However, profoundly neutropenic hosts with invasive aspergillosis may have less response to the class of echinocandins as compared to non-neutropenic patients [135].

Pharmacokinetics and Tissue Distribution

Preliminary plasma pharmacokinetic studies in rats and dogs showed dose-proportional increase in AUC and a half-life of approximately 4-6 h. The protein binding was more than 99% [136]. In a phase I study of FK 463 in healthy adult male volunteers, FK 463 was well tolerated up to 50 mg single dosing or 25 mg repeated dosing by all subjects. C\text{max} and AUC increased in proportion to the dose up to 50 mg. Plasma concentration attained steady state by day 4 with repeated dose [137].

Nikkomycin

Nikkomycin

Chitin is a linear polymer of β-(1,4)-linked N acetyl-glicosamine residues. It is synthesized on the cytoplasmic surface of the plasma membrane by chitin synthase [101]. The nikkomycins are potent competitive inhibitors of chitin-synthase. The chemical structure consists of a pyrimidine nucleoside linked to a short peptide moiety (Fig. 5).

The in vitro activity of nikkomycin Z is essentially confined to highly chitinous fungi such as C. immitis and B. dermatitidis. Nikkomycin Z showed promising activity in murine models of pulmonary blastomycosis, coccidiodomycosis, and histoplasmosis [138-140]. The compound was only modestly active against Candida spp. However, recent in vitro studies demonstrate synergistic activity against strains of Candida spp, C. neoformans, and A. fumigatus when nikkomycin Z was combined with fluconazole or itraconazole [141-143]. Based on its unique target, nikkomycin is a potential candidate for clinical development for endemic fungi and in particular in combination with other cell-wall active agents, or the antifungal triazoles.

Antifungal Compounds Targeting Protein Synthesis

Sordarins

Protein synthesis has always been considered a highly attractive target in the development of antimicrobial agents. However, application of this idea to the field of antifungal therapy is not an easy task since selectivity is hampered due to the eukaryotic nature of fungi and therefore to the great degree of similarity between the fungal and mammalian protein synthesis [144]. Sordarins, natural fungal products,
exert their antifungal effects by specifically inhibiting the protein synthesis elongation cycle in yeasts without affecting protein synthesis machinery in mammalian systems [144] (Fig. 6). The proposed mechanism of action of sordarins appears to be inhibition of elongation factor 2 [145]. Some sordarin derivatives, such as GM222712 and GM237354, display excellent in vitro activities against a wide range of pathogenic fungi, including Candida spp., C. neoformans, P. carinii, and certain filamentous fungi and emerging invasive fungal pathogens [146]. Pharmacokinetic studies, in vivo models, and safety data are pending and will delineate whether sordarins can be further developed.

**Recombinant Human Cytokines in the Management of Fungal Infection in Neutropenic Patients**

Reversal or amelioration of immunosuppression is a prerequisite for successful management of invasive fungal infections in any patient. This may include dose reduction or withdrawal of corticosteroids, transfusion of granulocytes, and use of cytokines (such as G-CSF and GM-CSF).

Utilization of recombinant hematopoietic cytokines has led to shortening of the duration of neutropenia and, thereby, to a shortening of the period of greatest risk for developing invasive fungal infections. On the other hand, they also allow for increased dose intensity of antineoplastic chemotherapy. Thus, the full clinical impact of this now ubiquitously employed modality on invasive fungal infections is unclear.

Laboratory studies with GM-CSF suggest that this recombinant cytokine may be active as adjunctive therapy in the management of invasive fungal infections. Interferon γ has been shown to work cooperatively with amphotericin in vivo against cryptococcosis [147]. G-CSF combined with fluconazole extended survival beyond that for fluconazole alone and reduced renal tissue counts below those for fluconazole alone in experimental disseminated candidiasis [148]. It was demonstrated in vitro that voriconazole could collaborate with G-CSF or GM-CSF-activated polymorphonuclear neutrophil or monocytes for significant increased candidacidal activity [149]. This finding suggests that antifungal agents would have additional efficacy in clinical settings in which G-CSF or GM-CSF is used. In a murine pulmonary invasive aspergillosis model, the combination of GM-CSF and voriconazole showed additive efficacy in neutropenic mice [150]. These encouraging results provide a foundation for further laboratory and clinical investigation of recombinant cytokines in the prevention and treatment of fungal infections in neutropenic patients.

**Conclusions**

Invasive fungal infections are a frequent and important complication in neutropenic cancer patients and in patients undergoing stem cell or marrow transplantation. The field of antifungal therapy is currently undergoing accelerated changes, and new antifungal agents with novel mechanisms of action will enter clinical practice. In parallel with laboratory evaluation of safety and efficacy, it is hoped that rationally designed clinical trials with these new compounds may ultimately lead to improved prevention and treatment of life-threatening fungal infections.

**References**


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Invasive Fungal Infections in Patients with Cancer


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