Lessons from animal studies for the treatment of invasive human infections due to uncommon fungi

Josep Guarro*

Mycology Unit, Medical School, IISPV, Rovira i Virgili University, 43201 Reus, Spain

*Tel: +34-977-759359; Fax: +34-977-759322; E-mail: josep.guarro@urv.cat

Clinical experience in the management of opportunistic infections, especially those caused by less common fungi, is, due to their rarity, very scarce; therefore, the most effective treatments remain unknown. The ever-increasing numbers of fungal infections due to opportunistic fungi have repeatedly proven the limitations of the antifungal armamentarium. Moreover, some of these fungi, such as *Fusarium* spp. or *Scedosporium* spp., are innately resistant to almost all the available antifungal drugs, which makes the development of new and effective therapies a high priority. Since it is difficult to conduct randomized clinical trials in these uncommon mycoses, the use of animal models is a good alternative for evaluating new therapies. This is an extensive review of the numerous studies that have used animal models for this purpose against a significant number of less common fungi. A table describing the different studies performed on the efficacy of the different drugs tested is included for each fungal species. In addition, there is a summary table showing the conclusions that can be derived from the analysis of the studies and listing the drugs that showed the best results. Considering the wide variability in the response to the antifungals that the different strains of a given species can show, the table highlights the drugs that showed positive results using at least two parameters for evaluating efficacy against at least two different strains without showing any negative results. These data can be very useful for guiding the treatment of rare infections when there is very little experience or when controversial results exist, or when treatment fails.

Keywords: opportunistic fungi, fungal infections, animal models, antifungal drugs, aspergillosis, candidiasis, fusariosis, scedosporiosis, zygomycosis, mycosis

Introduction

The therapeutic treatment of invasive mycoses caused by the commonest pathogenic fungi, i.e. endemic dimorphic fungi, agents of chromoblastomycosis, sporotrichosis, dermatophytosis, cryptococcosis, pneumocystosis, and infections by *Aspergillus fumigatus* and *Candida albicans*, are more or less well established and consensual guidelines for their treatment are regularly published. In addition, numerous reviews have been published on the use of animal models that have evaluated the treatment of those infections.1–3 However, in recent years the spectrum of fungi able to infect humans has changed dramatically and, although the mentioned pathogens are still the most important, numerous new agents are appearing, whose diagnosis and treatment are sometimes very complicated.4–9 Moreover, some of these fungi, such as *Fusarium* spp. or *Scedosporium* spp., are innately resistant to practically all the available antifungal drugs, which makes the development of new and effective therapies a prime pursuit. The incidence of infections by most opportunistic fungal pathogens, especially those caused by less common species, is low and the clinical experience on their management is very limited, meaning the more effective treatments remain unknown. There have only been a small number of case reports published concerning these rare fungal infections, and they often lack crucial details on the clinical course, diagnostic procedures, reliable identification of the causal agent, treatment details and, especially, clinical follow-up. This limits their usefulness in determining the efficacy of the therapy.

It is well known that in therapeutics the gold standard is the randomized clinical trial; however, in unusual opportunistic infections such trials are generally impossible to carry out, because of the small number of patients that are infected by any given pathogen at any one time, even across different countries and hospitals. Therefore, when it is difficult to conduct a randomized clinical trial, or even a clinical case series or an open trial, animal studies can be a good alternative. Animal models have proven to be useful predictors of clinical results in humans, in terms of efficacy, kinetics and toxicity.1–3 They are relatively inexpensive and quick to give results; a high number of animals can be used to perform appropriate statistical analysis and can mimic the pathogenesis of different human fungal infections. Therefore, animal studies might be particularly helpful for the management of uncommon fungal species for which clinical experience for guiding antifungal therapy is lacking. Most of the fungi included...
in this review are opportunists with low virulence and are only pathogenic for severely immunocompromised human patients. Therefore, in immunocompetent animals, the standard fungal inocula commonly fail to establish a lethal infection. In such cases, high inocula (≥10⁸ cfu per animal) are required and animals need to be immunosuppressed with cytotoxic agents or with corticosteroids.

This review provides an extensive overview of the studies that have used animal models for evaluating the efficacy of different therapeutic treatments for infections by a significant number of less common opportunistic fungi. The results of these studies have been discussed and compared with clinical results, where they are available, in order to assess the drugs that showed the best results against these rare mycoses.

Since all this information is spread across numerous articles, it is sometimes not available in the general databases, so the main objective of this review has been to bring together in a single paper almost all the available data on the response to different therapies in animal models of less-frequent opportunistic fungi.

**Methods**

A computerized search of the Medline, Scopus and Science Citation Index Expanded databases was carried out for animal studies reported in the literature, with (by cross-referencing) the terms ‘animal models’, ‘experimental antifungal therapy’, ‘antifungal drugs’, ‘host-response’, ‘fungal virulence’, ‘mice’, ‘rabbits’ and ‘guinea pigs’. Almost all the rare opportunistic fungal infections whose treatment has been evaluated in animal models are included in the review, with a table summarizing the different studies addressed to each of them (Table 1). Although the choice of the animal often depends on the pharmacokinetics and pharmacodynamics of the drug, in most of the studies on uncommon fungi, with a few exceptions that used guinea pigs or rabbits, the animals used were mice. Mice are not useful for studies on voriconazole because of rapid drug clearance, but it has been shown that there is an inhibitory effect of grapefruit juice on the cytochrome P450 enzymes involved in the metabolism of voriconazole, which increases the concentrations of such drugs in murine serum.¹² The efficacy markers most commonly used in the different studies are statistical prolongation of the survival of the infected animals and reduction of fungal burden as determined by quantitative organ sample cultures. Occasionally, additional markers of fungal load have also been used, such as galactomannan or β-glucan serum level reduction.

The efficacy of the different drugs tested in each study was scored in the corresponding tables (available as Supplementary data at JAC Online) as: high, when the results obtained with at least two markers were significantly positive with respect to controls (e.g. prolongation of survival and reduction of fungal load in the kidneys) and no negative result was obtained, or when only one marker was used with successful results in more than one strain; moderate, when the results obtained, with at least two parameters, were significantly better than for the controls and any negative result was obtained or when the results obtained with the only parameter tested were a significant improvement on those of the controls; low, when only one marker was positive and any negative result was also obtained; and variable, when more than one strain was used and against one or some of them the results were positive, and negative against the other(s).

A table is also included (Table 1) that summarizes the conclusions derived from the analysis of the reviewed studies and lists the drugs that showed the best results, categorized in three groups: (i) those that showed positive results when using at least two parameters to evaluate efficacy against at least two different strains, and that did not show any negative result; (ii) those that showed positive results using at least two parameters to evaluate efficacy against only one strain; and (iii) those that showed high efficacy in at least one study, in which at least two parameters were used or in which two or more strains were used, even if negative results were obtained in another study.

**Candidiasis**

The efficacy of different therapeutic treatments has been evaluated in recent years in animal models against eight species of Candida non-albicans: Candida dubliniensis, Candida glabrata, Candida guilliermondii, Candida krusei, Candida lusitaniae, Candida parapsilosis, Candida rugosa and Candida tropicalis.

**C. dubliniensis**

The incidence of C. dubliniensis in candidaemia episodes ranges from 0.7% to 7%.¹¹¹² However, in a recent study on bloodstream infections with uncommon Candida spp., C. dubliniensis was the commonest species, and was independently associated with recent intravenous drug use and chronic liver disease.¹³ This species shares many phenotypic similarities with C. albicans, which can create confusion in its identification. In animal models, only the efficacy of echinocandins has been tested¹⁶ (Table S1, available as Supplementary data at JAC Online). The three available echinocandins, micafungin, anidulafungin, and caspofungin, were tested against two isolates of C. dubliniensis, one of them showing a paradoxical effect in vitro and considerably high minimum fungicidal concentrations. The three drugs were similarly effective for the treatment of murine disseminated infections caused by this fungus, although they showed some variability depending on the strain tested. In general, all treatments reduced the tissue burden, but, in contrast, almost all of them showed a negative effect on the survival of immunocompetent mice infected with one of the two strains tested. The authors concluded that the paradoxical in vitro growth of C. dubliniensis does not preclude in vivo response to echinocandin therapy.¹⁴

**C. glabrata**

C. glabrata is the second leading cause of candidaemia in adults, representing up to 24% of candidaemia cases,¹⁵ and is particularly common in the northern hemisphere. This species is, of those included in this review, the most commonly tested in animal studies. In 13 different studies, all of them in mice, different therapeutic regimens were evaluated. With the exception of voriconazole, almost all the newer drugs, including several combinations of them, were tested (Table S2, available as Supplementary data at JAC Online). Amphotericin B has been the most commonly tested drug, being evaluated on 11 occasions.¹⁵⁻⁻²⁵ Caspofungin was tested nine times,¹⁵⁻⁻¹⁷,²⁰,²¹,²⁶⁻⁻²⁹ fluconazole on seven occasions,¹⁵,¹⁶,¹⁸,¹⁹,²¹,²⁴,²⁶ micafungin in four studies,¹⁷⁻⁻¹⁹,²² and posaconazole,²³,²⁶ anidulafungin,²¹,²⁸ and 5-fluorocytosine¹⁶,¹⁸ on two occasions each. Fluconazole displayed variable, but in general negative, results,¹⁸,²⁶ even against strains that had shown low fluconazole MICs.¹⁵ In general, amphotericin B demonstrated some efficacy, since this drug was able to prolong survival and to reduce cfu in the kidneys and spleen of mice infected with numerous strains, although not against all the
Table 1. Drugs and combinations of drugs that have demonstrated high efficacy against different opportunistic fungi in the animal studies reviewed

<table>
<thead>
<tr>
<th>Species</th>
<th>ABC</th>
<th>AFG</th>
<th>AMB</th>
<th>CAS</th>
<th>FLC</th>
<th>ITC</th>
<th>LAMB</th>
<th>POS</th>
<th>VRC</th>
<th>AMB+VRC</th>
<th>AMB+5FC</th>
<th>AMB+MFG</th>
<th>AMB+CAS</th>
<th>LAMB+TRB</th>
<th>MFG+FLC</th>
<th>MFG+VRC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apophysomyces elegans</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus nidulans</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus terreus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blastoschizomyces capitatus</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida dubliniensis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>+</td>
<td>(+)</td>
<td>(+)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida guilliermondii</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(+)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida krusei</td>
<td></td>
<td>(+)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida lusitaniae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida parapsilosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida rugosa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cladophialophora bantiana</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cunninghamella bertholletiae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exophiala dermatisidis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exophiala oligosperma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exophiala xenobiotica</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fonsecaea monophora</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fusarium oxysporum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fusarium solani</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fusarium verticilliioides</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lichtheimia corymbifera</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(+)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucor circinelloides</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucor ramosissimus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neoscytalidium dimidiatum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paecilomyces lilacinus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhinocladiella mackenziei</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhizopus microsporus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhizopus oryzae</td>
<td>(+)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scedosporium apiospermum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scedosporium aurantiacum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scedosporium boydii</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scedosporium prolificans</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trichosporon asahii</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SFC, 5-fluorocytosine; ABC, albaconazole; AMB, amphotericin B; AFG, anidulafungin; CAS, caspofungin; FLC, fluconazole; ITC, itraconazole; LAMB, liposomal amphotericin B; MFG, micafungin; POS, posaconazole; TRB, terbinafine; VRC, voriconazole; ++ drugs that showed positive results, using at least two parameters to evaluate efficacy, against at least two different strains and that did not show any negative result; + drugs that showed positive results using at least two parameters to evaluate efficacy against only one strain; (+), drugs that showed high efficacy in at least one study, with at least two parameters or against at least two strains and, in at least one other study, negative results were obtained.
strains tested. Liposomal amphotericin B improved slightly the results of amphotericin B, demonstrating a dose-dependent response and showing the best results at the highest doses (20 mg/kg/day). Caspofungin also showed good results; it was able to prolong survival and reduce the yeast cell load in the kidneys on different occasions, but not always in spleen. A small percentage of C. glabrata strains show high caspofungin MICs (>2 mg/kg) and high doses (>10 mg/kg) were required to obtain good results in the treatment of murine infections by those strains. In other studies, micafungin showed variable results and anidulafungin was able to reduce the fungal load in the kidneys in two different studies, although only this marker of efficacy was used. Posaconazole showed contradictory results, since it was able to reduce cfu in the kidneys on one occasion, but not on others. Numerous drug combinations have also been tested in animal studies and showed generally good results, although the results were not very different from the respective monotherapies. The combination of caspofungin with amphotericin B or liposomal amphotericin B showed synergy in reducing the fungal load in the kidneys in two different studies, however, only that marker of efficacy was evaluated. The combination of amphotericin B with each of the three echinocandins exerted similar efficacy, but only in the case of the combination with micafungin was more than one marker of efficacy used with positive results in all of the cases. Gumbo et al. demonstrated that once-weekly micafungin therapy is as effective as daily therapy in a murine model. Other authors testing caspofungin, another echinocandin, confirmed the efficacy of administering a high single dose once a week. For those studies, results may have been limited to infections caused by isolates with low susceptibility to echinocandins.

C. guilliermondii

C. guilliermondii, although a rare cause of candidiasis, is an emerging pathogen in Latin America. This species shows low susceptibility to fluconazole. C. guilliermondii has only been tested in one animal study (Supplementary data at JAC Online), which used three strains with high caspofungin MICs. However, even at doses as low as 1 mg/kg of body weight/day, caspofungin reduced the fungal load in the kidneys, which could be explained by the persistence of the drug in kidney tissue even when serum concentrations fall below the MIC.

C. krusei

C. krusei is a potentially multidrug-resistant pathogen, due to its intrinsic fluconazole resistance and low susceptibility to amphotericin B. This species ranked fifth among the Candida species involved in human infections. In five experimental studies, different treatments against C. krusei infections were evaluated, testing fluconazole, voriconazole, posaconazole, caspofungin, anidulafungin and amphotericin B (Table S4, available as Supplementary data at JAC Online). Amphotericin B was tested in two studies. In one study, it was not able to reduce the tissue burden in the kidneys, but in the other one the drug prolonged survival and reduced the tissue burden in the kidneys and spleen for two strains. Liposomal amphotericin B was tested successfully against murine infections by two strains; the drug prolonged survival, and reduced the fungal load in the kidneys and spleen for both strains. Although amphotericin B showed efficacy in animal models, there were numerous cases of failure with this drug in clinical practice. The two echinocandins tested, caspofungin (at doses >0.5 mg/kg) and anidulafungin (at 10 and 20 mg/kg), showed similar results, with both being able to prolong survival and reduce the fungal load in the kidneys. However, caspofungin did not reduce the fungal load in the spleen. Additionally, anidulafungin reduced serum β-glucan.

C. lusitaniae

C. lusitaniae is an uncommon species of Candida, being the sixth most frequent species of that genus to cause invasive infections. In the period 1997–2003, this species represented ≏0.6% of all cases of invasive candidiasis. This fungus has only been tested in a single animal study, where caspofungin was able to reduce the fungal load in the kidneys of mice infected with one strain of this species.

C. parapsilosis

C. parapsilosis is one of the most common non-albicans Candida species causing invasive candidiasis mainly in infants and neonates, especially in some regions of Latin America. This species is most frequently isolated from skin rather than mucosal surfaces and is typically associated with
catheters. To treat C. parapsilosis infections in the neutropaenic patient, fluconazole or liposomal amphotericin B have been recommended as initial therapy. Although C. parapsilosis seems to show less in vitro susceptibility to the echinocandins than most other Candida species do, this has not been confirmed in clinical trials. Recent molecular studies have demonstrated that C. parapsilosis sensu lato is a complex of species grouping the species C. parapsilosis, Candida orthopsilosis and Candida metapsilosis, but most of the isolates of these species seem to be equally susceptible to polyenes, voriconazole and caspofungin. In three animal studies, the activity of caspofungin and amphotericin B, both alone and in combination, was evaluated against four strains of C. parapsilosis (Table S5, available as Supplementary data at JAC Online). The results obtained demonstrated that at relatively high doses (5 mg/kg) the first drug was able to reduce cfu in the kidneys for all of the strains similarly to amphotericin B, however, lower doses of caspofungin (1 mg/kg) were not active against any of the three strains tested. There was also a positive interaction of both drugs.

C. rugosa

C. rugosa is a rare fungus, representing <1% of cases of invasive candidiasis. It has been involved in catheter-related infections in numerous countries, and isolated most often from blood and urine. This fungus shows low susceptibility to amphotericin B and fluconazole. With respect to the former drug, a clear discrepancy between clinical experience and animal response has been reported. In the only experimental study where this fungus was tested (Table S6, available as Supplementary data at JAC Online), the two strains used responded positively to amphotericin B, even at low doses (0.3 mg/kg); however, in a cluster of C. rugosa infections involving six patients hospitalized in an intensive care unit, four of them died despite receiving this drug. On the other hand, the moderate and low effect exerted by posaconazole and fluconazole, and voriconazole, respectively, in the mentioned murine study agrees with the reported low susceptibility of C. rugosa to these compounds.

C. tropicalis

C. tropicalis is the third or fourth most frequently isolated species of Candida in clinical practice. This species is particularly virulent in patients with neutropenia and those with haematological malignancies. Six different studies have evaluated the efficacy of micafungin, fluconazole, amphotericin B, caspofungin, voriconazole and posaconazole against C. tropicalis infections in mice (Table S7, available as Supplementary data at JAC Online). Micafungin was better than amphotericin B and fluconazole against an amphotericin B- and fluconazole-resistant strain. Barchiesi et al. tested several strains and demonstrated a variable pattern of tolerance of amphotericin B, which was isolate dependent. In general, caspofungin and voriconazole showed efficacy. The efficacy of posaconazole was evaluated against five strains of C. tropicalis that had shown in vitro MICs from <0.03 to >16 mg/L; the drug showed efficacy against all of the strains, with the exception of a strain with an MIC of >16 mg/L.

Infections by other yeasts

Blastoschizomyces capitatus

B. capitatus (formerly known as Geotrichum capitatum or Trichosporon capitatum) is an emerging opportunistic yeast that causes severe infections in immunocompromised patients, particularly in those with haematological malignancies. This fungus was tested in three different studies in order to evaluate the efficacy of amphotericin B, fluconazole, voriconazole, micafungin, fluconazole and their different combinations in a murine model of disseminated infection (Table S8, available as Supplementary data at JAC Online). Micafungin did not show efficacy in prolonging survival and reducing tissue burden; amphotericin B and fluconazole showed only moderate efficacy, since the results were strain dependent. The highest efficacy was exerted by fluconazole, being the most effective drug at prolonging the survival of mice and at reducing the fungal burden in the kidneys, spleen and liver. The combinations of amphotericin B with voriconazole, fluconazole or micafungin were not able to improve the efficacy over that of fluconazole. However, the combination micafungin plus fluconazole showed synergistic effects. The lack of efficacy of micafungin against this fungus was also proven in a recent clinical case, where a leukaemic patient receiving empirical micafungin therapy for neutropenic fever developed a fatal B. capitatus infection. The use of the combined therapies described above may be alternatives for the treatment of these infections, given that fluconazole-resistant strains causing nosocomial infections in cancer patients have been reported.

Trichosporon asahii

Trichosporonosis is a severe fungal infection that mainly affects haematological patients. T. asahii is the most frequently reported species causing disseminated trichosporonosis. Trichosporon is generally less susceptible to amphotericin B, the drug usually used empirically for the treatment of suspected yeast infections. Up to now, only two experimental studies have evaluated the efficacy of different treatments against animal infections by this fungus (Table S9, available as Supplementary data at JAC Online). In one of them, amphotericin B, fluconazole, micafungin and their combinations were tested. The best results were obtained with micafungin plus amphotericin B, which showed a synergistic effect for the two strains tested, and demonstrated a higher degree of efficacy in prolonging survival and reducing the kidney fungal burden than either agent alone. In the second study, guinea pigs were used to test amphotericin B and voriconazole. The former was effective against only one of the two strains, while voriconazole showed high efficacy against both. Although voriconazole has not always shown efficacy in the clinical setting, recent clinical cases have demonstrated its efficacy in the treatment of trichosporonosis caused by T. asahii. Other clinical cases confirmed the results obtained from the experimental therapies in animal models since the combination of liposomal amphotericin B and one echinocandin, caspofungin, showed good results, while fluconazole plus caspofungin were ineffective after the failure of amphotericin B.
Aspergillosis

Apart from *A. fumigatus*, which has been tested in numerous studies in different models, the antifungal susceptibility of only another three species of *Aspergillus* (*Aspergillus flavus*, *Aspergillus nidulans* and *Aspergillus terreus*) has been studied in animal models.

**A. flavus**

*A. flavus* is the second leading cause of invasive and non-invasive aspergillosis. Although in general the pattern of infections produced by this fungus is similar to that of *A. fumigatus*, *A. flavus* is rarely the aetiologic agent of chronic cavitary pulmonary aspergillosis. More commonly, it causes chronic granulomatous sinusitis, keratitis, cutaneous infections, wound infections and osteomyelitis following trauma and inoculation. Mixed infections by the two species mentioned have also been reported.

In addition, studies in different animal models seem to demonstrate that *A. flavus* is more virulent than *A. fumigatus*. Seven animal studies have been carried out with *A. flavus* to evaluate the efficacy of different drugs. Five antifungal drugs and two combinations were tested, i.e. amphotericin B, itraconazole, posaconazole, voriconazole, caspofungin, amphotericin B plus posaconazole and posaconazole plus caspofungin. The former two drugs were tested in four studies, caspofungin and posaconazole in two, and voriconazole in one.

Intranasal inoculation is probably the way of infecting mice that better mimics the natural route of infection in humans and would also be a more appropriate route than intravenous inoculation. However, the large size of the conidia of *A. flavus* may make it difficult for these spores to reach the alveoli of small experimental animals. No comparative studies have been conducted on the suitability of mice and larger animals to be infected by *A. flavus*. However, Chakrabarti et al., after failure with mice and rats, were able to develop a rabbit model that simulated paranasal sinus infections by *A. flavus* in humans. So far, all the animal studies that have evaluated the efficacy of drugs against this fungus have been carried out using mice, with the exception of one that used guinea pigs. The most common route of infection in these studies was intravenous.

Table S10 (available as Supplementary data at JAC Online) summarizes the results of the different animal studies conducted to evaluate different therapies for *A. flavus* infections, with the exception of Odds et al. The complexity of the experimental design in the latter study makes its results difficult to compare with those of other studies; therefore, it has not been included in the table. Amphotericin B showed contradictory effects. In one study, considerably high doses (5 mg/kg/day) were administered with poor results, but showed good results in another study. The clinical experience with the use of amphotericin B against these infections has been also contradictory, since in at least four clinical cases treatment with this drug failed. One was a case of secondary cutaneous infection by *A. flavus* in a leukaemia patient where treatment with high doses of liposomal amphotericin B failed. The other infections where this drug did not work were a case of an epidural abscess and osteomyelitis in a diabetic patient, a disseminated nodular cutaneous infection in a myeloid leukaemia patient, and a case of endocarditis. In contrast, a case of pulmonary infection by *A. flavus* in a leukaemia patient was successfully treated with liposomal amphotericin B and surgical pulmonary resection. Further, a case of invasive *A. flavus* pneumonia and endocarditis after bone marrow transplant was successfully treated with liposomal amphotericin B, with surgical intervention not being required.

Itraconazole showed good results in three studies, but in another it did not exert prophylactic effects. In the clinical setting, the experience with this drug has also been contradictory. Treatment with itraconazole failed in at least three cases, including a case of osteomyelitis in a diabetic patient, a lung infection in a renal transplant patient and a case of myositis after liver transplantation. In contrast, itraconazole cured several patients involved in an outbreak of *A. flavus* sternal wound infections after cardiac surgery in a Belgian hospital.

The efficacy of posaconazole has only been evaluated in reducing the fungal load in the lungs of infected mice. It showed efficacy in the two studies carried out, being more effective against *A. fumigatus* than *A. flavus*. However, this drug was not able to reduce the tissue burden in a prophylactic regimen. The clinical experience in the use of this drug for the treatment of *A. flavus* infections is very poor.

Although it has been only tested in one study, voriconazole is another promising drug that has demonstrated efficacy in reducing the fungal load in four organs at doses of 10 and 25 mg/kg, although the lowest dose was only able to prolong survival when administered prophylactically. The good response of *A. flavus* to voriconazole has been confirmed in several human infections. Clinical cases of endocarditis and CNS infection in an immunocompetent patient and disseminated nodular cutaneous infection in a leukaemic patient, which could not be resolved with liposomal amphotericin B, were treated successfully with voriconazole.

Although studies carried out under different testing conditions are difficult to compare, caspofungin has shown the best results in animal studies. In Warn et al., mice treated with caspofungin showed 100% survival and the fungal load was reduced in three different organs. Further, in Bowman et al. caspofungin showed a positive prophylactic effect in prolonging survival and in reducing the kidney burden. Clinical experience on the use of caspofungin in infections by *A. flavus* is very limited, although some efficacy has been demonstrated, since caspofungin showed clinical efficacy and tolerability in a lung infection in a renal transplant patient where itraconazole did not work.

In animal models, the combination of posaconazole with amphotericin B or caspofungin has not improved the results obtained with the monotherapies. On the other hand, no antagonism was observed either. There are no data available on the activity of such combinations in clinical practice.

**A. nidulans**

*A. nidulans* is the least common of the *Aspergillus* species included in this review. However, in numerous cases, localized and disseminated infections have been reported, both in humans and animals. Only one study that tested a single strain of *A. nidulans* has been published. In that study, the prophylactic effects of caspofungin and amphotericin B in the
treatment of a murine infection by this fungus were compared. Amphotericin B was inactive and of the six doses of caspofungin tested (0.03, 0.06, 0.125, 0.25, 0.5 and 1 mg/kg), only the 0.5 mg/kg dose was able to significantly prolong the survival of mice. The kidney fungal burden was significantly reduced by the six doses (0.03–1 mg/kg) of caspofungin tested. There is no clinical experience on the use of caspofungin alone to treat A. nidulans infections. However, in a recent case in a patient with a chronic granulomatous disease who developed a large popliteal abscess and a pulmonary infection, administering amphotericin B and voriconazole given alone or in combination with caspofungin and extensive surgical curettages failed, despite that fungus being susceptible in vitro to these drugs. Amphotericin B also failed in a case of an invasive A. nidulans infection in another patient with chronic granulomatous disease.95

A. terreus

A. terreus is an uncommon but emerging fungal pathogen that is usually resistant to amphotericin B.96 Only a few animal models of A. terreus infections have been developed (Table S11, available as Supplementary data at JAC Online). An intratracheal instillation model of murine and rabbit pulmonary aspergillosis showed the ability of A. terreus to produce disease.97 Seven studies on the antifungal response of this fungus in vivo have been published, all of them in mice apart from one, which used rabbits.96 Amphotericin B was tested in all those studies,96,98–102 in vitro with caspofungin in three,96,98,99 posaconazole in two,96,98 and micafungin in one.101 So far, anidulafungin and voriconazole have not been tested in animal models against A. terreus. Amphotericin B has generally shown very poor results, even at high doses (4.5 or 5 mg/kg/day)100 or when its liposomal formulation (up to 25 mg/kg/day) has been used.96,101 Posaconazole has prolonged survival and reduced the tissue burden in the brain and kidneys,96,99,100 although small doses (20 mg/kg/day) were not able to reduce the fungal load in the lungs of rabbits.96 In another study, itraconazole and micafungin prolonged survival but reduction of fungal load in organs was not related to the escalating doses used.101 Caspofungin has shown variable results, since in two studies it showed efficacy under different testing conditions96,99 and in others it was unable to reduce the fungal load of various organs of infected mice.98 In a case of disseminated infection by A. terreus, in a patient with prolonged neutropenia after stem cell transplant for myeloma, administering this drug together with surgical debridement resolved the infection.102

Posaconazole at a dose of 10 mg/kg has shown variable results, but at 20 mg/kg it has reduced the residual fungal burden in several organs, and improved survival in mice and rabbits. Amphotericin B formulations have not been effective.98,101 In a retrospective study in a US cancer centre, of the 300 patients with invasive aspergillosis identified, 32 were infected with A. terreus.104 Some of these patients experienced failure after receiving a lipid formulation of amphotericin B and were subsequently treated with posaconazole.104 Almost half (44%) of the patients treated with posaconazole responded successfully. In a retrospective study carried out at a hospital in Innsbruck, there was an 8% efficacy of deoxycholate amphotericin B in the treatment of A. terreus infections, 36% of liposomal amphotericin B, 50% of itraconazole, and 100% of voriconazole and caspofungin combined with voriconazole; although only two and one patients were treated with the latter two regimens, respectively.105 In a retrospective analysis of 60 cases of invasive A. terreus infections, 82% of the patients died and of the 11 patients with clinical improvement, only 2 were treated with amphotericin B monotherapy; 8 of the remaining 9 patients were treated with an azole.106

Although amphotericin B has failed in numerous clinical cases, its use together with itraconazole (sequentially or combined) has resolved several cases. A case of aortic aneurysm infection by this fungus was successfully treated with partial pneunomectomy, resection of the aneurysm with graft repair, and prolonged sequential administration of amphotericin B and itraconazole.107 A case of hepatitis caused by this fungus subsided after the addition of itraconazole to the combination of liposomal amphotericin B and granulocyte macrophage colony-stimulating factor (GM-CSF).108 A woman who developed acute necrotizing ulcerative gingivitis due to A. terreus during induction chemotherapy for acute myelogenous leukaemia failed to respond to treatment with amphotericin B, but the infection was resolved following the introduction of itraconazole.109

Fusariosis

The treatment of fusariosis in immunosuppressed patients is a frustrating task, due to the high resistance of Fusarium strains to the available drugs. With the different therapies commonly used in clinical practice, only anecdotal success has been reported.110 Numerous experimental studies have been carried out to evaluate different antifungal treatments, though generally with disappointing results.

Fusarium oxysporum

F. oxysporum, together with Fusarium solani and Fusarium verticillioides, are responsible for almost all cases of human fusariosis. F. oxysporum constitutes a complex of phylogenetic species that can be difficult to differentiate morphologically.111,113,114 These species have shown in vitro resistance to practically all the antifungal drugs.111,113,114 In an animal study published in 1998, the efficacy of amphotericin B and itraconazole was tested against two strains of F. oxysporum in guinea pigs and mice.75 In spite of the use of high inocula and immunosuppressed animals, the authors failed to develop a reproducible lethal infection in guinea pigs. The fungus was able to infect mice that were immunosuppressed, but the two drugs tested did not resolve the infection. In a more recent murine study, the efficacy of high doses of those drugs that have shown any in vitro activity, i.e. amphotericin B (3 mg/kg), voriconazole (60 mg/kg) and posaconazole (100 mg/kg), alone and in combination, was tested against two isolates of F. oxysporum (Table S12, available as Supplementary data at JAC Online). Some of the regimens evaluated showed efficacy against only one of the two strains tested, the combination of amphotericin B with posaconazole showing the best results, followed by posaconazole alone.115 Lewis et al.116 reported a lack of efficacy of this combination in a case of human fusariosis, although the species was not determined.
According to those authors, the failure resulted from the sub-therapeutic posaconazole levels achieved in serum due to the patient’s extremely poor diet. In contrast, voriconazole, which did not work in the experimental study, showed efficacy in a case of pneumonia caused by *F. oxysporum* in an immunocompetent host.117

**F. solani**

*F. solani* is the commonest species causing human fusariosis. Although all species of *Fusarium* show a high degree of *in vitro* resistance to antifungals, this species is generally the one that shows the highest MICs.114,118 Recent phylogenetic studies have shown that *F. solani* represents a complex of >45 phylogenetic species,119 although no significant differences in antifungal resistance have been found among them.118 Probably due to the lack of successful treatments for the infections caused by this fungus, it is one of the most tested in animal efficacy studies, evaluating many different therapeutic regimens. This fungus has been tested in eight different studies75,120–126 (Table S13, available as Supplementary data at JAC Online). While in all the studies carried out, using both immunocompromised or immunocompetent animals, amphotericin B did not show efficacy, in one study this drug was surprisingly able to prolong survival and reduce counts in the kidneys.122 In the same study, posaconazole showed similar efficacy to amphotericin B.122 In other studies where itraconazole, voriconazole,123,125 miconafungin,125 caspofungin126 and some combinations were tested, the results were generally unsatisfactory or only moderately satisfactory. Recently, Wiederhold et al.126 tested posaconazole in a murine model of systemic infection by *F. solani*, and demonstrated that efficacy was dose dependent, and that high dosages of posaconazole (50 mg/kg twice daily) were effective both as treatment and prophylaxis. In this latter regimen, the drug was administered 2 days prior to inoculation. However, such efficacy was only observed when animals were infected with a low inoculum (10⁴ conidia/mouse). Apart from the two mentioned studies with posaconazole, the different experimental studies have generally confirmed the poor results obtained in neutropenic patients infected with this fungus. Persistent neutropenia and corticosteroid therapy significantly affect the survival of such patients. Surprisingly, this drug failed in experimental studies against *F. oxysporum* and *F. verticillioides*, which showed lower MICs than *F. solani*. The good results obtained with posaconazole in the two studies mentioned above must be treated with caution since the good efficacy could be strain dependent. In addition, there are no clinical data that confirm such efficacy. In contrast, while voriconazole in experimental studies was ineffective127 or displayed only moderate efficacy,122 a positive response was observed in 42%127 and 69%128 of patients in two retrospective reviews of clinical cases where *F. solani* was the predominant species. Echinocandins showed poor efficacy in the two experimental studies performed up to now.124

**F. verticillioides**

*F. verticillioides* is one of the most common species of *Fusarium* that infects humans. In a recent study, this species was the most frequently isolated from deep-seated infections in Italy.113 This species was tested in three animal studies that evaluated amphotericin B, liposomal amphotericin B, terbinafine, posaconazole, voriconazole and combinations of amphotericin with the other three drugs129–131 (Table S14, available as Supplementary data at JAC Online). Generally, this species has shown a higher susceptibility in *in vitro* to antifungals than the other important species of the genus,113,114,112 although the amphotericin B MICs were always >1 mg/L, which correlates with the results from animal studies that usually report the failure of this drug. Liposomal amphotericin B was tested in two studies with only moderate results.129,130 Although a case of a nasal septum abscess in an immunosuppressed child was resolved by surgical intervention and the use of this drug,133 voriconazole also showed in *vitro* MICs that were always >1 mg/L, but this drug was able to resolve two clinical cases of disseminated infection in immunocompromised patients.134,135 However, it must be taken into account that in both cases liposomal amphotericin B had been also administered. Recently, murine disseminated infections by two strains of *F. verticillioides* were successfully treated when combining liposomal amphotericin B plus terbinafine.130 These data agree with the results of two clinical cases resolved by such a combination, although they were caused by two other species of *Fusarium*. One of them was a case of a patient with acute myelogenous leukaemia and disseminated infection by *F. oxysporum*, where previous treatment with amphotericin B had failed;136 the second case involved a patient with a disseminated infection by *Fusarium proliferatum* after allogeneic stem cell transplantation, where voriconazole treatment had failed.137 Terbinafine is not approved for the treatment of invasive fungal infections, but only for dermatomycosis or onychomycosis. After oral administration, this drug achieves high peak plasma levels that are rapidly redistributed to the skin and skin appendages.138 It seems doubtful, therefore, that a plasma concentration sufficient to treat deep fusariosis can be achieved. However, the role of terbinafine in combination with other drugs with different targets has not been explored. In the same murine model described before,130 the activity was evaluated of the azoles voriconazole and posaconazole alone at high doses and each of them combined with amphotericin B. The results of that study were modest, overall: voriconazole prolonged survival for two strains, but was not able to reduce the tissue burden; posaconazole was almost ineffective; and the two combinations did not generally improve the results.131 However, the combination liposomal amphotericin B plus voriconazole has worked well in a recent case of disseminated infection by this fungus.135

**Scedosporiosis**

**Scedosporium apiospermum**

*S. apiospermum* is an emerging fungus, traditionally considered the anamorph (asexual state) of *Pseudallescheria boydii*, which has become the third most common mould in the clinical setting in recent years after *Aspergillus* and *Fusarium*. Recently, the fungi causing scedosporiosis have gone through important taxonomic changes, and molecular studies have shown that *P. boydii* is indeed a complex of species113 with different virulence and response to antifungals,142,144 and that *P. boydii* and
S. apiospermum are two different species. The most clinically relevant species included in this group are Scedosporium boydii, S. apiospermum and Scedosporium aurantiacum. Scedosporium prolificans is a related fungus that is genetically different from the species of this complex. Since these species have only been differentiated fairly recently, it is difficult to assess which species are represented in the older experimental studies. Seven animal studies have evaluated the efficacy of different drugs, five in mice and two in guinea pigs (Table S15, available as Supplementary data at JAC Online). In our centre, we have now identified molecularly the isolates tested in those studies, with the exception of that used in two previous studies, and determined than they belong to the current S. apiospermum. In the study of Odds et al., two strains of a fungus identified as P. boydii were tested against amphotericin B and itraconazole in guinea pigs and mice. For both isolates, despite almost all animals surviving to the end of the experiment, neither amphotericin B nor itraconazole treatments led to any significant increase in negative cultures from infected organs. Amphotericin B, administered intravenously or intraperitoneally, was tested in four studies, in mice or guinea pigs, showing general low efficacy. In the study of González et al., posaconazole showed efficacy, but, surprisingly, so did fluconazole, which is a drug that does not usually work against moulds. However, these data have to be treated with caution considering the doubtful identification of the isolate, which makes it very difficult to evaluate the different treatments in the reported clinical cases. Nevertheless, a more recent study confirmed the good activity of posaconazole against two strains of S. apiospermum. Another drug that performed well against different strains of this fungus was voriconazole, although in guinea pigs it was only able to prolong survival and reduce the fungal load in the brain against one of the two strains tested. The voriconazole MIC for the strain that responded to treatment was low (0.5–1 mg/L), while for the second one it was high (8 mg/L).

S. aurantiacum

This new species is probably the most virulent of the P. boydii species complex and also the most resistant to antifungals. Although occasional cases have been reported from different regions of the world, this species is relatively common in Australia. Since it is a new species, the efficacy of the different drugs against this fungus has only been evaluated in one study (Table S16, available as Supplementary data at JAC Online), in which amphotericin B was ineffective, and the efficacy of posaconazole and voriconazole was strain dependent. In clinical practice the combination of voriconazole and surgery resolved a case of osteomyelitis in an immunocompetent patient. This fungus has emerged as a human pathogen in the last 20 years. The infections that it causes are characterized by a high rate of mortality, especially in immunocompromised patients, and by a poor response to the available antifungals. The treatment of infections caused by this species is unknown, but, to date, only five murine studies have been carried out to evaluate the efficacy of several drugs, i.e. amphotericin B, liposomal amphotericin B, caspofungin, micafungin and some combinations of them (Table S18, available as Supplementary data at JAC Online). In general, all the therapies tested showed only poor or moderate activity against this fungus. In several clinical cases of successfully recovered scedosporiosis, cytokines were included in the treatment, but an experimental study administering granulocyte colony-stimulating factor did not improve the results obtained with high doses of liposomal amphotericin B when both were administered in combination.

In another study where double and triple combinations of antifungal drugs were tested, the combinations of micafungin plus amphotericin B or voriconazole showed some efficacy, being the only treatments tested that were able to prolong survival and reduce the numbers of cfu in the kidneys and brain. The drug that has so far demonstrated the highest activity against this fungus is albicidin. In a rabbit model, doses of 50 mg/kg of that drug showed a 100% survival and were able to reduce numbers of cfu in the five organs tested. However, no more studies on the efficacy of this drug have been conducted, either in animals or in humans. In a recent study, 107 patients with scedosporiosis were treated with voriconazole and a successful therapeutic response was achieved in 57% of them. In a subset of 36 of these patients infected with S. prolificans the success rate was 44%.

Infections by other moulds

Cladophialophora bantiana

C. bantiana is a melanized fungus that causes severe infections, mainly in immunocompetent hosts. Cerebral abscesses are among its more severe complications, with a high mortality rate. The recommended antifungal treatment involves the use of amphotericin B combined with a triazole and, if the patient's condition allows it, flucytosine. However, infections are rarely resolved by antifungal treatments alone and require surgical removal of the abscesses, too. Long-term survival from cerebral abscesses has been reported only when complete surgical resection was possible. The treatment of the infections caused by this fungus was experimentally studied >20 years ago, using murine models and the drugs available at that time, i.e. flucytosine, amphotericin B, fluconazole and terbinafine (Table S19, available as Supplementary data at JAC Online).
Although all of these drugs, with the exception of terbinafine, were able to prolong survival, the best results were obtained with flucytosine.\textsuperscript{161} In another study, flucytosine reduced mortality, but brain cultures remained positive to the end of the study.\textsuperscript{162} More recently, Al-Abdely \textit{et al.}\textsuperscript{163} used two different models, i.e. immunosuppressed mice infected intravenously and non-immunosuppressed mice infected intracerebrally. In the first model, posaconazole and itraconazole prolonged survival and reduced the fungal load in the brain, while amphotericin B was only able to prolong survival. In the latter model, posaconazole showed dose-dependent responses using the two mentioned markers of efficacy. These responses were observed for short, delayed and prolonged therapy, but posaconazole did not sterilize brain tissue in spite of continuous therapy for 8 weeks. In a more recent study, amphotericin B, micafungin, voriconazole, posaconazole and flucytosine were tested alone and in combination in immunocompetent mice.\textsuperscript{164} Among the monotherapies, only posaconazole and flucytosine were able to prolong survival. Posaconazole combined with micafungin or flucytosine improved survival, too, but the results were not better than those obtained with posaconazole alone. The triple combination of posaconazole, micafungin and flucytosine improved survival with respect to the monotherapies, but all the animals died during the experiment. However, when treatment with this triple therapy was extended to 30 days, half of the animals survived for ≥10 months.\textsuperscript{164}

There are scarce data on the use of different antifungals in the treatment of \textit{C. bantiana} infections. The use of deoxycholate amphotericin B or liposomal amphotericin B has shown variable results.\textsuperscript{165} In experimental studies, amphotericin B seems to show worse results than triazoles. Although posaconazole is the triazole that has shown the best results in murine models,\textsuperscript{163,164} in the clinical setting both posaconazole and voriconazole have shown positive results.\textsuperscript{155,166} Itraconazole, which also showed experimental efficacy, could be useful for less severe infections, i.e. mycetoma.\textsuperscript{167} Flucytosine has also shown activity against this fungus, but its high toxicity in bone marrow limits its use. The most suitable treatment for severe \textit{C. bantiana} infections is very far from being resolved and more effort is required, especially considering the high mortality rates associated with them (up to 70%).\textsuperscript{160} The use of a single antifungal is not enough and a combination of them, when resection of the lesions is not possible, seems to be the most adequate approach.

**Exophiala dermatitidis**

\textit{E. dermatitidis} is a melanized yeast-like fungus with marked predilection for the CNS. Infections by this fungus are predominantly described in Asia, although several cases have been also reported in other geographical regions.\textsuperscript{168} A peculiar characteristic of the infections by this fungus is the ability to involve young, otherwise healthy patients.\textsuperscript{169} In a review of 21 cases of systemic \textit{Exophiala} spp. infections, a mortality of 48% was reported.\textsuperscript{168} \textit{E. dermatitidis} being the most common species. Four experimental studies have been carried out in mice to evaluate different treatments against infections by this fungus\textsuperscript{161,170–172} (Table S20, available as Supplementary data at \textit{JAC} Online). The two oldest of these studies tested the drugs available at the time, i.e. flucytosine, itraconazole, fluconazole, terbinafine and amphotericin B. Therapies with fluconazole, fluconazole and their combination showed some efficacy against this infection, although only mortality rates were evaluated. More recently, posaconazole at different concentrations was tested, showing efficacy in prolonging survival and reducing cfu counts in the brain, spleen and kidneys.\textsuperscript{171,172} In general, that triazole performed better than amphotericin B and itraconazole. On the basis of the experimental data available, posaconazole seems to be a promising drug for the treatment of phaeohyphomycosis, including for patients with CNS infections; however, clinical data are lacking, to date. This drug demonstrated efficacy in a case of disseminated infection by another species of the genus, \textit{Exophiala spinifera}.\textsuperscript{173}

**Exophiala oligosperma**

\textit{E. oligosperma} was the third most common species of the genus in clinical samples in the USA.\textsuperscript{174} In a recent study, the efficacy of posaconazole, amphotericin B and itraconazole was evaluated against two strains of this species\textsuperscript{171} (Table S21, available as Supplementary data at \textit{JAC} Online). The efficacy of the three drugs was considered as only moderate, since in spite of all of them prolonging survival, they were not able to significantly reduce the fungal load in all of the organs tested. This was probably due to this species not being highly virulent and, with the inocula used, the fungal load, especially in the kidneys and brain, was not high enough to be able to detect a significant reduction.\textsuperscript{171} Since this species is relatively new, clinical experience in the management of the infections caused by it is very limited. However, a case of olecranon bursitis has been published, the patient being successfully treated with aspiration and intrabursal amphotericin B.\textsuperscript{175}

**Exophiala phaeomuriformis**

\textit{E. phaeomuriformis} is one of the species of \textit{Exophiala} of clinical interest. In a retrospective study of the fungi from clinical samples isolated in a reference centre in the USA, this fungus represented 6.6% of the isolates belonging to that genus.\textsuperscript{174} The antifungal response of one isolate belonging to this species was investigated against caspofungin, amphotericin B and posaconazole (Table S22, available as Supplementary data at \textit{JAC} Online). All three antifungals evaluated improved the survival of immunocompromised mice, but only the latter two reduced the fungal burden.\textsuperscript{176}

**Exophiala xenobiotica**

This species was the second most common one of the genus in clinical samples in the USA.\textsuperscript{174} In the experimental study described above,\textsuperscript{171} the same drugs (posaconazole, itraconazole and amphotericin B) were tested against two strains of this species (Table S23, available as Supplementary data at \textit{JAC} Online). The results were similar for the three drugs, i.e. they showed moderate efficacy. In one recent clinical case, oral itraconazole showed efficacy in the resolution of a subcutaneous infection by this species in a patient with non-Hodgkin lymphoma.\textsuperscript{177}
**Fonsecaea monophora**

The melanized fungus *F. monophora* has been reported as a causal agent of cerebral phaeohyphomycosis and chromoblastomycosis. This fungus has recently been segregated from *Fonsecaea pedrosoi*, a traditionally well-known pathogen. Although *F. monophora* and *F. pedrosoi* have a similar morphology, they seem to differ in the range of infections that they cause: *F. pedrosoi* seems strictly associated with chromoblastomycosis, whereas *F. monophora* is more variable and shows some neurotropism. In a recent study, the therapeutic experience with the use of posaconazole in clinical practice.179 There is no experience with the use of posaconazole in clinical practice.

**Neoscytalidium dimidiatum**

The dematiaceous fungus *N. dimidiatum* is the aetiological agent of chronic superficial infections in human skin and nails, mycoses, and subcutaneous infections. Less rarely, it also causes invasive infections affecting different organs in patients with predisposing factors such as corticosteroid therapy, diabetes and solid organ transplant. The classical antifungal drugs have shown good in vitro activity against this fungus. In a recent article, the activity of amphotericin B, voriconazole and posaconazole has been investigated against two strains of this fungus, and the three drugs showed high efficacy (Table S25, available as Supplementary data at JAC Online). A few clinical cases of deep infections were resolved with amphotericin B treatment.182

**Apophysomyces elegans**

*P. lilacinus* is an emerging fungus repeatedly reported in recent years to cause mainly superficial infections, although several deep-seated infections have also been reported. Although the appropriate treatment of these infections is unknown and the fungus has generally been resistant in vitro to the antifungal drugs, the efficacy of antifungal therapy in experimental models has been tested in only two studies (Table S26, available as Supplementary data at JAC Online). It was demonstrated that posaconazole and voriconazole show efficacy both in prolonging survival and in reducing fungal load in organs. Surprisingly, a dose–effect relationship was not observed with posaconazole; the results obtained with the 50 mg/kg dose were better than with either the 75 or 100 mg/kg dose. To explain this, a murine pharmacokinetic study suggested that posaconazole absorption could be lower when higher doses were administered.187 Several case reports have confirmed the potential use of both drugs in the clinical setting, voriconazole also being useful for treating keratitis. Both triazoles have been suggested as first-line therapies in transplant patients. Posaconazole has also shown efficacy in salvage therapy.189

**Rhinocladiella mackenziei**

*R. mackenziei* is a fungus recently recognized as a human pathogen and is endemic in the Middle East. This fungus causes cerebral infections, with a mortality rate of almost 100% in infections that remain untreated; even in patients treated with surgery and chemotherapy, mortality is still ~65%. Al-Abdely et al. studied the in vivo response of two strains of *R. mackenziei* to amphotericin B, itraconazole and posaconazole (Table S27, available as Supplementary data at JAC Online). Posaconazole significantly prolonged survival and reduced the brain fungal burden, while itraconazole reduced the brain fungal load in mice infected with one strain but not the other; amphotericin B had no effect on brain fungal concentrations. There is little clinical experience with infections by this fungus, and the patient has died in most cases despite therapy with amphotericin B, itraconazole and 5-fluorocytosine alone or in combination. However, in two cases the condition of the patient improved considerably after administering posaconazole.

**Mucormycosis**

Treatment of infections by members of Mucorales is based on four critical factors: early diagnosis; removal of predisposing factors; surgical debridement; and appropriate antifungal therapy, with liposomal amphotericin B being the drug of choice. Posaconazole is considered a reasonable option for patients who are refractory to or intolerant of polyenes, being used as salvage therapy in several clinical trials where 14%–37% of the patients showed a complete response. However, the response to these drugs varies among the different species and even strains of zygomycetes, as demonstrated different animal studies.
Cunninghamella bertholletiae

C. bertholletiae is not the commonest species of Mucorales causing human infection, but the diseases produced by this species are probably the most aggressive and have the poorest outcome of those caused by the species of that fungal order.

In only two experimental studies have different antifungal therapies been evaluated against this fungus (Table S29, available as Supplementary data at JAC Online). In the oldest study, only a low dose of amphotericin B (0.25 mg/kg) was tested,200 which showed only minimal improvement in survival, comparable to the poor result of this treatment in real human cases. More recently, two murine models using neutropenic or diabetic mice were developed that were used to test itraconazole, amphotericin B and posaconazole against several strains of that fungus.201 Posaconazole and amphotericin B showed high efficacy, although under some test conditions the former performed better. Itraconazole showed only moderate efficacy; the dose 50 mg/kg twice daily worked under some conditions, but not under others. In spite of the good results obtained with amphotericin B in this study, the clinical results have not always been favourable.202,203 The high efficacy shown by amphotericin B and posaconazole in the study of Pastor et al.,201 further justifies studies on the use of a combined therapy with these two drugs, a treatment that has already shown efficacy in a recent clinical case of infection by this fungus.204

Lictheimia corymbifera

L. corymbifera (formerly Absidia corymbifera) has been reported together with Rhizopus oryzae, Rhizopus microsporus and Mucor circinelloides as the most common species causing zygomycotic,197 but its antifungal susceptibility in vivo has only been tested in four studies198,205–207 (Table S30, available as Supplementary data at JAC Online). In two of those studies, the drugs were evaluated prophylactically.206,207 The drugs tested were amphotericin B, itraconazole, terbinafine and posaconazole. In one study using high doses of amphotericin B (5 mg/kg), which has shown some toxicity in other studies, this drug showed excellent activity.206 In the above-mentioned animal studies, the results were similar to those obtained against other zygomycetes, i.e. amphotericin B, even at very high doses, showed the best results. Posaconazole showed efficacy in one case, being able to prolong survival and to reduce the number and size of infection foci in the brain and kidneys.207 In the study by Dannaoui et al.,205 only the highest dose of posaconazole (100 mg/kg) was able to prolong survival, but not to reduce lesions in brain.

Mucor spp.

The two most common species of Mucor causing human infections are M. circinelloides and Mucor ramosissimus.196 Both species were tested in one study against posaconazole, amphotericin B and itraconazole,208 which is the only study performed against species of this genus (Tables S31 and S32, available as Supplementary data at JAC Online). Both amphotericin B and posaconazole showed efficacy in prolonging survival and reducing the tissue burden in the kidneys. Posaconazole showed a dose–response relationship. There is some evidence of the efficacy of amphotericin B in clinical practice in the treatment of infections caused by these species,209,210 however, there are no data available on the clinical use of posaconazole against these species.

R. microsporus

R. microsporus is, after R. oryzae, the second most common species of Mucorales causing human infections. Only three studies have been devoted to this species, testing deoxycholate amphotericin B, liposomal amphotericin B, itraconazole, terbinafine and posaconazole198,205,211 (Table S33, available as Supplementary data at JAC Online). In two of these studies the drugs were evaluated prophylactically, and in one study the mice tested were non-immunocompromised198 and in the other two they were rendered neutropenic. Itraconazole and terbinafine were ineffective in non-immunocompromised mice, despite high tissue levels of the first in the kidneys, and of the latter in the kidneys and brain.198

In another study with neutropenic mice, itraconazole was again ineffective, while posaconazole showed a dose–effect relationship. Posaconazole at 40 mg/kg was as effective as amphotericin B at 1 mg/kg in prolonging survival, but it was not able to reduce active infections in organs versus control animals.205 In a more recent study in immunosuppressed mice, the efficacy of posaconazole was evaluated, administered once or twice daily against four strains of R. microsporus. Liposomal amphotericin B at 10 mg/kg was the most effective treatment for the two strains with intermediate susceptibility to posaconazole. For the two posaconazole-susceptible strains, liposomal amphotericin B at the same dose and posaconazole at 20 mg/kg twice daily showed similar efficacy. In that model, administering posaconazole twice a day proved to be more effective at prolonging survival and reducing the tissue burden than the once–a-day regimen, and correlated with higher posaconazole levels in serum.211 In a recently reported case of intra-abdominal infection by R. microsporus in a child with acute leukaemia, the patient survived after aggressive combined surgical, antifungal (liposomal amphotericin B, caspofungin and posaconazole) and iron chelation therapy.212

R. oryzae

R. oryzae is the most frequent species of Mucorales involved in human infections196 and is, after C. glabrata, of the species included in the present review, the second most commonly tested in animal studies. Up to now, 14 different animal studies have evaluated the efficacy of several drugs against R. oryzae animal infections75,198,207,213–222 (Table S34, available as Supplementary data at JAC Online). Although, occasionally, other drugs were also tested, the most commonly evaluated drugs have been different formulations of amphotericin B and posaconazole. In most of the studies, neutropenic murine models were used, but non-immunocompromised205 and diabetic ketoacidotic models213,215,216,221 were used, too. On some occasions, prophylactic therapies were also tested.205,207,221

Although amphotericin B and posaconazole have, in general, proven to be the most effective drugs in the treatment of murine infections by R. oryzae, there are also some significant
studies that seem to show the contrary (Table S34). In Odds et al., amphotericin B was able to prolong survival in mice infected with two strains of R. oryzae, but not to obtain cure. Negative organs in mice and guinea pigs. Ibrahim et al. compared liposomal amphotericin B and amphotericin B lipid complex, and demonstrated that both showed similar efficacy in neutropenic animals but not in diabetic ketoacidotic mice where the latter drug was less effective. However, no treatment was able to reduce the brain fungal burden in neutropenic animals. Posaconazole, even at 40 mg/kg, failed to prolong survival in non-immunocompromised mice, and amphotericin B at 1 mg/kg and liposomal amphotericin B at 5 and 10 mg/kg also failed in a ketoacidotic diabetic model. In Barchiesi et al., the protective benefit of posaconazole against R. oryzae was inoculum dependent; an inoculum of 1 × 10^5 spores/mouse showed efficacy, but not 2 × 10^6 spores/mouse. Prophylaxis with posaconazole and amphotericin B was not effective in terms of organ sterilization.

Posaconazole has shown efficacy in clinical practice for treating infections caused by Rhizopus spp., and although some strains were not identified to species level, it is likely that some of them were R. oryzae. However, the actual role of posaconazole in human infections is difficult to assess, as most of these patients had previously been treated with amphotericin B or their lipid formulations. It is also difficult to ascertain whether these success rates were related to the patients’ immune status and/or other conditions, or to the antifungal susceptibility of the strains involved in these infections. In animal studies this latter issue is generally difficult to determine since in most of the cases, only one or two strains are tested. The possible strain-dependent response to posaconazole was tested in a recent study. A panel of 50 clinical strains was split into two groups on the basis of in vitro data. For the group with most strains (85%), the strains had low posaconazole MICs. Mice infected with these strains showed higher rates of survival (30%–40%), and posaconazole was able to reduce the fungal load in the kidneys and, less regularly, in the brain of animals infected with these strains. For the second group (15% of the strains), the strains had intermediate posaconazole MICs, mice infected with the strains had lower survival rates (10%–20%), and posaconazole treatment resulted in variable and no reductions in the fungal loads in the kidneys and brains, respectively.

Because the response of the murine infections with R. oryzae to either of the two most efficacious drugs, i.e. liposomal amphotericin B and posaconazole, is not always good, Ibrahim et al. evaluated the efficacy of the combined therapy in mice. The combination of the drugs was no better than using liposomal amphotericin B alone, and the results obtained with posaconazole monotherapy were not better than the controls. Another similar study confirmed that the combination of posaconazole and liposomal amphotericin B was no better than the polyene alone, but allowed the doses of amphotericin B to be reduced with no loss of efficacy.

Treatments with echinocandins have also been evaluated in several animal studies, but only modest results were obtained. Caspofungin showed a limited activity against this fungus and an inverse dose–response effect. A low dose (0.5 mg/kg twice daily) rather than higher doses of caspofungin improved the survival of mice with diabetic ketoacidosis infected with a small inoculum, but not with a large inoculum. Micafungin and anidulafungin were ineffective. The effect of combination therapy with polyenes and echinocandins was effective in prolonging survival, but in organ clearance it varied depending on the drugs tested, on the fungal inoculum size and on the technique used to measure the fungal burden. Prophylactic combination therapy using caspofungin and amphotericin B lipid complex was no more effective than prophylactic amphotericin B lipid complex alone.

The use of the cytokines interferon-γ (IFN-γ) and GM-CSF, which had shown efficacy as adjunctive agents in the treatment of several clinical cases of zygomycosis, was evaluated by Rodriguez et al. GM-CSF enhanced the efficacy of liposomal amphotericin B, significantly prolonging survival and reducing the tissue burden. IFN-γ was ineffective and the combination of this cytokine with liposomal amphotericin B did not improve the results obtained with the polyene alone.

Other drugs, such as ketoconazole, saucerconazole and itraconazole, have also been tested in a few experimental studies, but generally with negative results.

Conclusions

Table 1 provides an interpretative summary of the results of the animal studies included in this review, highlighting those drugs that have shown the highest efficacy. Although the data have to be looked at with caution, since they are based on only a small number of studies, useful information is at least provided on the in vivo antifungal susceptibility of some rare fungal infections whose best treatment is unknown. It is not unusual that clinicians faced with an infection by a rare fungus must initiate therapy based only on in vitro susceptibility data, but, in such cases, data obtained from animal studies, if there are any, are a step forward. If the therapies shown in Table 1 have worked in animals, they may be useful in human infections. At the same time, it is very doubtful that those that have not shown any activity in animal models will have efficacy in humans, especially considering that the doses administered to animals are generally very high.

Although other fungi have also been tested in animal studies, the species included in Table 1 are only those against which the referenced drugs showed high efficacy on the basis of the criteria defined in the ‘Methods’ section. Against the species included in this review, which represent the most common opportunistic pathogens, the drugs that have shown the best results have been posaconazole and amphotericin B. Posaconazole showed high efficacy against experimental infections caused by 19 different fungi and amphotericin B against 12 species. Caspofungin and voriconazole showed high efficacy against seven species each. It is also worth mentioning that against several important pathogens, such as S. prolificans and F. oxysporum, no appropriate treatment has been found, in spite of numerous therapies having been tested. In the case of other important pathogens, such as R. oryzae, some therapies that have been based mainly on the use of posaconazole or amphotericin B have shown high efficacy, but not in all of the studies carried out. This emphasizes the wide variability in the response to antifungals that the different strains of a given species can show. In addition, it implies that several isolates need to be tested in
order to obtain conclusive results on the antifungal susceptibility of the different fungal species. Although there is little clinical experience in the use of combinations of drugs in the treatment of fungal infections, animal studies have shown that, in some cases, their efficacy is superior to that of the monotherapies.

Acknowledgements
I am grateful to Dr J. Capilla for his expert advice.

Funding
The study was supported in part by the Rovira i Virgili University.

Transparency declarations
None to declare.

Supplementary data
Tables S1–S34 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References
30 Pfaller MA, Diekema DJ, Mendez M et al. Candida guilliermondii, an opportunistic fungal pathogen with decreased susceptibility to


Polak A. Combination therapy of experimental candidiasis, cryptococcosis, aspergillosis and wanjelliosis in mice. Chemotherapy 1987; 33: 381–95.


Ibrahim AS, Bowman JC, Avanesian V et al. Caspofungin inhibits Rhizopus oryzae 1,3-β-D-glucan synthase, lowers burden in brain measured by quantitative PCR, and improves survival at a low but not


