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New and emerging treatments for fungal infections

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Although several new antifungal drugs have been licensed in the last 5 years, some patients remain difficult to treat. The main reasons for this include intrinsic or acquired antifungal resistance, organ dysfunction preventing the use of some agents and drug interactions. In addition, some drugs penetrate poorly into sanctuary sites including eye and urine, and others are associated with considerable adverse events. Here, we review the preclinical and clinical development progress with four new antifungal agents: isavuconazole, ravuconazole, albaconazole and aminocandin. Isavuconazole and ravuconazole are extremely similar, with a broad spectrum of activity, a very long half-life and large volume of distribution and good *in vivo* data supporting their efficacy in invasive aspergillosis and candidosis. Both compounds are in early Phase 3 development. Albaconazole has also shown very potent activity against species of *Candida*, *Cryptococcus* and *Aspergillus*. It was well tolerated and effective in women with vaginal candidosis. Aminocandin is an intravenous-only echinocandin with *in vivo* activity against *Candida* spp. and *Aspergillus* spp. Its extended half-life probably permits dosing less frequently than once a day. Overall these new antifungal agents in development offer extended half-lives, possibly reduced drug interaction profiles and good tolerance. Their antifungal spectrum is narrower than posaconazole and probably similar to voriconazole (isavuconazole and ravuconazole) and caspofungin (aminocandin). Licensure and determination of their place in clinical practice requires randomized clinical studies, which are or will be underway.

Keywords: isavuconazole, BAL-8557, ravuconazole, albaconazole, aminocandin, posaconazole

Introduction

Treating invasive fungal infections is becoming more and more interesting. In the 1980s, our options to treat patients with deadly fungal infections were restricted by the number and toxicity of available compounds; since then major efforts have resulted in more effective antifungal drugs. Physicians of the new millennium can now count on three licensed echinocandins, the first new antifungal drug class introduced for more than 15 years. A further breakthrough has been the licensing of new potent azoles such as voriconazole and posaconazole. However, despite the arrival of these new effective drugs, some therapeutic problems remain, in particular new pathogenic fungal species, slow microbiological diagnosis, variable drug bioavailability, some toxicity, lack of either oral or intravenous (iv) preparations, significant drug interactions for some agents and development of resistance or breakthrough infections. Some new agents are in development that address some of these issues. The aim of this review is to summarize the *in vitro*, *in vivo* and clinical data available on three azoles (isavuconazole, ravuconazole and albaconazole) and one echinocandin (aminocandin) in advanced stages of development. A summary of the findings is presented in Table 1.

New triazoles

Isavuconazole

BAL-8557 (isavuconazonium) is the water-soluble pro-drug of BAL-4815 (isavuconazole) (Figure 1). BAL-8557 comprises a [*N*-(3-acetoxypropyl)-*N*-methylamino]-carboxymethyl group linked through an ester moiety to the triazole nitrogen in isavuconazole.[1] After oral or iv administration, BAL-8557 is rapidly cleaved into isavuconazole, in a reaction catalysed by plasma esterases of rats, monkeys and humans.[2] Very low levels of the cleavage product (BAL-8728) remain detectable in the serum. In animals, isavuconazole undergoes slow elimination that is related to low plasma clearance and extensive tissue distribution.[1,2] Levels of drug exposure are high (AUC$_{0-24}$ 14–40 μg·h/mL with 50–100 mg/day).[1] Plasma protein binding of isavuconazole reaches 98% in humans, and binding occurs almost exclusively to serum albumin.[2] Owing to the rapid conversion of the water-soluble pro-drug, this new azole does not require the addition of cyclodextrin to increase/achieve solubility, as is necessary for itraconazole and voriconazole iv solutions. Isavuconazole is mainly inactivated by slow

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Table 1. New antifungal drugs on the frontline

Drug	Group	Mechanism of action	Via	Spectrum	Company	Synonyms	Advantages	Disadvantages
Isavuconazole	triazole	inhibition of ergosterol synthesis	oral and iv	*Candida* spp.,[a] *Aspergillus* spp.,[b] dermatophytes[c]	Basilea Pharmaceutica Ltd	BAL-8557 BAL-4815 BAL-8349 BAL-8728 RO-0098557 WSA (water-soluble azole)	broad spectrum water-soluble (no need for cyclodextrin) long-acting (allows once-daily up to once-weekly dosing) drug tolerability favourable so far limited drug interactions	most data available as meeting abstracts
Ravuconazole	triazole	inhibition of ergosterol synthesis	oral and iv	similar to BAL-8557 and also: *Cryptococcus* spp.,[a] *Chaetomium* spp., *Trypanosoma cruzi*	Eisai Co. Ltd	BMS-207147 ER-30346 BMS-379224	long-acting drug very similar to isavuconazole	potential for cross-resistance with other azoles
Albaconazole	triazole	inhibition of ergosterol synthesis	oral	Candida spp.,[a] *Aspergillus* spp.[b] *Paecilomyces spp.,* *Chaetomium* spp., *Cryptococcus* spp.,[a] *Malassezia* spp., *Trypanosoma cruzi*	Laboratorios Uriach & Cía. S.A.	UR-9825	broad spectrum good pharmacokinetics excellent oral bioavailability	low concentrations achieved in the cerebrospinal fluid potential for cross-resistance with other azoles
Aminocandin	echinocandin	inhibition of 1,3-β-glucan-synthase	iv	*Candida* spp.,[a] *Aspergillus* spp.[b]	Novexel	HMR-3270 IP-960	low toxicity less drug-interactions than the azoles potent anti-*Aspergillus* activity long-acting more active than micafungin and caspofungin against *C. parapsilosis*	iv only spectrum more limited if compared with the new azoles less active against *C. parapsilosis* and *C. guilliermondii* than the azoles

[a]Including fluconazole-resistant strains.
[b]Including itraconazole-resistant strains.
[c]Including terbinafine-resistant strains.
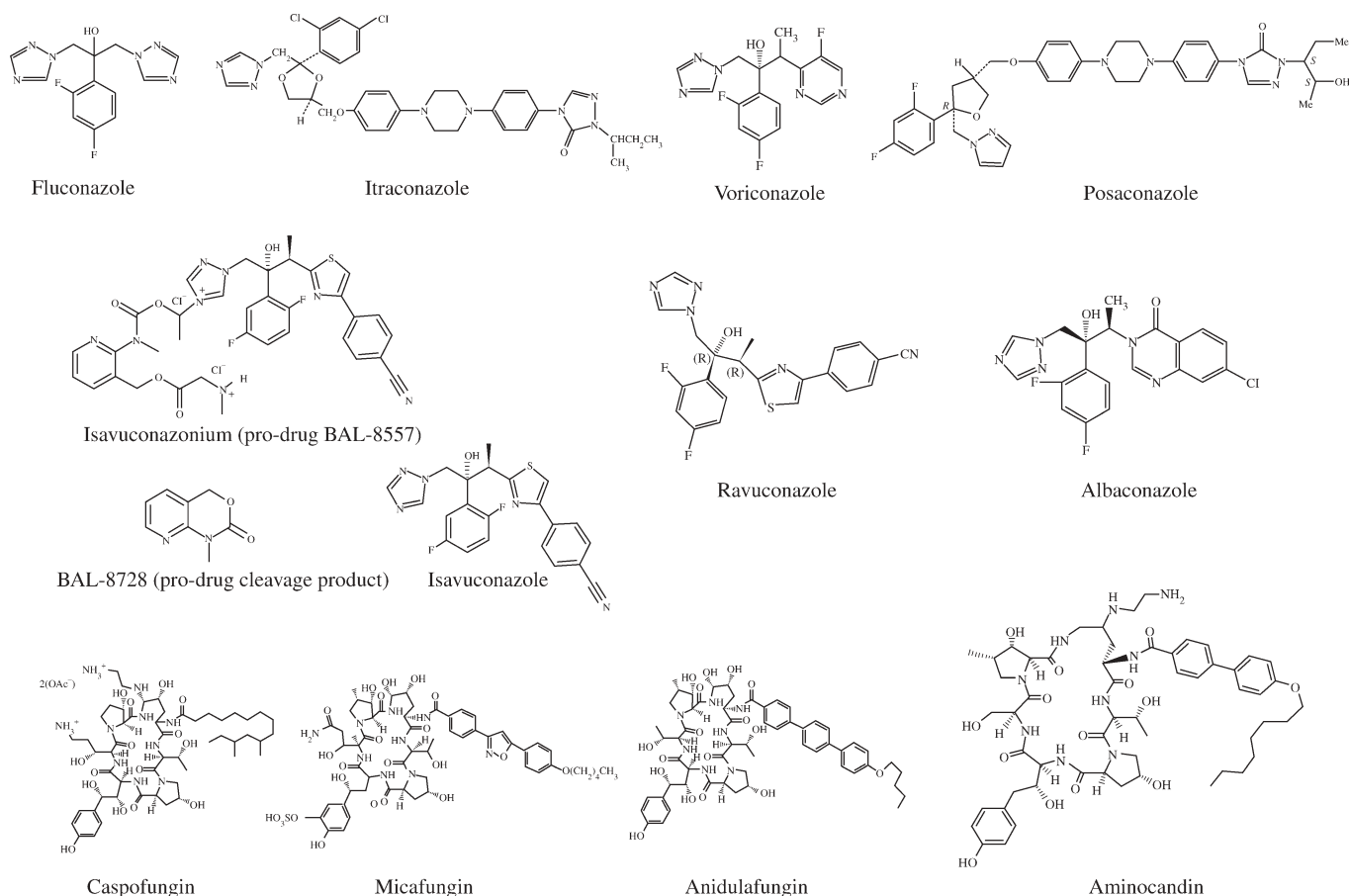
New antifungal treatments

Figure 1. Chemical structures of the licensed and new azoles and echinocandins.

CYP3A4-mediated metabolic clearance, and faeces are its main route for elimination.

In vitro studies. Isavuconazole demonstrated potent *in vitro* activity against *Aspergillus* species in a study testing 118 clinical isolates of *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus terreus* and *Aspergillus niger*.[3] The study included 16 *Aspergillus* isolates resistant to itraconazole, caspofungin or amphotericin B (some with an increased MIC of posaconazole). The geometric mean MIC value of BAL-4815 was 0.62 mg/L (range, 0.125–2.0 mg/L). In comparison with isavuconazole, lower mean MICs were observed for itraconazole (0.4 mg/L; range, 0.06 to >8.0 mg/L), voriconazole (0.4 mg/L; range, 0.125–8.0 mg/L), caspofungin (0.3 mg/L; range, 0.125–4.0 mg/L) and amphotericin B (0.5 mg/L; range, 0.06–4.0 mg/L). For all isolates, isavuconazole, itraconazole, voriconazole and amphotericin B were fungicidal [minimum fungicidal concentrations (MFCs) within two dilutions of the MIC] in 98.3%, 71.1%, 96.6% and 96.6% of instances, respectively.

Isavuconazole was compared with voriconazole and fluconazole *in vitro* against 231 *Candida* isolates with decreased susceptibility to fluconazole.[4] Overall, MIC and MIC_{50} values of isavuconazole were lower than those of voriconazole for the majority of *Candida* species tested, including *Candida glabrata* and *Candida krusei* ($P < 0.05$). Similar results were obtained in a second study that tested isavuconazole against 63 *Candida* isolates.[5] As before, the tendency for higher potency than

fluconazole against isolates of *C. glabrata* was observed. However, *C. glabrata* isolates for which MICs of isavuconazole were elevated (2–4 mg/L) have been recently described.[6] The *in vitro* activity of isavuconazole was again demonstrated in a study involving 296 *Candida* isolates.[7] Based on MIC_{50} results, isavuconazole was more active (0.004 mg/L) than amphotericin B (0.5 mg/L), itraconazole (0.008 mg/L), voriconazole (0.03 mg/L), flucytosine (0.125 mg/L) and fluconazole (8 mg/L).

Potent activity of isavuconazole has been demonstrated against the dermatophytes *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Trichophyton tonsurans*, *Epidermophyton floccosum* and *Microsporum canis*.[7] Terbinafine is generally very active against these species (mean MIC of 0.01 mg/L). The mean MIC of isavuconazole was 0.1 mg/L for all strains, including six *T. rubrum* strains for which MICs of terbinafine were elevated (mean MIC of 5.4 mg/L). However, neither of these agents showed fungicidal activity against the majority of the strains tested.[7]

The activity of isavuconazole against the Zygomycetes has also been investigated. In comparison with itraconazole, ravuconazole and voriconazole, isavuconazole was the only drug where all MICs were ≤4 mg/L, with mean MICs of 0.8 and 1.2 mg/L after 24 and 48 h, respectively.[8] Isavuconazole has shown limited activity against isolates of *Sporothrix schenckii* (MIC 2–8 mg/L) and *Fusarium* spp. (MIC 1–16 mg/L).[9]

Animal studies. Warn *et al.*[10,11] compared the activity of the oral pro-drug of BAL-4815 with that of oral itraconazole, oral

voriconazole and iv caspofungin in a neutropenic murine model of disseminated *A. flavus* infection (note that for the purpose of simplification, BAL-8557 is herein referred to as isavuconazole, not isavuconazonium). Isavuconazole in the range of 10–25 mg/kg started 4–24 h post-infection resulted in a 54–850-fold reduction in the mean template tissue burden, as determined by quantitative PCR. Similar results were observed for the different treatment regimens.

The dose–response and effect of dose fractionation on the tissue burden of subcutaneous treatment with isavuconazole was investigated in a mouse model of disseminated candidosis.[12] As the effect of isavuconazole occurred independently of the schedule of drug administration, the AUC/MIC ratio seemed to best link drug exposure with effect. Increasing efficacy with isavuconazole was noted over a wide therapeutic range (1.5–36 mg/kg). The pharmacodynamic importance of the AUC/MIC ratio was also shown in a mouse model of *Candida albicans* infection treated intraperitoneally with isavuconazole.[13]

Phase 1/2 studies. First pharmacokinetic data of isavuconazole in humans were obtained in a single-ascending-dose cohort of healthy male individuals after iv and oral administration.[2] BAL-8557 doses given orally were 100, 200 or 400 mg equivalents of isavuconazole (corresponding to 180.5, 361 or 722 mg of BAL-8557). Doses given iv were 50, 100 or 200 mg equivalents of isavuconazole. Maximum plasma concentrations of isavuconazole were observed 1.5–3 h after oral drug intake or at the end of the 1 h iv infusion. Mean elimination half-lives were long; 56–77 h after oral administration and 76–104 h after iv administration. Accordingly, the volume of distribution was large (155–292 and 304–494 L after oral and iv administration, respectively) and systemic clearance was low (1.9–2.8 and 2.8–5.0 L/h after oral and iv use, respectively). Increments on isavuconazole dose resulted in AUC values being slightly higher than proportionally to the dose, indicating a moderate deviation from dose linearity. The pharmacokinetic parameters of isavuconazole showed low to moderate inter-subject variability ($<$40%) in these volunteer studies, which is in contrast with what has been observed with voriconazole in patients (up to 100-fold).

The effect of loading doses of isavuconazole was investigated in a multiple-dose pharmacokinetic study in 24 healthy male subjects.[14] Participants were randomly assigned to four treatment cohorts to receive multiple oral doses or multiple 1 h constant-rate iv infusions of BAL-8557. Loading doses of BAL-8557 were equivalent to 100 and 200 mg of isavuconazole, followed by once-daily maintenance doses of 50 and 100 mg, respectively. Plasma concentrations of isavuconazole increased rapidly after administration of BAL-8557 and reached a maximum 2.0–3.5 h after drug intake and 0.7–1.0 h after the start of iv infusion. In all groups, serum levels were dose-proportional after multiple doses with no indication of induction or inhibition of its own metabolism and excellent bioavailability. Treatment with 100 mg daily resulted in plasma levels of $>$1.7 mg/L for the entire dosing interval,[14] which is in excess of the MFC for the vast majority of *Aspergillus* isolates tested. Based on the Monte Carlo simulations with *Candida* spp. isolates susceptible to isavuconazole (MIC 0.06 mg/L), isavuconazole is likely to exceed target AUCs sufficient to ensure clinical efficacy for patients treated with BAL-8557.[15]

The results of a Phase 2 study comparing three dosing regimens of BAL-8557 (equivalent to 50 and 100 mg/day and 400 mg/week of isavuconazole) with fluconazole (100 mg daily) in the treatment of oesophageal candidosis were recently presented.[16] Patients received treatment for 14–21 days and all groups received a loading dose on day 1. Endoscopically confirmed clinical cure was achieved for 95% to 98% of patients treated with BAL-8557 (all three doses) and in 95% of patients in the fluconazole arm. Statistical non-inferiority to comparator was confirmed for all BAL-8557 dosing regimens. Co-administration of isavuconazole with rifampicin, a potent CYP3A4 inducer, resulted in a 35-fold increase in systemic clearance of isavuconazole, with an associated 4- and 40-fold decrease in C_{max} and AUC of isavuconazole, respectively.[17] Dosing with isavuconazole had no significant effect on exposure of both warfarin (a substrate of CYP2C9)[18] and cyclosporine (a CYP3A4 substrate).[19] Overall, isavuconazole seems to cause less drug interaction than itraconazole or voriconazole.

Phase 3 studies. An ongoing non-randomized trial aims to determine the safety and efficacy of escalating dosing regimens of iv BAL-8557 in the prophylaxis of patients undergoing chemotherapy for acute myeloid leukaemia (clinicaltrials.gov NCT00413439). The study investigates the safety and tolerability of two different dosages, the efficacy in prevention of fungal diseases and the pharmacokinetics of the antifungal drug. Another Phase 3 trial will evaluate the safety and efficacy of BAL-8557 versus caspofungin followed by voriconazole in the treatment of invasive *Candida* infections (NCT00444366). Two other clinical studies are being conducted with isavuconazole (NCT00413218 and NCT00412893). The first compares the safety and efficacy of isavuconazole versus caspofungin followed by voriconazole in the treatment of invasive *Candida* infections. The second compares isavuconazole and voriconazole in invasive aspergillosis.

Toxicity. The toxicity profile of BAL-8557 in animals was consistent with that of other azoles; BAL-8557 revealed no mutagenic, allergenic, phototoxic or irritant potential.[2] In the single-ascending-dose study in healthy volunteers, all doses were well tolerated, and no severe or serious adverse events occurred.[2] In the multiple-dose pharmacokinetic study,[14] the most frequent adverse events were headache, nasopharyngitis and rhinitis (severe for only one volunteer). One subject on oral high-dose of BAL-8557 also had mild transient abnormal liver function on day 14 of therapy. No clinically relevant changes in vital signs or electrocardiogram were observed. In the Phase 2 clinical trial for the treatment of oesophageal candidosis, BAL-8557 was safe and well tolerated, with an adverse event profile comparable to that of fluconazole.[16]

Ravuconazole

Ravuconazole (BMS-207147, ER-30346) is very similar in structure to BAL-8557 (Figure 1), the only difference being that the latter has a 2,5-difluoro-substituted benzene moiety in place of the 2,4-dihalo substituent present in ravuconazole as well as other azoles.[1] Like BAL-4815, ravuconazole is characterized by a long elimination half-life (76–202 h) and high protein binding (98%).[20] Ravuconazole is the only clinically studied triazole with a long half-life comparable to that of isavuconazole. The iv pro-drug of ravuconazole is named BMS-379224 (ravuconazole di-lysine phosphoester). In contrast to the cyclodextrins, the

lysine and phosphate residues are readily cleared and do not accumulate in the presence of renal insufficiency.

In vitro studies. The potent *in vitro* activity of ravuconazole against *Aspergillus* spp. has been demonstrated in several studies.[21–26] Cuenca-Estrella *et al.*[25] tested the *in vitro* activity of ravuconazole against 575 clinical strains of *Aspergillus* species and found that the average geometric mean MICs ranged from 0.3 (against *Aspergillus nidulans*) to 1 mg/L (against *A. flavus*). The magnitude of this inhibitory effect was comparable to that of amphotericin B, itraconazole and voriconazole. Only six strains of *A. fumigatus* (1.9%) had ravuconazole MICs $\geq$4 mg/L, including two isolates for which the MICs were $\geq$8 mg/L (0.6%). The prevalence of *in vitro* resistance to ravuconazole (MICs of $\geq$4 mg/L) among other *Aspergillus* species was 3%. In another investigation, ravuconazole inhibited the growth of 96.8% of all *Aspergillus* species at MIC $\leq$1 mg/L, including 98.8% and 100% of *A. fumigatus* and *A. flavus* isolates, respectively.[21]

Ravuconazole is also highly active *in vitro* against *Candida* spp., *Cryptococcus neoformans* and other yeasts, including fluconazole-resistant isolates.[27–37] Results from the SENTRY study confirmed the enhanced potency of ravuconazole against *Candida* species, including fluconazole-resistant isolates.[27] MIC$_{90}$ values for the most prevalent *Candida* species were: *C. albicans* $\leq$0.008 mg/L; *Candida parapsilosis* 0.12 mg/L; *C. glabrata* 1 mg/L; *Candida tropicalis* 0.12 mg/L; and *C. krusei* 0.5 mg/L; similar results were obtained with voriconazole. Ravuconazole is 2–64 times more active *in vitro* against *Candida* species than itraconazole, fluconazole and amphotericin B.[26,28] Against *C. neoformans*, ravuconazole is 64-fold more potent than fluconazole (MIC$_{90}$ 0.5 mg/L versus 32 mg/L, respectively).[27] Almost all clinical isolates of *C. neoformans* were susceptible to ravuconazole, voriconazole and posaconazole (MICs <1 mg/L).[29]

Cuenca-Estrella *et al.*[25] showed the activity of ravuconazole against 923 clinical isolates of non-dermatophyte filamentous fungi. *In vitro* resistance to ravuconazole (MICs $\geq$4 mg/L) occurred at the following rates: *Paecilomyces* spp. 23.1%; *Penicillium* spp. 26.5%; *Acremonium* spp. 36.4%; *Fusarium* spp. 90.7%; and *Scopulariopsis* spp. 93.5%. Although only 56.2% of the *Mucorales* strains tested were susceptible to ravuconazole, particular potency was seen against *Rhizopus oryzae* (MIC$_{50}$ 1.0 mg/L). Minassian *et al.*[30] also investigated the *in vitro* activity of ravuconazole against the Mucorales, with six out of seven strains of *Mucor* showing MICs of $\geq$4 mg/L. Among other Mucorales, ravuconazole was active against *Absidia corymbifera* (37.5%), *Cunninghamella* spp. (66.7%), *Rhizopus* spp. (100%) and *Rhizomucor* spp. (100%). Ravuconazole has been invariably inactive against *Scedosporium prolificans*, and only one-third of *Scedosporium apiospermum* strains have shown susceptibility.[25,30] Poor activity is also described against the species of the *Pseudallescheria boydii* complex.[31] Ravuconazole has demonstrated good activity against the black fungus *Exophiala dermatidis* (MIC$_{90}$s of 0.5 mg/L).[32] Despite the limited activity against *Fusarium* spp., ravuconazole showed synergy when combined with amphotericin B against 6 of 11 isolates tested in one study.[33] Ravuconazole is also active *in vitro* against *Chaetomium* spp. (MICs of 0.06–1 mg/L), an emerging aetiology of disseminated infection in neutropenic patients.[34]

Fluconazole, voriconazole and ravuconazole all displayed up to 2-fold less activity in the SENTRY study when results from the year 2003 were compared with the period 1997–1999.[27] Cross-resistance in the group of azoles is of major concern, especially due to the overuse of agents such as fluconazole and itraconazole in the last few decades. Alves *et al.*[35] showed cross-resistance among azoles for isolates of C. *glabrata*, with MICs as high as 8 mg/L for ravuconazole and albaconazole. Pfaller *et al.*[36] showed that resistance to ravuconazole could be predicted for isolates of C. *glabrata* when a high level of resistance to fluconazole was present (MICs of $\geq$64 mg/L). Cross-resistance between itraconazole, voriconazole, posaconazole and ravuconazole has also been demonstrated in a patient who died of chronic pulmonary *Aspergillus* infection.[37] Studying 17 isolates of *Histoplasma capsulatum* recovered from patients who failed therapy with fluconazole, Wheat *et al.* observed no increase in MIC when isolates were tested with posaconazole or ravuconazole (MICs 0.007 mg/L). However, 42% of the isolates of *H. capsulatum* recovered from patients who failed fluconazole exhibited a 4-fold or greater increase in MIC of voriconazole.[38] As presented before, C. *glabrata* isolates with high isavuconazole MICs (2–4 mg/L) have been recently described.[7] Owing to the similarity in molecular structure seen with isavuconazole and ravuconazole (Figure 1), cross-resistance between these drugs is also expected to occur, and may carry over to voriconazole as well.

Animal studies. In a murine neutropenic model of invasive aspergillosis,[39] oral ravuconazole delayed mortality significantly when compared with itraconazole, all at 10 mg/kg. However, no difference was demonstrated when ravuconazole and itraconazole at 40 mg/kg were compared. The half-life of ravuconazole (4 h) was ~3 times longer than that of itraconazole (1.4 h). Oral ravuconazole was evaluated afterwards in a neutropenic guinea pig model of invasive aspergillosis.[40] Animals received ravuconazole (5, 10 and 25 mg/kg daily), oral itraconazole (2.5 and 5.0 mg/kg twice daily) or intraperitoneal amphotericin B (1.25 mg/kg). Compared with no treatment, all regimens decreased mortality by the sixth day after challenge ($P < 0.003$). Although 100% survival occurred only for animals treated with ravuconazole at 5 or 10 mg/kg/day or itraconazole at 10 mg/kg daily, all three doses of ravuconazole improved survival and also reduced the tissue burden of *Aspergillus*. Ravuconazole 25 mg/kg/day and itraconazole 10 mg/kg/day were found to sterilize brain and lung tissues more effectively than the lower doses of ravuconazole, the low dose of itraconazole or amphotericin B.

Testing the iv formulation of ravuconazole *in vivo*, Petraitiene *et al.*[41] observed concentration- and time-dependent effects of ravuconazole on the hyphae of *A. fumigatus*. Rabbits treated with ravuconazole at 2.5 mg/kg showed minimal benefit, whereas those treated with 5 and 10 mg/kg showed reductions approaching that obtained with amphotericin B at 1 mg/kg/day. Rabbits treated with ravuconazole at 5 and 10 mg/kg but not at 2.5 mg/kg also showed a significant reduction of the mean pulmonary infarct score in comparison with the untreated controls ($P < 0.001$). A significant dose-related effect in galactomannan levels and in the reduction of computed tomography-measured pulmonary injury was also observed. There were no significant benefits of treatment with the loading dose and maintenance regimen over treatment with only the maintenance regimen. Data from the same group also revealed that the combination of ravuconazole and micafungin was synergistic in the treatment of

experimental pulmonary aspergillosis in persistently neutropenic rabbits,[42] whereas the combination of ravuconazole and liposomal amphotericin B was antagonistic.[43] In a comparative study with ravuconazole in immunosuppressed rabbits with invasive aspergillosis,[44] efficacy was better than anidulafungin where improved survival and decreased *Aspergillus* antigenaemia were observed, along with an elimination of organisms from tissues.

In a murine neutropenic model of disseminated C. *albicans* infection, Andes *et al.*[45] investigated the outcomes of ravuconazole therapy with a total dose range of more than 1000-fold, five dosing intervals and two treatment durations (24 and 72 h). The organisms studied required ravuconazole MICs of 0.016–0.12 mg/L for inhibition of growth. Peak serum levels and the AUC increased in a relatively linear fashion with dose escalation and serum elimination half-life ranged from 3.9 to 4.8 h. Ravuconazole administered at doses of 10 and 40 mg/kg produced levels of total drug in serum above the MIC for *Candida* organisms for 16 and 27 h, respectively. Free-drug time above MIC for these dose levels was, however, considerably shorter (0 and 9.4 h, respectively). Treatment efficacies with the five dosing intervals studied were similar, supporting the argument for the AUC/MIC ratio as the PK/PD parameter predictive of efficacy. Moreover, the AUC/MIC ratio was also strongly predictive of treatment outcomes. The peak/MIC ratio was also an important parameter in this study, showing a strong relationship with microbiological effect (R^2 85%). Ravuconazole concentrations in tissues are 2–6 times higher than the corresponding blood concentrations.[39,46] Ravuconazole was also effective in murine models of mucosal candidosis,[39,47] being more efficacious than fluconazole when both drugs were given at 25 mg/kg.[47]

In a healthy murine model of intracranial cryptococcosis, both ravuconazole and fluconazole given orally significantly reduced the number of *Cryptococcus* colonies in brain tissue, in comparison with either itraconazole or no treatment.[39] Ravuconazole and itraconazole both at 50 mg/kg were equally effective in experimental histoplasmosis.[48] In terms of MICs, ravuconazole was ∼4 times more active than itraconazole against *H. capsulatum* (MICs of 0.02 and 0.1 mg/L, respectively). Ravuconazole has also showed very potent *in vitro* activity against the parasite *Trypanosoma cruzi*,[49] with MICs of 0.3 mg/L. In mice, oral ravuconazole induced a dose-dependent effect on the growth rate of *T. cruzi* epimastigotes. In the experimental model of acute Chagas' disease suppressive rather than curative activity was observed with daily dosing of ravuconazole. This was markedly improved by giving ravuconazole twice a day, which is consistent with the short terminal half-life of ravuconazole in mice (4 h). Both ravuconazole and ketoconazole were unable to induce parasitological cures in a murine model of chronic Chagas' disease.

Protein binding of ravuconazole in animals is >95%, which is similar in degree to what occurs for other azoles such as isavuconazole, posaconazole and itraconazole. On the contrary, protein binding of voriconazole is 58%, and only 11% for fluconazole.[50] Although it is usually assumed that free-drug levels better predict drug potency than total drug levels, the importance of these parameters is not so obvious. Because of the lower degree of protein binding for fluconazole, studies evaluating PK/PD parameters for fluconazole have evaluated total drug levels, while many investigations performed with the new azoles have focused on free-drug

concentrations. Discrepancies among studies might be at least in part explained by this fact.

Phase 1/2 studies. In an ascending-dose study, single oral doses of ravuconazole were given to healthy subjects (50, 100, 200, 400, 600 and 800 mg).[51] An approximately dose-proportional increase in ravuconazole plasma levels was observed for doses of 50–400 mg, although a less than dose-proportional increase was noted for doses >400 mg in fasted state. A 2–4-fold increase in systemic bioavailability was observed when ravuconazole was co-administered with a high-fat meal.[51] In a study with daily oral dosing for 14 days, a 10-fold accumulation was noted,[52] which is in accordance with the drug half-life (4–8 days).[51,52] Single parenteral doses of 25–600 mg of BMS-379224, the water-soluble pro-drug of ravuconazole, were well tolerated and demonstrated linear plasma pharmacokinetics.[53] Ravuconazole did not induce CYP3A isozymes in these studies, although in one study ravuconazole was found to result in decreased exposure to the antiretroviral nelfinavir.[54] As ravuconazole is a less potent inhibitor of CYP3A4 compared with the licensed triazoles, it is speculated that ravuconazole has a lower potential for drug interactions.[55] However, no information is available about interaction with other liver cytochrome enzymes such as CYP2C9 or 2C19, with which voriconazole interacts.

In a Phase 2 trial, HIV-infected patients with oropharyngeal candidosis were randomized to receive ravuconazole 50 mg once daily for 5 days, 200 mg once daily for 5 days and 400 mg as a single dose or placebo.[20] Dosing was 2 h before or 2 h after a meal. The most effective regimen was 200 mg once daily for 5 days (85% of the subjects being cured or improved). Ravuconazole (400 mg once daily) was also compared with fluconazole (200 mg once daily) in immunocompromised individuals with *Candida* oesophagitis, both for 21 days in a double-blind manner.[56] Rates of cure were similar between groups (86% and 78%, respectively). Ravuconazole cure rate improved to 93% if individuals taking rifampicin were excluded, since rifampicin reduced ravuconazole levels by over 50%. In another Phase 1/2 trial,[57] the effect of three 12-week-dosing regimens of ravuconazole was investigated in patients suffering from onychomycosis: 200 mg daily; 100 mg weekly; and 400 mg weekly. Effective cure was reported in 56% of patients receiving 200 mg/day, while the results for the other treatment options did not differ from placebo. Nearly all (95%) patients in the 200 mg/day group had a clinical response to ravuconazole.

Partial results from a Phase 1/2 prophylactic trial in patients undergoing allogeneic stem cell transplantation (NCT00064311) have been recently presented.[58] Three dosage cohorts of eight patients each received daily ravuconazole orally at 400, 600 or 800 mg once a day from within 48 h of the transplant preparative regimen until 48 h after recovery from neutropenia. Linear dose–responses in plasma concentrations were observed, with a plasma half-life of 22–36 h after a single dose. C_{max} and AUC_{0-24} following a single 800 mg dose were 2-fold higher than after a single 400 mg dose.

Phase 3 studies. No data are yet available.

Toxicity. No toxicity was described in animal studies where ravuconazole was tested. Ravuconazole was well tolerated in the single-ascending-oral-dose study;[51] all adverse events were mild or moderate and resolved prior to discharge. Headache was the

most frequent adverse event. Side effects were noted in 73% of patients involved in the Phase 2 trial for the treatment of onychomycosis.[57] Headache and abdominal pain were the most frequent severe adverse events recorded. Only adverse events considered to be drug-related were reported, and the incidence of these was similar for patients treated with ravuconazole or placebo. Three out of 115 patients discontinued medications because of adverse events, including dizziness, abnormal thinking, urinary incontinence, diarrhoea and anaemia. No subject discontinued therapy because of laboratory abnormalities. In the randomized study comparing ravuconazole and fluconazole in patients with oesophageal candidosis,[56] the most common drug-related adverse events occurring in the ravuconazole arm were abdominal pain (8%), diarrhoea (6%), pruritus (6%) and rash (6%). There were no discontinuations related to laboratory abnormalities.

Albaconazole

Albaconazole (UR-9825) is a new triazole (7-chloro derivative compound; Figure 1) with a potent, broad spectrum of antifungal activity, good pharmacokinetics and excellent oral bioavailability (nearly 80% in rats and 100% in dogs).[59]

In vitro studies. Capilla *et al.*[60] reported the *in vitro* activity of albaconazole against 77 medically important filamentous fungi. Potent activity was demonstrated against species of *Aspergillus* (MICs 0.06–0.5 mg/L), *Paecilomyces* (MIC_{90} 0.125 mg/L) and *Chaetomium* (MIC_{90} 2 mg/L). Albaconazole was more active than amphotericin B against all fungi tested except for *Fusarium solani* (MICs 4 to >16 mg/L) and *Scytalidium* species (MICs 2 to >16 mg/L).

Ramos *et al.*[61] compared the *in vitro* activity of albaconazole with that of fluconazole and itraconazole against a panel of 283 isolates of *Candida* spp. Albaconazole was the most active of the drugs, with MIC_{90} values in the range ≤0.0002–0.12 mg/L. *Candida* isolates for which MICs of fluconazole were ≥16 mg/L were still susceptible to albaconazole (MIC_{90} 0.06–1 mg/L) although high MICs where required for *C. tropicalis* (64 mg/L). Albaconazole was found to be very active against *C. neoformans* isolates including an isolate for which the fluconazole MIC was 64 mg/L.[62] The albaconazole MICs for the majority of cryptococcal isolates were between 0.002 and 0.2 mg/L, indicating 100-fold higher activity *in vitro* than fluconazole. Like voriconazole, albaconazole is very active against isolates of *Cryptococcus gattii*,[63,64] including fluconazole-resistant strains.

In a study with 70 strains of *Malassezia* spp.,[65] albaconazole showed an *in vitro* activity similar to that of ketoconazole, itraconazole and voriconazole (MIC <0.06 mg/L for all isolates). All strains were resistant *in vitro* to flucytosine (MIC >64 mg/L) and fluconazole MICs ranged from 0.25 to 4 mg/L. Albaconazole has demonstrated no *in vitro* activity against *Fusarium* spp. (MIC_{90}s 16–32 mg/L).[33] However, an additive effect was observed for most of the *Fusarium* species tested when albaconazole was combined with amphotericin B. Against *Paecilomyces* spp.,[66] albaconazole MIC_{90}s ranged from 0.5 mg/L (*Paecilomyces variotii*) to 4 mg/L (*Paecilomyces lilacinus*). *In vitro* activity against *Chaetomium* spp. has also been demonstrated (MICs of 0.12–1 mg/L).[34] Albaconazole is also very potent against the protozoan parasite *T. cruzi*. The observed

MIC of 0.03 μM is 33-fold lower than that required with the reference compound ketoconazole.[67] Like ravuconazole, albaconazole demonstrated poor *in vitro* activity against the species of the *P. boydii* complex,[31] where best results occurred with voriconazole and posaconazole.

Animal studies. Albaconazole was tested orally in a steroid-immunosuppressed rat model of disseminated aspergillosis and compared with amphotericin B.[59] Albaconazole prophylaxis protected rats infected with conidia of *A. fumigatus* in a dose-related fashion, with 50 mg/kg twice daily giving 100% protection. Similar results were obtained with amphotericin B at 2 mg/kg iv daily.

In a murine model of systemic candidosis, oral albaconazole displayed an activity comparable to that of fluconazole when given for 5 days at 5 mg/kg twice daily.[68] As albaconazole drug levels declined very rapidly in mice and became undetectable 6 h post-administration (half-life estimated to be 1 h), better results were observed when the drug was given twice daily in mice models.[59]

In a rabbit model of cryptococcal meningitis,[62] the efficacy of oral albaconazole was also similar to that of fluconazole. Different dosages of albaconazole (5, 20 and 80 mg/kg/day) resulted in similar efficacy. Albaconazole serum concentrations obtained 1–2 h after drug intake for doses of 5, 20 and 80 mg/kg daily were 1.18, 3.59 and 4.14 mg/L, respectively. Albaconazole could be detected in cerebrospinal fluid only at higher dosages (80 mg/kg/day), with drug concentrations in the cerebrospinal fluid reaching 15% of serum levels. Albaconazole has also been investigated *in vivo* in an animal model of Chagas' disease. Dogs were used instead of mice due to the short terminal half-life of albaconazole in mice (<0.5 h).[69] Although albaconazole given at 1.5 mg/kg/day was very effective in suppressing the proliferation of the parasite in the animals infected, no parasitological cure was observed among them, even when a longer treatment period (150 doses) was used.

Albaconazole was evaluated in oral doses of 15, 25 and 50 mg/kg daily for 10 days in an immunocompetent rabbit model of *S. prolificans* systemic infection.[70] The mortality of the animals treated with albaconazole at 15 mg/kg/day was similar to that of the untreated animals. Animals treated with albaconazole at 25 mg/kg/day or amphotericin B at 0.8 mg/kg/day showed 50% survival, and all animals treated with albaconazole at 50 mg/kg daily survived. Higher albaconazole dosages were also more effective in reducing tissue burden.

Phase 1/2 studies. In a Phase 1 study involving 72 healthy volunteers,[71] albaconazole was rapidly absorbed. C_{max} values were reached in 2–4 h, and the drug was widely distributed throughout body fluids. C_{max} and AUC of albaconazole were dose-proportional for doses between 5 and 80 mg (half-life 30–56 h), and substantially increased exposure occurred at modest dose increases from 160 to 400 mg, indicating non-linear pharmacokinetics at higher doses.

A Phase 2 trial compared the therapeutic efficacy of a single dose of albaconazole at 10, 40, 80, 160 and 320 mg, in comparison with fluconazole, in 64 women affected by acute non-complicated *Candida* vulvovaginitis.[72] Rates of therapeutic cure were 25%, 91%, 85%, 89%, 80% and 67%, respectively. A single dose of albaconazole ≥40 mg seems to be more efficacious than fluconazole at 150 mg.

Phase 3 studies. No data are yet available.

Toxicity. Low toxicity was observed when albaconazole was administered to rats at 250 mg/kg once daily or 100 mg/kg every 12 h for 28 days.[59] Minimal toxicity was described when dogs infected with *T. cruzi* were treated with albaconazole,[69] and no signs of toxicity were observed in rabbits receiving 15–50 mg/kg daily against *Scedosporium* infection.[70] No serious adverse effects were reported in clinical studies involving albaconazole.[71,72]

Echinocandins

Aminocandin

Caspofungin was the first echinocandin to be licensed, followed by micafungin and recently anidulafungin. These drugs have occupied an important place in the therapeutic arsenal against invasive *Candida* infections and refractory aspergillosis. Clear advantages of echinocandins over other antifungal drugs include low toxicity (since their site of action—fungal cell walls—is not present in humans) and limited drug interactions. Aminocandin (HMR-3270) is a semi-synthetic fermentation product from *Aspergillus sydowi*. Its chemical structure has some similarities with the other members of the echinocandin class (Figure 1). The degree of protein binding has been >99% for both mice and in humans, which differs slightly from caspofungin (97%) and anidulafungin (85%), but probably not micafungin (99.9%). Like other echinocandins, aminocandin is a lipopeptide that is not metabolized by the liver and unlike the azoles is not a substrate, inhibitor or inducer of the cytochrome P450 enzymes.[73] Unlike other echinocandins, aminocandin's long half-life makes infrequent dosing feasible.

In vitro studies. Aminocandin has demonstrated potent activity against both *Aspergillus* spp. (including itraconazole-resistant strains) and *Candida* spp. (including species resistant to azoles and amphotericin B).[74–76] Warn *et al.*[76] demonstrated the *in vitro* activity of aminocandin against 80 isolates of *Aspergillus* comprising four different species (*A. fumigatus*, *A. flavus*, *A. terreus* and *A. niger*). The minimum effective concentration (MEC) was recorded for aminocandin as the drug concentration in which the predominant growth was reduced, whereas MICs were determined for amphotericin B and itraconazole. For all isolates, geometric mean MIC/MEC values and ranges were (mg/L): aminocandin 0.23 and 0.06–2; amphotericin B 0.4 and 0.06–4; and itraconazole 0.3 and 0.06 to >8. *A. flavus* (average MICs of 0.6 mg/L) and *A. niger* (0.3 mg/L) were less susceptible to aminocandin than the other *Aspergillus* species.

In a study involving 110 isolates of *Candida* comprising nine different species,[74] aminocandin showed *in vitro* activity similar to that of amphotericin B, while both drugs performed better than fluconazole. For all isolates, geometric mean MIC values and ranges (mg/L) were: aminocandin 0.02 and 0.002–1; amphotericin B 0.03 and 0.008–0.5; and fluconazole 8.6 and ≤0.125 to >128. The species most susceptible to aminocandin were *C. glabrata* (0.008 mg/L), *C. albicans* (0.012 mg/L) and *C. krusei* (0.02 mg/L), while *C. parapsilosis* (0.4 mg/L) and *Candida guilliermondii* (0.25 mg/L) were significantly less susceptible. Aminocandin showed twice the inhibitory effect of caspofungin and micafungin against a strain of *C. parapsilosis*

resistant to both caspofungin and micafungin (MICs of 64 mg/L).[77] The clinical implications of these findings are yet to be determined.

Isham and Ghannoum[78] demonstrated that while the MIC ranges of aminocandin against yeasts were similar (0.03–4 mg/L), results for filamentous fungi were species-specific. Aminocandin was very active *in vitro* against *A. fumigatus* (MIC$_{90}$ 0.5 mg/L) but not active against *Scedosporium* spp., *Fusarium* spp. and the Mucorales (MIC$_{90}$ 8, >256 and >16 mg/L, respectively). Amphotericin B and voriconazole demonstrated similar activities (MIC$_{90}$ 4 mg/L) against the *Fusarium* and *Scedosporium* isolates tested, whereas neither caspofungin nor micafungin showed activity against isolates from these genera. Amphotericin B showed the most potent activity against the Mucorales (MIC$_{90}$ 0.5 mg/L). Aminocandin was less active *in vitro* than voriconazole against yeasts in this study but similar to amphotericin B and caspofungin. The MIC$_{90}$ of aminocandin for all yeasts was 3-fold lower than that of micafungin, again suggesting that differences in the activities against non-*C. albicans* strains might exist among members of this drug class.

Animal studies. The *in vivo* activity of aminocandin against itraconazole-susceptible and -resistant *Aspergillus* species was demonstrated in a murine neutropenic model of disseminated aspergillosis.[79] Mice received iv aminocandin (0.25–5 mg/kg/dose), amphotericin B (5 mg/kg/dose), oral itraconazole (25 mg/kg/dose) or solvent control for 9 days. Aminocandin at doses of ≥1 mg/kg was highly effective in reducing mortality and organ burden but fungicidal activity was observed only with higher doses (5 mg/kg). In terms of both tissue burden and survival, aminocandin was at least as effective as similar doses of micafungin and caspofungin in another murine model of aspergillosis.[80] Dose fractionation studies with aminocandin indicate that the response is peak driven and adequate therapy may be achieved using less frequent high-dose regimens.[12,81]

Andes *et al.*[82] characterized the *in vivo* pharmacodynamic proprieties of aminocandin administered to neutropenic mice infected with *C. albicans* for which the MIC of aminocandin was 0.5 mg/L. The study evaluated a number of dosages and schedules to identify the pharmacodynamic parameter that optimally links drug exposure with effect. Peak serum levels were achieved within 2 h and linear pharmacokinetics were documented. A concentration-dependent fungicidal effect was observed when drug levels in serum were >4 times the MIC. Post-antifungal effects were also dose-dependent, lasting 8–80 h. Treatment outcomes most strongly correlated with the peak/MIC ratio (R^2 98%) and moderately correlated with the AUC/MIC ratio (R^2 79%). Shortening the dosing interval decreased drug efficacy. A 4-fold-lower total drug dose was necessary to produce a net fungistatic effect, with the once-every-6-day dosing compared with the every 36 h dosing regimen. The elimination half-life did not change significantly with the doses studied, ranging from 22.1 to 23.2 h. These findings are consistent with the pharmacodynamic properties of caspofungin, which appears to exhibit concentration-dependent (as opposed to time-dependent) killing.[83,84] More experimental work is required to further understand the pharmacokinetics and pharmacodynamics of aminocandin as a means of optimizing the clinical use of this agent.

Warn *et al.*[85] compared the activity of aminocandin with that of amphotericin B and fluconazole in a neutropenic murine

model of disseminated candidosis caused by a fluconazole-resistant strain of *C. tropicalis*. The MIC values for this isolate were: amphotericin B 0.004 mg/L; fluconazole >128 mg/L; and aminocandin 0.06 mg/L. Aminocandin at ≥2.5 mg/kg/day and amphotericin B were superior in terms of survival to aminocandin ≤0.25 mg/kg/day, fluconazole and controls ($P \leq 0.05$). Aminocandin 1 mg/kg/day was superior to fluconazole and controls ($P \leq 0.026$). The only treatment to clear organ burdens totally was amphotericin B, which resulted in clearance in 40% of mice. Lowther *et al.*[86] compared the *in vivo* efficacy of aminocandin against fluconazole-susceptible and -resistant strains of *C. albicans*. Aminocandin at 0.7 mg/kg protected 100% of the mice infected by fluconazole-susceptible *C. albicans* and was significantly more efficacious ($P < 0.05$) than fluconazole at 2 mg/kg. For mice infected with the fluconazole-resistant strain, a single 0.5 mg/kg iv dose of aminocandin was more efficacious than a 0.5 mg/kg iv dose of amphotericin B or fluconazole at 5 mg/kg. Aminocandin demonstrated good fungicidal activity in significantly reducing the renal burden. In another animal model of fluconazole-resistant candidosis,[87] 100% survival was observed for mice treated with caspofungin (0.5 mg/kg intraperitoneally once daily) or aminocandin at any given dose (5 or 10 mg/kg iv once or twice weekly). Dosing of aminocandin twice a week did not significantly lower the average tissue fungal burden compared with the same doses given once a week. There was no significant difference between the fungal burden for all aminocandin treatment groups and amphotericin B (0.5 mg/kg iv every other day) or caspofungin treatment groups.

Phase 1/2 studies. Single iv doses of 75–300 mg of aminocandin were administered to 12 healthy, male volunteers in a single-blind, randomized, placebo-controlled trial.[88] The C_{max} and AUC of aminocandin increased in proportion to its dose and significant dose-dependent fungicidal titres against *Candida* and *Aspergillus* species were observed over the range evaluated. The elimination half-life of the drug was 48–58 h and the volume of distribution was 23.5–26.2 L. The 300 mg dose retained antifungal activity even up to 168 h post-administration.

Phase 3 studies. No data are yet available.

Toxicity. No infusion-related histamine reactions occurred in the Phase 1 study, and the tolerated dose levels were 7-fold higher than the anticipated clinical dose (http://www.indevus.com).

Conclusions

Although isavuconazole, ravuconazole and aminocandin offer extended half-lives compared with other members of their respective class, it is not clear that they carry other distinguishing characteristics. Albaconazole's antifungal spectrum appears slightly different from those of the other triazole compounds, although it has not been formally tested against the Mucorales. Completion of the clinical trial programme of each agent will be needed to determine their place in clinical management. None offer a new class of antifungal, or a different mechanism of action, which is required to improve the spectrum of activity. The new azoles have some of the problems associated with azoles such as CYP-mediated drug interactions, azole cross-resistance and drug disposition issues, whereas aminocandin has a limited spectrum of activity like the other echinocandins, with only an iv formulation possible.

Transparency declarations

A. C. P.: none to declare. In the past 5 years, D. W. D. has received grant support from Astellas, Merck, Pfizer, F2G, OrthoBiotech, Indevus, Basilea, the Fungal Research Trust, the Wellcome Trust, the Moulton Trust, The Medical Research Council, the National Institute of Allergy and Infectious Diseases and the European Union. He has been an advisor/consultant to Basilea, Vicuron (now Pfizer), Schering Plough, Indevus, F2G, Nektar, Daiichi, Sigma Tau, Astellas, Gilead and York Pharma. He has been paid for talks on behalf of Astellas, Merck, GSK, Chiron, AstraZenca and Pfizer. He holds founder shares in F2G Ltd and Myconostica Ltd, both university spin-out companies. F2G is engaged in identifying novel antifungal targets and compounds and developing new chemical classes for the treatment of life-threatening antifungal drugs. Myconostica is engaged in commercializing molecular diagnostics for infectious diseases, including invasive fungal infections.

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