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REVIEW

New facets of antifungal therapy

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ABSTRACT

Invasive fungal infections remain a major cause of morbidity and mortality in immunocompromised patients, and such infections are a substantial burden to healthcare systems around the world. However, the clinically available armamentarium for invasive fungal diseases is limited to 3 main classes (*i.e.*, polyenes, triazoles, and echinocandins), and each has defined limitations related to spectrum of activity, development of resistance, and toxicity. Further, current antifungal therapies are hampered by limited clinical efficacy, high rates of toxicity, and significant variability in pharmacokinetic properties. New antifungal agents, new formulations, and novel combination regimens may improve the care of patients in the future by providing improved strategies to combat challenges associated with currently available antifungal agents. Likewise, therapeutic drug monitoring may be helpful, but its present use remains controversial due to the lack of available data. This article discusses new facets of antifungal therapy with a focus on new antifungal formulations and the synergistic effects between drugs used in combination therapy.

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Introduction

Approximately 1.2 billion individuals worldwide suffer from fungal infections, and the occurrence of these infections has significantly increased in recent years due to a rise in the number of immunocompromised patients, such as patients with AIDS or those with cancer, organ transplant, or autoimmune disease who require immunosuppressive therapy.[1,2] Unlike superficial infections that cause local, benign, or self-limiting diseases, invasive fungal infections (IFIs) are deep-seated and include bloodstream and systemic infections as well as infection of specific organs. IFIs are frequently caused by yeast pathogens such as *Candida* and *Cryptococcus*; filamentous fungi such as *Aspergillus, Fusarium*, or *Mucor*; or less frequently dimorphic fungi, including *Coccidioides, Blastomyces*, or *Histoplasma*.[3-6] Currently, only 3 main classes of antifungals are approved for treatment of patients with IFIs: polyenes, triazoles, and echinocandins. These agents target ergosterol, lanosterol 14-α-demethylase, and β-1,3 glucan synthase, respectively.[7]

Because our current antifungal therapies have only modest efficacy with significant toxicities, newer antifungal formulations have been developed that ideally will reduce the occurrence of adverse effects associated with the original formulations.[8,9] The newest antifungal drug, isavuconazonium sulfate, is now commercially available in the United States and Europe. Isavuconazonium sulfate is a new member of the triazole class and provides an additional option for the treatment of aspergillosis and mucormycosis in adult patients.[10-12]

Due to the slow pace of novel antifungal drug development, combination therapy has been suggested as an alternative approach to increase fungicidal potency, combat emerging drug resistance, and improve spectrum of activity. Unfortunately, combination antifungal therapy has been shown to improve outcomes in few clinical scenarios.[13] Furthermore, adverse drug effects and drug interactions are more likely with combination therapy.

In this article, we review the most recent antifungal formulations and discuss antifungal combination therapy from a clinical perspective.

Antifungals currently in clinical use

Azoles

Azoles inhibit the fungal cytochrome P450 enzyme, lanosterol 14α-demethylase (CYP51), a key enzyme in ergosterol synthesis.[14] Unlike mammalian membranes, which are rich in cholesterol, ergosterol is the predominant sterol in the cell membrane of fungi. Thus, targeting ergosterol synthesis results in selectivity against fungi. The azoles also have higher affinity for fungal P450

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enzymes than the mammalian counterparts, adding to their selectivity.[15] Nevertheless, azoles do affect human cytochrome P450 (CYP) enzymes, resulting in significant drug interactions.[16] Interestingly, the human CYP isoforms that are affected vary depending on the azole, underlining the importance of evaluating each patient for potential drug interactions prior to azole use.

The first imidazole-based antifungal drugs, miconazole and ketoconazole, became available for systemic use in 1978 and 1981, respectively.[17,18] Ketoconazole became the standard drug used to treat candidiasis and infections caused by dimorphic fungi (Table 1). However, ketoconazole is associated with significant liver toxicity. Greenblatt *et al.* demonstrated that approximately 1 in 500 patients were at risk of liver injury after ketoconazole administration.[14] In the early 1990s, when the triazoles became available for systemic use, they rapidly supplanted ketoconazole.

Resistance to azole antifungals can be intrinsic (primary) or evolved. *Candida krusei* has strong intrinsic resistance to fluconazole, whereas *Candida glabrata* has intrinsic reduced susceptibility to fluconazole and, with increasing frequency, is evolving high-level fluconazole resistance.[19,20] The widespread use of fluconazole may be contributing to the increased incidence of *Candida* infections with evolved resistance and/or infections with intrinsically resistant non-*albicans Candida*. The antifungal drug resistance mechanisms of azoles include: (1) decreased effective drug concentration due to the activation of efflux transporters such as *CDR1* and *CDR2* in *C. albicans*, or overexpressing the drug target Erg11; (2) alteration of drug targets, such as *erg11* mutation, which has been shown to decrease the affinity of the target to azoles.[21] Prior to effective anti-retroviral therapy, patients with AIDS often required treatment with very long courses of triazoles to treat or suppress oropharyngeal candidiasis, resulting in a clinically significant increase in evolved resistance to triazoles among *Candida* species.[20] However, the current rate of evolved azole resistance remains low in intrinsically susceptible *Candida* species with the exception of *C. glabrata*. Rates of high-level resistance to azoles in *C. glabrata* have been steadily increasing, which is of particular concern because many of these isolates are also resistant to echinocandins.[19] Recently, resistance of *Aspergillus* to azoles has been described. The evolution of voriconazole resistance in *Aspergillus* appears to be due in part to the use of agricultural fungicides.[22] Unfortunately, cross-resistance among azoles is relatively common and develops rapidly in *Candida* species.[19] Therefore, if a *Candida* isolate is resistant to fluconazole, development of resistance to newer-generation triazoles during treatment should be expected, even if the organism appears to be "susceptible" *in vitro*. If a *Candida* isolate is fluconazole-resistant, it is unlikely that use of later-generation triazoles would be of significant clinical benefit.

Because the target of triazoles is a cytochrome P450 enzyme and the triazoles are substrates for human CYP3A, CYP2C19, and CYP2C9 enzymes.[23,24], concurrent treatment with triazoles and other drugs that are substrates for the CYP450 enzymes may lead to significant drug interactions. One particularly challenging drug interaction occurs because patients who are on calcineurin and/or mTOR inhibitors are typically at high risk for IFI and often require treatment with triazoles. By inhibiting the clearance of immunosuppressive agents, triazoles can cause accumulation of the immunosuppressive agent and prolonged immunosuppression[25] Individual azoles have varied drug interactions based on their individual binding affinity for the CYP450 isozymes. For example, azole-induced QT interval prolongation is of significant clinical concern. Meanwhile, fluconazole and voriconazole can affect the QT interval of patients and cause Torsade de Pointes (TdP).[26,27], an uncommon but dangerous cardiac arrhythmia. However, this adverse effect is rare in patients treated with posaconazole[28,29] Therefore, antifungal drugs should be carefully used, and patients with TdP should be therapeutically monitored.

First generation triazoles: Fluconazole and itraconazole

Fluconazole and itraconazole, the first-generation triazoles, became available in the early 1990s (Table 1). Both have a substantially improved safety profile compare with the imidazoles. Fluconazole, which is highly water soluble and available in both oral and intravenous formulations[18], is the only member of the first- and second-generation triazoles with high, reliable bioavailability and minimal variation in absorption. It is also the only triazole drug that is excreted unchanged in the urine, making it the treatment of choice for *Candida* urinary tract infections.[30] Importantly, fluconazole enters the cerebral spinal fluid (CSF) well.

Fluconazole has activity against many *Candida* species, but *C. krusei* and some strains of *C. glabrata* are inherently resistant. It is also highly active against *Cryptococcus neoformans*, but has no activity against filamentous fungi.[24] Fluconazole is currently used as first-line therapy for mucocutaneous candidiasis, empiric therapy for candidemia in non-neutropenic patients with mild-moderate illness, and for "step-down" treatment of candidemia in clinically stable patients with an isolate that is likely fluconazole-susceptible.[31] Recently, a meta-analysis demonstrated that non-neutropenic patients with invasive candidiasis who were treated with an echinocandin had lower mortality rates than patients treated with

Table 1. New antifungal formulations or agents approved by US Food and Drug Administration.

Agent	Original Formulation(s)	Year	New Formulation(s)	Year	Indications	Ref.
Azoles						
Ketoconazole	Oral tablet*	1981	*N/A*		Blastomycosis, coccidioidomycosis, histoplasmosis, chromomycosis and paracoccidioidomycosis	9,34
Fluconazole	Intravenous injection Oral tablet	1990	Oral suspension	1993	Invasive and mucosal candidiasis, cryptococcal meningitis, and prophylaxis of *Candida* infections	17,34,123
Itraconazole	Oral capsule	1992	Oral solution	1997	Coccidioidomycosis, blastomycosis, histoplasmosis, onychomycosis, mucosal candidiasis, sporotrichosis, paracoccidioidomycosis, chromomycosis, and dermatomycosis Alternative agent: Aspergillosis	17,34
Voriconazole	Intravenous injection Oral tablet Oral suspension	2002	*N/A*		Aspergillosis, invasive and mucosal candidiasis Alternative agent: Fusariosis and Scedosporiosis	34,105,123
Posaconazole	Oral suspension	2006	Delayed-release oral tablet Intravenous injection	2013 2014	Mucosal candidiasis and prophylaxis of invasive fungal infections	34,105,123
Isavuconazole	Intravenous injection Oral capsule	2015	*N/A*		Invasive aspergillosis and mucormycosis	11,49
Echinocandins						
Caspofungin	Intravenous injection	2001	*N/A*		Invasive and mucosal candidiasis; empiric antifungal therapy in patients with fever and neutropenia, Alternative agent: Aspergillosis	81,123
Micafungin	Intravenous injection	2005	*N/A*		Invasive and mucosal candidiasis, Prophylaxis of *Candida* infections	81,123,124
Anidulafungin	Intravenous injection	2006	*N/A*		Invasive and mucosal candidiasis	81,123
Polyenes						
Amphotericin B deoxycholate	Intravenous injection	1958	AmB lipid complex	1995	Aspergillosis, cryptococcosis, blastomycosis, invasive candidiasis, Coccidioidomycosis, histoplasmosis, mucormycosis, sporotrichosis, phaeohyphomycosis	71,105,123
			AmB colloidal dispersion	1996		
			Liposomal AmB	1997		

Note. *The oral tablet was initially approved but later withdrawn in 2013; *N/A*: not available

triazoles or amphotericin B.[32] Fluconazole is also used as a primary therapy for the treatment of pulmonary cryptococcosis and consolidation therapy for patients with cryptococcal meningitis after induction therapy with amphotericin B.[24] In resource-limited regions that lack the ability to safely treat patients with amphotericin B, fluconazole is the mainstay of anti-cryptococcal therapy. Fluconazole is also frequently used in high-risk patients to provide prophylaxis against *Candida* infections. As one would expect given its spectrum of activity, it is not as effective as second-generation triazoles at preventing aspergillosis.[24]

Unlike fluconazole, itraconazole has generally lower bioavailability that is complicated by substantial variation in absorption.[24] Furthermore, itraconazole has poor central nervous system (CNS) penetration and urinary metabolites are inactive.[24] Thus, clinical use of itraconazole is primarily limited to treatment of fungi that do not cause CNS disease. Itraconazole is currently available in 2 formulations: oral capsule and oral solution (Table 1). The oral solution is superior to capsules because of improved bioavailability, but it is not tolerated as well as the capsules.[33] Importantly, the solution should be taken on an empty stomach, while capsules should be taken with food to maximize absorption. An intravenous formulation was once FDA-approved, but its approval was withdrawn in 2007 due to cardiac toxicity.[34]

Itraconazole has a much wider spectrum of activity than fluconazole. It is active against fluconazole-susceptible *Candida* species, *Cryptococcus*, and many dimorphic fungi including *Coccidioides*, and has some activity against filamentous fungi such as *Aspergillus*.[35] Importantly, itraconazole is not active against *Fusarium* species or Zygomycetes. Due to its less favorable pharmacokinetics and more prominent drug-drug interactions, itraconazole has widely been replaced by second-generation triazoles for most clinical uses. Currently, itraconazole is still used to treat patients with dimorphic mycoses, including coccidiomycosis, blastomycosis, and histoplasmosis.[4-6] Interestingly, itraconazole, with known safety and tolerability, has the potential to be developed into an anti-cancer agent.[36,37] However, the drug interactions of

itraconazole and existing anti-cancer agents remain unclear and require further investigation.

Second generation triazoles: Voriconazole and posaconazole

The second-generation triazoles were developed with the goal of improving pharmacokinetics and spectrum of activity and decreasing drug-drug interactions. The chemical structure of voriconazole is similar to fluconazole, whereas posaconazole is more closely related to itraconazole.[30] Variation in blood levels is an issue with both voriconazole and posaconazole. The predominant source of variability for voriconazole is due to individual variations in metabolism, whereas absorption of the posaconazole oral suspension from the GI tract is highly variable.[30] Both voriconazole and posaconazole have poor solubility in water. Thus, intravenous solutions require the addition of cyclodextrins to improve solubility. Although neither voriconazole nor posaconazole are renally eliminated, the cyclodextrin component is and it accumulates in patients with renal failure.[30]

Voriconazole is available in both intravenous and oral forms (Table 1). The oral forms have excellent bioavailability.[24] Similar to fluconazole, it has excellent CNS penetration. Voriconazole is primarily metabolized by the cytochrome P450 enzyme CYP2C19.[24] The safety profile of voriconazole is excellent, but treatment-related visual disturbances occur in approximately 20% of patients. Voriconazole has a substantially broader spectrum of activity than the first-generation azoles. Its activity against *Candida* species and *Cryptococcus* mirrors that of fluconazole, but voriconazole also has activity against dimorphic fungi, *Fusarium*, and *Scedosporium*, and most importantly, it has very high activity against most *Aspergillus* species.[24] However, it is not active against Zygomycetes. Voriconazole has become the first-line treatment for invasive aspergillosis as it has better efficacy and substantially fewer drug-related toxicities than amphotericin B.[38] Voriconazole is FDA-approved for treatment of mucocutaneous and systemic candidiasis, but it is not frequently used for these indications because it is not substantially better than fluconazole for these diseases. Voriconazole is commonly used for prophylaxis against yeast and mold infections in high-risk patients such as those undergoing bone marrow transplantation.[30] One possible difficulty with the use of voriconazole for prophylaxis against IFIs is that it does not protect against Zygomycetes.

Posaconazole was originally available only in oral suspension form. However, the bioavailability of the oral suspension depends on food intake. Ingestion of high-fat meals or nutritional supplements is required for good absorption.[24] In 2013, a new oral delayed release tablet formulation was introduced (Table 1) that is given once daily; its bioavailability is independent of food intake.[39,40] The delayed release oral tablet is considered a more reliable option for the prophylaxis or treatment of IFIs. Studies have suggested that the posaconazole oral tablet has higher azole plasma levels[41], better absorption[42], and improved bioavailability[43] than that of oral suspension. Furthermore, an intravenous formulation of posaconazole was developed and FDA-approved in 2014 for patients who are unable to take oral medications.[39,44] The phase 1B trial results showed that the intravenous formulation of posaconazole was well tolerated in patients at high risk for IFIs.[44]

Posaconazole has a very broad spectrum of antifungal activity.[24,30] Like voriconazole, it is active against fluconazole-susceptible isolates of *Candida, Cryptococcus*, dimorphic fungi, and *Aspergillus*. In addition, posaconazole is active against many Zygomycetes. Among the azoles, only posaconazole and itraconazole have activity against these difficult-to-treat fungi. Posaconazole also has better efficacy than fluconazole for the prophylaxis of systemic fungal infections.[39,45] One major limitation of posaconazole is that it does not penetrate into the CSF well.[24] This raises concerns that it may not be suitable for treatment of invasive *Aspergillus* infections and disseminated candidiasis, both of which can cause CNS disease.

Isavuconazonium sulfate

Isavuconazonium sulfate is a water soluble pro-drug of the triazole isavuconazole that was approved by the FDA in 2015 for the treatment of invasive aspergillosis and invasive mucormycosis.[46] The pro-drug is rapidly metabolized into isavuconazole by plasma esterases after intravenous administration. The oral capsule (Table 1) formulation of isavuconazonium sulfate hydrolyzes and converts to the active form in the gut lumen.[47] Some studies have reported that the high bioavailability of the oral capsule is minimally affected by food intake.[48,49], but this requires more evidence to support its clinical relevance. Besides, initial studies suggest that blood levels are substantially more consistent than for voriconazole or posaconazole[24] Although the tissue distribution of isavuconazole has not yet been fully evaluated, it is highly protein bound and thus expected to have low levels in the CSF, although it may reach clinically useful concentrations in the brain parenchyma.[11] Active isavuconazole is not excreted in the urine. Because of the high water solubility of the isavuconazole prodrug (isavuconazonium sulfate) relative to voriconazole and posaconazole, isavuconazole does not require cyclodextrin, an agent with potential nephrotoxicity, to increase its solubility.[50] However, isavuconazole is a substrate and inhibitor of

CYP3A4, so co-administration with a strong CYP3A4 inhibitor or inducer is a pharmacokinetic concern.[48]

Isavuconazole exhibits *in vitro* activity against azole-susceptible *Candida* species, *C. neoformans, Cryptococcus gattii*, dimorphic fungi, *Aspergillus*, and, importantly, many other molds including *Alternaria*, some Zygomycetes, and some species of *Scedosporium*.[11] Pfaller *et al.* showed that the vast majority of 21 *Candida* species were inhibited by isavuconazole with MIC $\leq$ 0.25 μg/mL, with the exception of *C. glabrata, C. krusei*, and *C. guilliermondii* with MIC $\geq$ 1 μg/mL[51]. Clinical trials of isavuconazonium sulfate for systemic candidiasis and other IFIs caused by *Aspergillus* and rare fungi are now complete, although many of the results have been released only as abstracts or FDA briefing documents.[11,51,52] Isavuconazonium sulfate was found to be non-inferior to voriconazole for the treatment of patients with proven or probable invasive fungal disease caused by filamentous fungi, including *Aspergillus*. Although it is not as active as voriconazole, isavuconazole has activity against some Zygomycetes.[11,24] The *in vitro* studies showed that isavuconazole exhibits potent antifungal activity against many Mucorales including *Mucor, Rhizomucor, Rhizopus*, and *Absidia*. However, the susceptibility of these Mucorales to isavuconazole varies largely.[49] In spite of that, the open-label clinical trial for licensing demonstrated that patients who received isavuconazole had similar mortality to patients who received amphotericin B or posaconazole.[49]

Therapeutic drug monitoring for triazole therapy

As one of the most prominent clinical difficulties in the use of itraconazole, voriconazole, and posaconazole is variability in plasma drug levels, many investigators have suggested that monitoring drug levels could optimize efficacy and/or decrease toxicity.[53] Unfortunately, the majority of studies investigating therapeutic drug monitoring (TDM) for triazoles have been retrospective descriptive studies that do not address the question of whether the use of TDM can improve patient outcomes. Furthermore, many of these studies rely on drug concentration measurements taken at random times with respect to the time since the most recent drug dose or the time since initiation of therapy.[32,54] Finally, the laboratory methods needed to accurately measure triazole concentrations typically require that samples be sent to specialized reference laboratories. Thus, results are often not available until 5 to 7 d after samples are obtained. Therefore, antifungal TDM is typically restricted to patients who require fairly long-term antifungal therapy.[53,55]

The relationship between drug concentration and therapeutic efficacy is clearest for itraconazole. After one to 2 weeks of therapy, patients with trough levels >0.5 to 1 μg/mL are more likely to have treatment success.[53,56] There is no strong correlation between itraconazole levels and adverse events. Although itraconazole use has decreased significantly since voriconazole and posaconazole became available, it remains the treatment of choice for infections such as coccidioidomycosis, and in these patients, itraconazole TDM is indicated.

Posaconazole drug levels are typically a function of absorption of the drug from the GI tract.[24,30]; the newer oral delayed release tablet formulation has substantially better, and more reliable, absorption than earlier formulations[39,40] A series of studies have shown a relationship between posaconazole levels and efficacy.[54,57,58] While these studies varied in their rigor with respect to TDM methods and definitions of efficacy, the body of data has led to recommendations of target steady-state trough posaconazole concentrations of > 700 ng/mL for prophylaxis and, provisionally, >1250 ng/mL for treatment of invasive fungal disease.[54,59] In a recent study, Cornely *et al.* found that 90% of adult patients taking the newer delayed release tablets for prophylaxis of fungal infections had steady-state trough levels >700 ng/mL and there was no correlation between drug levels and adverse events.[60] Thus, TDM is unlikely to provide clinical benefit to patients taking the delayed release formulation of posaconazole for prophylaxis of fungal infections. It is not yet clear whether there will be a role for TDM in patients taking posaconazole for treatment of invasive fungal disease.

In contrast to itraconazole and posaconazole, voriconazole levels are related more to variation in metabolism than absorption. Genetic polymorphisms in CYP2C19 are common and strongly affect voriconazole blood concentrations[30], and an increasing body of retrospective data suggests that there is a correlation between voriconazole levels and efficacy and toxicity with improved outcomes occurring in patients with trough voriconazole levels >1 mg/L[56]. A prospective, randomized, blinded study was recently performed comparing outcomes in patients who were managed with TDM and voriconazole dose adjustment to those without TDM.[61] This study showed improved clinical response in the TDM/dose adjustment group, with complete or partial response in 81% of the TDM group but only 57% of the control group. However, this study was a single-center study performed in Seoul, Korea. Given known variations in drug metabolism between Asian and non-Asian populations, results may differ in other patient populations. Interestingly, a recent meta-analysis of voriconazole TDM found that patients with voriconazole levels >1.0 mg/L were more likely to have a successful clinical response, but there was no difference in survival between patients who

had therapeutic and subtherapeutic levels.[62] Taken together, the accumulating data suggest that TDM for voriconazole therapy may be clinically useful, but we do not currently have enough data to clearly define the clinical scenarios in which it would be most useful.[56,63] One special population for whom voriconazole TDM may be quite helpful is children with IFIs.[64] Typically, much less is known about the pharmacokinetics of drugs in pediatrics, but children often have increased drug clearance compare with adults. This makes them more vulnerable to sub-therapeutic dosing and thus they may be more likely to benefit from TDM.

Polyenes

The polyene antifungal amphotericin B was the first antifungal agent used for IFI treatment. With broad-spectrum fungicidal activity against yeasts and filamentous fungi, amphotericin B has been widely used clinically to treat systemic *Candida, Cryptococcus, Aspergillus*, and many other IFIs.[65] Despite its long use, the exact mechanisms of action of amphotericin B remain unclear.[66] In the most traditional model, amphotericin B kills fungal cells by forming pores in ergosterol-containing membranes. More recent studies have proposed a variety of other possible mechanisms. For example, amphotericin B directly binds to ergosterol and leads to electron transfer in the cell membrane, thus creating oxidative stress and reactive oxygen species.[67,68] Whatever the exact mechanism of action is, amphotericin B kills fungal cells with some specificity for fungal rather than mammalian cells. This specificity is related to the increased ergosterol content in fungal membranes in contrast to cholesterol, which is the major sterol in mammalian cell membranes. Nevertheless, toxicity toward cholesterol-containing cells occurs and leads to the significant adverse effects. Amphotericin B can cause serious nephrotoxicity as well as electrolyte abnormalities and severe infusion-related reactions such as hypomagnesium, chills, fever, and rigors.[66,69]

In view of this high toxicity but excellent efficacy, a drug structure or formulation modification was needed. Amphotericin B lipid complex (ABLC) and liposomal amphotericin B (L-AmB) are combinations of amphotericin B and lipids in a specific ratio that improve the safety profile.[70,71] (Table 1). Interestingly, different amphotericin B formulations possess distinct pharmacological properties and adverse effects. For example, Wade *et al.* found that patients who received ABLC had a higher risk of experiencing nephrotoxicity compared with those receiving L-AmB[72] Although the safety and toxicity of these new formulations are much improved compared with the old formulation, the toxicity of amphotericin B on the kidneys and infusion-related organs still remains a clinical concern.[73]

A landmark study recently reported the possibility of manufacturing less toxic amphotericin B derivatives.[74] The prototype of these derivatives is amphotericin B with a C'2 hydroxyl group deletion, which allows it to only bind to fungal ergosterol and not mammalian cholesterol.[75] *In vivo* toxicity and therapeutic experiments using a systemic candidiasis murine model demonstrated that amphotericin B methyl urea (AmBMU) was less toxic and more effective than the traditional deoxycholate amphotericin B formulation.[74] These results contribute to the exciting progress in antifungal drug development linked to the gold standard antifungal agent amphotericin B.

Resistance to polyene antifungals is still quite rare in the clinic, mostly because the fitness cost of developing modifications for survival is high.[76] Meanwhile, pathogens may be vulnerable and unable to evade the host immune system. Pathogens gain polyene resistance by decreasing ergosterol content in cell membranes and increasing catalase activity.[77] Meanwhile, mutation of *ERG3*, a gene involved in ergosterol biosynthesis in *C. albicans*, may lead to the accumulation of other sterols and thus reduce the affinity of amphotericin B to ergosterol in the fungal cell membrane. Utilization of other sterols instead of ergosterol and reduction of oxidative stress can change the physiology of pathogens and cause drug-resistant isolates.[78]

Echinocandins

The echinocandins are the newest class of antifungal agents and are currently widely used for the treatment of IFIs. Caspofungin, micafungin, and anidulafungin are echinocandin-class antifungals that have been approved for intravenous administration by the FDA and the European Medicines Agency[79] (Table 1). Echinocandins are cyclic lipopeptide molecules derived from natural products that inhibit fungal β-1,3 glucan synthase, a major enzyme complex functioning in cell wall synthesis.[79-81] Similar to polyenes and azoles that target fungal ergosterol and its biosynthesis pathway, echinocandins have a unique drug target that is only present in fungi but not in mammalian cells, and thus these agents are much less toxic to humans. Echinocandins have several additional merits, including fungicidal activity against *Candida* species[82], reduced emergence of drug-resistant isolates[79], and most importantly, an improved safety profile and fewer drug interactions.[82] Unfortunately, echinocandins also have a high molecular weight and are not stable in acid, so they are not

amenable to oral use.[79] Oral glucan synthase inhibitors are now under development.

Although the echinocandins are generally very safe drugs, unexplained cardiac-associated adverse events such as arrhythmias and cardiac failure have occurred in some patients after the administration of caspofungin.[80] In addition, Fink *et al.* reported a fatal hemodynamic instability adverse event after anidulafungin administration[83], and in *ex vivo* testing, caspofungin and anidulafungin decreased left ventricular contractility.[80] Taken together, these data imply that echinocandins should be used cautiously in patients with preexisting cardiac dysfunction, though additional studies are required.

Echinocandins exhibit potent fungicidal activity against most *Candida* species.[31,79] In fact, in the new 2016 Infectious Diseases Society of America candidiasis guideline, echinocandins are the primary drugs of choice for invasive candidiasis.[31] In general, *Candida parapsilosis* isolates tend to have lower susceptibility to echinocandins *in vitro* but clinically the echinocandins are usually effective against this species.[24] Unfortunately, clinical reports of echinocandin-resistant *Candida* isolates are increasing.[84] Of particular concern is a group of *C. glabrata* isolates that are resistant to both azoles and echinocandins.[51] However, the vast majority of *Candida* isolates are currently highly susceptible to echinocandins.

Generally, echinocandins exhibit fungistatic activity against *Aspergillus* and are typically used only as alternative or second-line therapies against invasive aspergillosis.[24] Echinocandins are not active against *Cryptococcus*, dimorphic fungi, or Zygomycetes. Interestingly, echinocandins have antifungal activity against the cyst form but not the vegetative form of *Pneumocystis jirovecii*, a human fungal pathogen that causes pneumonia.[85] Because the vegetative form is a major component of disease, echinocandins are not used clinically to treat *Pneumocystis*.

Resistance to echinocandin antifungals is mostly due to mutations of *FKS*.[86] Fks is a subunit of glucan synthase and the drug target of echinocandins. Two conserved regions of *FKS*, Ser 645 and Phe 641, can mutate, leading to increased tolerance or resistance to antifungals.[87] In general, failure of echinocandin treatment for common *Candida*-causing candidiasis is rare, except for *C. glabrata*, a well-known multidrug-resistant species. A similar mechanism was implicated in the emerging echinocandin resistance in molds.[77]

Echinocandins have a unique structure and target a fungal-unique pathway, and are currently the safest antifungal drugs available. These agents are neither substrates nor inhibitors of CYP450, thus making clinical drug-drug interactions relatively rare.[23] Though caspofungin, micafungin, and anidulafungin possess similar antifungal activities, the differences in their backbone structures lead to distinct pharmacokinetics.[88] Caspofungin may affect the plasma concentration of cyclosporine A and tacrolimus.[88] However, Saner *et al.* demonstrated that co-administration of caspofungin with either of these 2 immunosuppressants in liver transplant patients resulted in an acceptable safety profile with no hepatotoxicity.[89] In addition, based on an open-label clinical trial in healthy adults, micafungin may increase exposure to amphotericin B about 30%; thus, it may not well tolerated during co-treatment in human host.[90] Overall, most of the drug-drug interactions between echinocandins and other drugs are not serious when compare with those associated with the azoles.

Antifungal agents in clinical trials

Several antifungal agents are currently being evaluated in clinical trials. We have selected 2 promising candidates and summarized their progress below.

VT-1161

VT-1161, a tetrazole developed by Viamet Pharmaceuticals, is a novel ergosterol synthesis inhibitor targeting fungal CYP51 (lanosterol 14 α-demethylase) that has been in phase 2 clinical trials for treatment of vaginal candidiasis since 2013.[91,92] Warrilow *et al.* demonstrated that VT-1161 tightly binds to *C. albicans* CYP51 and thus inhibits cellular function, and that it also weakly inhibits human enzymes such as CYP2C9, CYP2C19, and CYP3A4.[93] The lack of interference with human enzymes suggests that VT-1161 may potentially have fewer negative drug-drug interactions, thus overcoming a major issue of the triazoles.[34] In addition, VT-1161 retains high *in vitro* potency against several *C. albicans* isolates that are clinically fluconazole-resistant. In a murine model of vaginal candidiasis, Garvey *et al.* demonstrated that VT-1161 was equivalent to fluconazole for treatment of vaginitis due to fluconazole-susceptible *C. albicans* and significantly superior to fluconazole for the treatment of vaginal candidiasis due to fluconazole-resistant organisms.[94] These results suggest that VT-1161 has considerable potential to be an efficacious and safe antifungal agent.

SCY-078

Echinocandins have potent fungicidal activity against *Candida* species through the inhibition of the fungal enzyme β-1,3 glucan synthase.[95] Currently, echinocandins are only available in intravenous formulations.[82] SCY-078 (formerly MK-3118) is a potential candidate for an oral glucan synthase inhibitor that is currently in

phase 2 clinical trials.[96] The mechanism of action of SCY-078 is similar to that of the echinocandins, but SCY-078 has a different chemical structure and possesses excellent oral bioavailability.[82,95,97,98] SCY-078 exhibits broad-spectrum antifungal activity against several *Candida* species and even some echinocandin-resistant isolates.[98] Moreover, it is also effective against some filamentous fungi, including *Aspergillus fumigatus, Paecilomyces variotii*, and *Scedosporium prolificans*.[95,98] The pharmacokinetics and pharmacodynamics of SCY-078 after oral treatment have been evaluated in a neutropenic murine model of disseminated candidiasis. The 1-log kill doses of SCY-078 were numerically lower than those of conventional intravenous echinocandins[97], indicating that SCY-078 is a promising antifungal agent. A clinical phase 1 study showed that SCY-078 was generally well tolerated. Adverse effects associated with SCY-078 included diarrhea, abdominal pain, and headache.[81]

Combination therapy

Due to the emergence of drug-resistant fungi and the limited efficacy of monotherapy, the therapeutic strategy of combining several current antifungal drugs with different mechanisms of action has often been considered. The only combination therapy that is supported by well designed, randomized clinical trials is the use of amphotericin B with flucytosine for the treatment of cryptococcal meningitis.[99,100] Because fungal infections typically have poor outcomes and treatment frequently results in adverse effects, clinicians are compelled in some cases to abandon conventional antifungal therapy for salvage therapy.[101] In the absence of effective monotherapy, combination therapies are frequently used as a "last ditch attempt" to treat potentially life-threatening IFIs. Individual case reports or case series describing success with combination therapy are common, but such reports are highly susceptible to publication bias and should be interpreted cautiously. In order to find the best options to improve outcomes and minimize risk, clinicians need to evaluate the *in vitro* and *in vivo* efficacy and drug interactions of antifungal drug combinations.

Combination therapy against candida

Invasive *Candida* infections can usually be treated with azoles, echinocandins, or amphotericin B monotherapy. As with other infections, case reports using combination therapy have been published[102-104], but there are no data to indicate that combination therapy is necessary for treatment of candidiasis.

Combination therapy against aspergillus

The clinical practice guidelines for the treatment of invasive aspergillosis recommend voriconazole over other antifungal drugs as a primary therapy, while amphotericin B, itraconazole, posaconazole, itraconazole, caspofungin, and micafungin serve as alternative therapies.[105] If patients are refractory to primary therapy or are predicted to fail monotherapy, clinicians may opt for combination therapy. In a recent large clinical study comparing voriconazole monotherapy versus combination therapy with voriconazole and anidulafungin, combination treatment did not significantly improve overall survival compare with monotherapy.[106] In this study, the primary endpoint was all-cause mortality at 6 weeks; 27.8% of patients on monotherapy and 19.5% of patients on combination therapy died, but this difference did not reach statistical significance. One tempting interpretation of these data are that combination therapy did have a benefit, but the study was underpowered. This must be balanced against the finding that other studies have also failed to demonstrate an improvement with combination therapy. Furthermore, most studies of combination therapy find an increase in adverse drug effects with combination therapy. Much of the available data on combination therapy comes from retrospective or non-comparative studies. For example, Raad *et al.* reported results from combination therapy of voriconazole and caspofungin vs. voriconazole alone based on a retrospective chart review. Combination therapy did not enhance the survival rate of patients compared with monotherapy, but adverse events were higher in the combination group.[107] Likewise, Lellek *et al.*, in an uncontrolled retrospective salvage therapy study, reported that patients with aspergillosis who failed to respond to primary therapy had a favorable response with combination therapy using posaconazole and caspofungin, but no comparison data for monotherapy were provided.[108] Although the precise use and success of combination therapy for aspergillosis remain uncertain, the potentially dire outcomes of invasive aspergillosis continue to drive consideration of combination treatment by clinicians at the bedside.

Combination therapy against cryptococcus

Treatment of cryptococcal meningitis is the only circumstance for which combination antifungal therapy is well supported with prospective randomized clinical trials. The fluorinated pyrimidine flucytosine (5-FC) is a seldom used antifungal drug that interferes with nucleic acid synthesis.[24] It is active against *Cryptococcus* and *Candida*, but it is not used as a monotherapy because

drug resistance readily develops. It also causes significant bone marrow and liver toxicity.[109] Thus, the clinical use of flucytosine is typically limited to combination therapy for treatment of cryptococcal meningitis. Co-administration of amphotericin B and flucytosine is more efficacious than amphotericin B alone, and this fungicidal regimen is included in clinical practice guidelines for invasive *Cryptococcus* management.[100,110,111] Day *et al.* demonstrated that combination therapy of amphotericin B plus flucytosine for cryptococcal meningitis was more effective than amphotericin B alone or with fluconazole.[112] Judging by the decreased mortality and high rate of clearance of yeast in CSF, the combination of amphotericin B and flucytosine is an excellent therapeutic strategy against cryptococcosis and is the standard of care for induction therapy.[99,100,112] Nevertheless, treatment with amphotericin B and flucytosine requires a high level of supportive medical care not feasible in countries with limited medical resources, suggesting that an alternative approach must be developed for these areas.[113] Furthermore, flucytosine has significant toxicity and limited availability and high cost, even in the United States.

As fluconazole and amphotericin B are 2 major antifungal agents that can be obtained easily, the feasibility of combination therapy using these agents has been evaluated. A clinical trial performed by Loyse *et al.* on cryptococcal meningitis in HIV patients demonstrated that there was no significant difference in the early fungicidal activity of amphotericin B in combination with flucytosine, fluconazole, or voriconazole.[113] Thus, the fluconazole and amphotericin B combination provides another potential option for treating *Cryptococcus* infection if flucytosine is not available or not tolerated by the patient.[111,113,114]

Combination therapy against zygomycetes

Mucormycosis is an IFI that can be caused by any of the Zygomycetes, including *Mucor, Rhizopus, Rhizomucor*, and *Apophysomyces*. Due to extremely high mortality, management of mucormycosis has become a critical issue in the clinic. Currently, only amphotericin B, posaconazole, and isavuconazole have sufficient activity against these organisms to be used clinically.[49,115,116] As with treatment of invasive aspergillosis, ineffective monotherapy and serious side effects of amphotericin B have prompted clinicians to attempt alternative strategies, including combination therapy. Two retrospective analyses of combination therapy for treatment of mucormycosis infections have recently been published. Kyvernitakis *et al.* reviewed charts of 106 patients with hematologic malignancy and mucormycosis and found no difference in mortality 6 weeks after therapy between monotherapy and combination therapy.[117] In contrast, Reed *et al.* reviewed the charts of 41 patients with mucormycosis; 34% of these patients had malignancy, 10% had organ transplantation, and 83% had diabetes mellitus. In this group of patients, they found that treatment was successful 30 d after hospital discharge in 100% of patients given combination therapy vs. 45% of patients on monotherapy.[118] These data are particularly difficult to interpret when one considers that just 7 patients were given combination therapy, only one of whom had a malignancy. These reports illustrate the difficulties in interpretation of retrospective clinical data and highlight the quandary faced by clinicians caring for patients with mucormycosis.

Combination therapy against coccidioides

Coccidioides immitis and *Coccidioides posadasii* are the species that cause coccidioidomycosis, leading to symptoms such as pneumonia, fever, and skin nodules. In some individuals, infection progresses into a chronic disease.[119,120] The current practice guidelines advocate itraconazole, fluconazole, or amphotericin B alone as therapeutic regimens.[4] However, some cases are refractory to monotherapy. Few studies on combination therapy for *Coccidioides* have been reported. One case in 2006 described a patient with coccidioidomycosis who received caspofungin and fluconazole co-treatment with good efficacy instead of the recommended monotherapy with amphotericin B.[121] Levy *et al.* demonstrated several successful examples of combination therapy with voriconazole and caspofungin in pediatric patients with *Coccidioides* infection.[122] Although these case studies do not provide enough guidance on when to use combination therapy with this infection, refractory cases may warrant consideration of combination treatment.

Combination therapy as prophylaxis

Prophylaxis is important in high-risk patients, including immunocompromised, neutropenic, organ transplant, and chemotherapy patients. Currently, fluconazole, posaconazole, voriconazole, and micafungin have been proven to be effective prophylactic agents against IFIs in high-risk patients.[123-125] It is possible that combination prophylaxis would confer better protection from disease while decreasing the development of drug resistance. Krishna *et al.* demonstrated that posaconazole in combination with micafungin given to healthy volunteers was well tolerated and the pharmacokinetics of the 2 drugs were not affected.[126] Hiemenz *et al.* found that a combination of micafungin and fluconazole in immunocompromised bone marrow/stem cell transplant recipients

was well tolerated for up to 4 weeks after transplant in a randomized, double-blinded dose escalation study.[127] Although the number of patients was low, a smaller percentage of patients in the combination prophylaxis group developed a suspected fungal infection. This evidence suggests the feasibility of successful combination prophylactic therapy with posaconazole and micafungin. More trials are needed to determine whether the possible benefits of combination prophylaxis outweigh the risks.

Conclusions

Over the past half-century, antifungal drugs have been developed to combat IFIs. However, IFIs are still associated with high morbidity and mortality, increased length of hospital stay, and high healthcare costs. This is partly due to the limited antifungal armamentarium, challenges in the timely diagnosis of pathogens, and adverse drug-drug interactions. Fortunately, newer formulations or antifungal agents (*e.g.*, isavuconazole) have entered the market (Table 1), providing clinicians with more options for the treatment of IFIs. In addition, combination therapy provides a potential strategy to increase the efficacy of 2 or more drugs, especially for drug-resistant fungal isolates, when fungicidal therapy is needed. Because each currently available antifungal drug has limitations in terms of the pharmacokinetics and pharmacodynamics profiles, spectrum activity, drug-drug interactions, and variability in absorption, TDM may be applied in patients receiving these antifungals. In the meantime, additional classes of antifungal drugs are needed to combat emerging fungal infections and drug-resistant isolates.

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