Mucormycosis: its contemporary face and management strategies

Hsin-Yun Sun, Nina Singh

Several countries have seen rising frequencies of mucormycosis among patients with haematological disorders, malignancies, or diabetes mellitus, and among transplant recipients. Growing numbers of immunocompromised hosts, widespread use of antifungal agents inactive against mucormycosis, or other unidentified factors, could be contributing to this situation. The predominant clinical manifestations of mucormycosis vary from host to host. Additionally, risk factors specific to different subgroups have been identified, such as leukaemia, allogeneic haemopoietic stem-cell transplant, voriconazole prophylaxis, diabetes, and malnutrition. We summarise the current state of knowledge of characteristics and risk factors and discuss topical developments in therapeutic methods and strategies in the management of mucormycosis.

Lancet Infect Dis 2011; 11: 301–11

Infectious Diseases Section, VA Pittsburgh Healthcare System, Pittsburgh, PA, USA (H-Y Sun MD, Prof N Singh MD); **Department of Internal Medicine, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan** (H-Y Sun); **and Department of Medicine, Division of Infectious Diseases, University of Pittsburgh, Pittsburgh, PA, USA** (Prof N Singh)

Correspondence to: Prof Nina Singh, Infectious Diseases Section, VA Medical Center, University Drive C, Pittsburgh, PA 15240, USA
nis5@pitt.edu

Introduction

With the advent of effective antifungal agents against *Candida* and *Aspergillus* spp, a growing population of immunocompromised hosts, improved diagnostic tools, and possible selection pressure from widespread use of broad-spectrum antifungal agents, mucormycosis has emerged as an important infection. Mucormycosis and entomophthoramycosis were previously encompassed by the term zygomycosis.[1] Changes in high-level taxonomy in reference to molecular phylogenetic analyses have, however, led to the class name Zygomycota being replaced by Glomeromycota.[2] In this new classification, all the agents of mucormycosis have been placed under the subphylum Mucormycotina and the agents of entomophthoramycosis are now in the subphylum Entomophthoramycotina. Since the phylum Zygomycota no longer exists, the disease name zygomycosis has become obsolete.[3]

Mucormycotina are characterised by large, ribbon-like hyphae with only occasional septae (aseptate fungi). These fungi cause mucormycosis, which comprises severe and potentially life-threatening infections, particularly in immunocompromised hosts. Improvements in culture-based morphological and molecular identification of fungi in the past two decades have led to expansion of the number of species known to cause mucormycosis. Infections are, however, primarily caused by those in the order Mucorales and the family Mucoraceae.[4]

Mucormycosis was reported in the Transplant Associated Infectious Surveillance Network study to occur in 9% of haemopoietic stem-cell transplant (HSCT) recipients and 2·1% of solid-organ transplant recipients.[5,6] Increases in incidence of mucormycosis have been reported in several countries in the past few years.[7–14] In developed countries, such as the USA, France, and Austria, mucormycosis mainly occurs in transplant recipients and neutropenic patients, although in France the frequency is rising among patients with diabetes mellitus.[10–13] In India the frequency has risen from 13 cases per year in 1990–99 to 36 cases per year in 2000–04, and 50 cases per year in 2006–07,[7–9] and the highest-risk group is patients with uncontrolled diabetes.[15]

In immunocompetent patients, *Apophysomyces elegans* has emerged as an important pathogen for mucormycosis, leading to primarily cutaneous and rhino-orbital-cerebral disease with infections and generally occurring after traumatic inoculation.[16–18] Additionally, several studies from India have reported isolated renal mucormycosis due to *A elegans*, mostly in immunocompetent young adults.[7–9] Although rare, mucormycosis outbreaks—defined as more than two cases in 2–6 months—have been reported to occur in hospitals.[19]

In this Review we summarise the current state of knowledge about features of and risk factors for mucormycosis, and provide updates on potential therapeutic options and strategies for the management of this infection.

Characteristics of mucormycosis

Clinical manifestations

Invasive mucormycosis is characterised by the rapid development of tissue necrosis as a result of vascular invasion and subsequent thrombosis. Disease may manifest as rhino-orbital-cerebral, pulmonary, cutaneous, gastrointestinal, or disseminated forms.[1] In a review of 929 patients with mucormycosis assessed in 1940–2003, diabetes was the most frequent underlying condition (36%).[20] The predominant clinical manifestations differ from host to host. Patients with diabetes most frequently present with rhino-orbital-cerebral manifestations (66%), followed by pulmonary (16%) then cutaneous (10%) disease, whereas patients with no underlying condition most frequently have cutaneous manifestations (50%). Patients receiving deferoxamine most frequently have pulmonary manifestations (28%), then rhinocerebral (26%), then disseminated disease (23%); in injecting drug users the order is cerebral (62%) then cutaneous (11%) disease, and in solid-organ transplant recipients it is pulmonary (37–53%) and rhino-orbital-cerebral disease (31%).[20–22]

Rhino-orbital-cerebral disease may present as rhinosinusitis, sinusitis, rhino-orbital, or rhinocerebral disease.[1] Necrosis or eschar in the nasal cavity or on the palate, trigeminal and facial cranial nerve palsy,

ophthalmoplegia and loss of vision signify disease progression. Intracranial complications include epidural and subdural abscesses and cavernous and, less frequently, sagittal sinus thrombosis, but frank meningitis is unusual.

Symptoms of pulmonary mucormycosis include fever refractory to antibiotics, cough, pleuritic chest pain, and dyspnoea. Invasion of blood vessels can lead to fatal haemoptysis. Clinical characteristics of pulmonary mucormycosis and aspergillosis are indistinguishable.[23,24] Patients with leukaemia and pulmonary aspergillosis versus those with pulmonary mucormycosis, however, are more likely to have concomitant sinusitis, have previously used prophylactic voriconazole, and have more than ten nodules and pleural effusion on chest CT.[23] The reverse halo sign might be an early radiographic finding of pulmonary mucormycosis.[24]

Cutaneous mucormycosis can be acquired by direct spore inoculation or via burns or traumatic wounds.[1] Erythema and induration of the skin typically progresses to necrosis with a black eschar.[1]

Gastrointestinal mucormycosis is rare,[20,25] and only 25% of cases are diagnosed antemortem owing to its non-specific presentation.[1] Predisposing disorders include extreme malnutrition, premature birth, and immunosuppression.[26] Neutropenic patients might present with fever, typhlitis, and haematochezia.[26]

Disseminated mucormycosis has been reported in patients with cerebral (48% of cases), cutaneous (39%), and pulmonary (20%) disease.[20]

Risk factors

Traditional risk factors for mucormycosis include long-term neutropenia, high-dose glucocorticoid therapy, hyperglycaemia, diabetes with or without ketoacidosis, iron overload, and use of deferoxamine for chronic iron overload in patients undergoing haemodialysis.[1] Although deferoxamine acts as an iron chelator for human beings, it is, paradoxically, an exogenous iron supplier (xenosiderophore) to Mucormycotina and can, therefore, predispose the host to mucormycosis. However, children with haemaglobinopathies who are receiving deferoxamine for iron overload seldom develop mucormycosis.[27] Thus, additional factors related to renal failure and deferoxamine might lead to the development of mucormycosis.[27] Patients who receive deferoxamine for iron overload or persistent neutropenia are at risk of disseminated disease.[1]

Specific risk factors for mucormycosis in different host populations have been identified. For example, leukaemia, allogeneic HSCT, voriconazole prophylaxis, diabetes, and malnutrition are cited as being independent risk factors for mucormycosis.[10] In solid-organ transplant recipients, renal failure, diabetes mellitus, and previous use of voriconazole, caspofungin, or both, are associated with mucormycosis. HSCT recipients who receive corticosteroids for graft-versus-host disease (GVHD) are at risk of disseminated disease.[1] The immunosuppressive agent tacrolimus seems to offer protection against mucormycosis.[28]

Numerous cases of breakthrough mucormycosis in voriconazole recipients have been reported.[29–33] In patients with haematological diseases or HSCT recipients receiving voriconazole for prophylaxis against fungal infection, empirical therapy of febrile neutropenia, or both, breakthrough mucormycosis occurred in 0–8·9%, 0·5%, and 4·3%, respectively.[31,32,34–39] In a case-control study, previous use of voriconazole, caspofungin, or both, was significantly associated with breakthrough mucormycosis in solid-organ transplant recipients.[28] Of note, breakthrough mucormycosis has also been reported in patients with haematological disorders or malignancy and who are receiving prophylactic azole therapy with drugs other than voriconazole (fluconazole, itraconazole, or ketoconazole).[40]

Several hypotheses have been proposed for the occurrence of breakthrough mucormycosis after exposure to voriconazole.[41] Since incidence of mucormycosis was increasing even before widespread voriconazole use, changes in host immunosuppression status are thought to be responsible, and voriconazole use is thought merely to have accelerated the trend.[41] Another theory is that virulence of Mucormycotina has increased after exposure to voriconazole. In experimental studies, survival was worse in animals infected with a *Rhizopus oryzae* strain exposed to voriconazole than in those infected with a strain not exposed to this drug.[42] Furthermore, notable angioinvasion, inflammation, and expression of genes involved in stress response and tissue repair have been seen in mouse lungs infected with voriconazole-exposed *R oryzae*.[42]

Treatment

Successful treatment of mucormycosis requires early diagnosis, reversal of underlying risk factors, reduction, if possible, of immunosuppression, prompt administration of antifungal therapy, and surgical debridement when applicable.[43] Delayed initiation of an amphotericin-B-based regimen (>6 days after diagnosis) has been reported to be associated with doubled mortality at 12 weeks.[44] In patients with diabetes, with or without ketoacidosis, efforts should be made to restore euglycaemia and normal acid-base status. Advances in conventional and non-conventional therapeutic approaches are discussed.

Conventional antifungal agents

Polyenes

The mortality associated with mucormycosis worldwide declined from 84% in the 1950s to approximately 40% in the 1990s.[20] Nevertheless, outcomes remain dismal, particularly in HSCT recipients and in patients with disseminated disease, in whom mortality remains at 91% and 95–100%, respectively.[20] Polyenes are the preferred therapeutic agents for mucormycosis. In patients receiving amphotericin B deoxycholate as

	Study type	Underlying cause	Pharmacotherapy	Surgery (n/total [%])	Response* (n/total [%])	Mortality (n/total [%])
Sun et al, 2010[45]	Review	SOT	First-line AmBd alone or combined First-line lipid AmB alone or combined	NA NA	NA NA	4/26 (15) 28/47 (60)†
Ruping et al, 2010[33]	Registry	Cancer	First-line L-AmB monotherapy 210 mg/kg daily First-line AmBd monotherapy First-line posaconazole monotherapy First-line combined posaconazole and L-AmB Posaconazole salvage therapy	NA NA NA NA NA	16/17 (94) 1/4 (25) 4/6 (67) 4/7 (57) 5/8 (63)	14/34 (41) 3/17 (18) 3/4 (75) 3/6 (50) NA
Chakrabarti et al, 2009[9]	Retrospective/ prospective	Diabetes	First-line AmBd monotherapy 0·7–1·0 mg/kg daily First-line L-AmB monotherapy 1–4 mg/kg daily	23/33 (70) 9/12 (75)	16/33 (49) 7/12 (58)	17/33 (52) 5/12 (42)
Kara et al, 2009[46]	Retrospective	Haematological disease	First-line AmBd monotherapy 1 mg/kg daily First-line/salvage L-AmB monotherapy 3 mg/kg daily	4/4 (100) 14/14 (100)	0/4 (0) 8/14 (57)‡	NA NA
Shoham et al, 2009[47]	Retrospective	Haematological disease	First-line L-AmB monotherapy 3–14 mg/kg daily	13/28 (46)	9/28 (32)	17/28 (61)
Chamilos et al, 2008[44]	Retrospective	Haematological disease	First-line AmBd monotherapy 0·5–1·0 mg/kg daily First-line lipid AmB monotherapy 4–15 mg/kg daily First-line L-AmB monotherapy Posaconazole salvage therapy	NA NA NA NA	NA NA NA NA	8/12 (67) 14/20 (70) 24/38 (63) 5/15 (33)
Reed et al, 2008[48]	Retrospective	Diabetes	First-line AmBd monotherapy mean 1 mg/kg daily or L-AmB monotherapy mean 5 mg/kg daily First-line ABLC monotherapy mean 5 mg/kg daily First-line combined caspofungin/ABLC or L-AmB mean 5 mg/kg daily	15/15 (100) 4/4 (100) 7/7 (100)	13/19 (68) 7/22 (32) 4/5 (80)	NA NA NA
Singh et al, 2007[28]	Prospective	SOT	First-line AmBd monotherapy First-line ABLC monotherapy First-line L-AmB monotherapy First-line posaconazole monotherapy	2/5 (40) 4/8 (50) 12/17 (71) 2/5 (40)	3/5 (60) 5/8 (63) 16/17 (94) 3/5 (60)	NA NA NA NA
Cordonnier et al, 2007[49]	Prospective	NA	L-AmB	NA	5/6 (83)	NA
Greenberg et al, 2006[50]	Retrospective	Haematological disease	Posaconazole 800 mg daily salvage monotherapy or combined therapy	18/24 (75)	19/24 (79)	9/24 (38)
van Burik et al, 2006[51]	Retrospective	Haematological disease	Posaconazole 800 mg daily salvage monotherapy or combined therapy	64/91 (70)	55/91 (60)	35/91 (38)
Roden et al, 2005[20]	Review	Diabetes	AmBd Lipid AmB	NA NA	NA NA	208/532 (39) 36/116 (31)
Pagano et al, 2004[52]	Retrospective	Haematological disease	First-line AmBd monotherapy 1 mg/kg daily First-line L-AmB 3 mg/kg daily	NA NA	7/39 (23) 7/12 (58)‡	NA NA
Gleissner et al, 2004[53]	Retrospective	Haematological disease	First-line AmBd monotherapy First-line/salvage L-AmB monotherapy	NA NA	NA NA	38/62 (61) 8/24 (33)†
Herbrecht et al, 2001[54]	Prospective	HSCT	Salvage ABCD monotherapy 4·8 mg/kg daily	13/21 (64)	12/20 (60)	1/21 (5)
Walsh et al, 1998[55]	Prospective	NA	Salvage ABLC monotherapy <5–>15 mg/kg daily	NA	17/24 (71)	NA
Oppenheim et al, 1995[56]	Prospective	NA	Salvage ABCD monotherapy 6 mg/kg daily	NA	4/4 (100)	NA

SOT=solid organ transplantation. AmBd=amphotericin B deoxycholate. AmB=amphotericin B. NA=data not available. Lipid AmB=lipid formulations of amphotericin B. L-AmB=liposomal amphotericin B. ABLC=amphotericin B phospholipid complex. HSCT=haemopoietic stem-cell transplantation. ABCD=amphotericin B colloidal dispersion. *Partial or complete. †Receipt of lipid AmB was significantly associated with reduced mortality. ‡Receipt of L-AmB was independently associated with partial or complete response.

***Table 1*: Summary of outcomes in the treatment of mucormycosis with different agents and regimens**

primary therapy, combined complete and partial response rates range from 0 to 60%, and all-cause mortality varies from 39% to 75%; in patients receiving lipid formulations of amphotericin B as primary or salvage therapy, combined complete and partial response rates are 32–100%, and overall mortality ranges from 5% to 61% (table 1).

Data from direct comparisons of the efficacy of lipid formulations of amphotericin B and amphotericin B deoxycholate for the treatment of mucormycosis are sparse. Several studies suggest that lipid formulations of amphotericin B, particularly liposomal amphotericin B, are associated with improved response rates (partial and complete) or survival.[28,33,45,46,52,53] In a global clinical registry primarily comprising patients with malignant diseases (64%), liposomal amphotericin B significantly improved response rates and survival.[33] One study reviewing mucormycosis outcomes in patients with haematological or oncological disorders reported higher survival with liposomal amphotericin B than with amphotericin B deoxycholate (67% *vs* 39%, p=0·02).[53] Two additional studies demonstrated that liposomal amphotericin B significantly correlated with recovery from mucormycosis in patients with haematological malignancies.[46,52] In a prospective, matched case-control study, the success rate in solid-organ transplant recipients receiving liposomal amphotericin B was increased by four times (odds ratio [OR] 4·0, 95% CI 0·63–26·0).[28] Additionally, in a review of solid-organ transplant recipients with rhino-orbital-cerebral mucormycosis, lipid formulations of amphotericin B were independently and significantly associated with

improved survival even when controlled for CNS involvement and the era of disease management (OR 0·09, 95% CI 0·02–0·50; p=0·006).[45]

The advantages of lipid formulations of amphotericin B, particularly liposomal amphotericin B, over amphotericin B deoxycholate in the treatment of mucormycosis could result from better brain penetration, reduction of fungal burden, and immunomodulatory effects, and fewer nephrotoxic effects. For instance, in animal models of coccidioidal or cryptococcal meningitis, liposomal amphotericin B significantly reduced fungal burden in the brain and improved survival compared with amphotericin B deoxycholate.[57–59] Liposomal amphotericin B also yields better results than certain other lipid formulations. For example, five times higher concentrations of liposomal amphotericin B were seen in the brains of rabbits with candidosis than of amphotericin B phospholipid complex.[60] In the clinical setting, lipid formulations of amphotericin B have been independently associated with lower mortality in solid-organ transplant recipients with cryptococcal meningitis than have other forms of amphotericin B.[61] Likewise, in a murine model of diabetes and haematogeneous *R oryzae* infection, liposomal amphotericin B was associated with significantly improved overall survival compared with amphotericin B deoxycholate.[62] Additionally, mice with diabetes, ketoacidosis, and with disseminated mucormycosis treated with liposomal amphotericin B had better survival and clearance of fungi from the brain than those treated with amphotericin B phospholipid complex.[63] This finding echoes lower success rates and higher clinical failure rate in patients treated with amphotericin B phospholipid complex than those treated with other polyenes.[48] However, in two mouse models of neutropenia—one with invasive pulmonary aspergillosis and one with invasive pulmonary mucormycosis—5 mg/kg amphotericin B phospholipid complex daily delivered higher concentrations of amphotericin B to the lung tissues than did 5 mg/kg liposomal amphotericin B daily, and early control of infection and improved fungal clearance were achieved.[64,65] At higher doses, though, no differences were observed between the two lipid formulations.

Survival after infection with a Mucormycotina organism requires a tightly controlled immune system that straddles a fine line between successful eradication of the invading pathogen and limitation of the damage to self-tissues from a dysregulated immune response. An imbalance in either direction could lead to detrimental outcomes.[66] In addition to improved CNS penetration, unique immunomodulatory characteristics of specific antifungal agents might be responsible for the differences observed in outcomes.[67] Although amphotericin B deoxycholate increases the expression of inflammatory cytokines, the lipid polyenes downregulate or have no effect on expression.[67] A lesser effect on inflammation might lower the risk of inflammatory tissue pathology and be advantageous to the host.[68]

Lastly, lipid formulations have good safety profiles. Side-effects, particularly nephrotoxic effects, related to amphotericin B deoxycholate have been associated with increased mortality, prolonged duration of hospital stay, and increased total cost of health care.[69,70] Despite various definitions of nephrotoxic effects, several studies have shown that liposomal amphotericin B has a better safety profile than amphotericin B deoxycholate.[69] The hydrophobicity of lipid polyenes enables effective drug delivery to the sites of infections and can lower the risk of nephrotoxic effects. Overall, liposomal amphotericin B and amphotericin B phospholipid complex have similarly low nephrotoxicity, but in one randomised study, nephrotoxic effects were less frequently seen in patients receiving the former.[69–71]

Triazoles

Posaconazole is the first azole possessing a broad spectrum of activity against Mucormycotina. *Rhizopus* spp and *Cokeromyces recurvatus* have higher minimum inhibitory concentrations (MICs) for this drug than other species, and *Mycocladus* spp are the most susceptible.[72] Nevertheless, activity in a clinical setting depends not only on the MIC but also on the achievable drug concentrations. A review summarised 23 published case reports of patients with mucormycosis who received posaconazole as salvage therapy, with or without amphotericin B, and a partial or complete response was reported in 19 (83%) patients.[73] Responses to posaconazole salvage therapy were seen in one report in eight (33%) of 24 with malignancy and 11 (46%) of 24 with haematological diseases,[50] and in another in 42 (46%) of 91 and 13 (14%) of 91, respectively.[51] Data on the use of posaconazole monotherapy as the first-line treatment are sparse. Two studies reported partial responses in three (60%) of five and complete responses in four (67%) of six patients,[28,33] but these data must be interpreted with caution owing to the very small numbers of patients.

Posaconazole is less active than amphotericin B as a treatment for neutropenia in mice infected with *R oryzae*;[74,75] isolates from this species are the most frequently recovered in cases of mucormycosis. Additionally, compared with placebo, posaconazole monotherapy does not improve outcomes in mice with neutropenia or diabetes and ketoacidosis that are also infected with *R oryzae*.[76,77] Thus, posaconazole is not recommended as a primary therapy for mucormycosis.[43] Furthermore, combined posaconazole and amphotericin B deoxycholate or liposomal amphotericin B offered no survival benefit over amphotericin B deoxycholate or liposomal amphotericin B alone.[76,77]

In two randomised trials of posaconazole as an antifungal prophylactic in HSCT recipients with GVHD and patients with chemotherapy-related neutropenia, no cases of mucormycosis were seen.[78,79] Breakthrough mucormycosis, however, was also virtually non-existent in the comparator groups (0·33% in both studies). In

other reports, development of mucormycosis during posaconazole prophylaxis has been reported in seven cases, among whom detailed information is available for four.[33,80–82] All four patients were HSCT recipients and had been treated with intensified immunosuppression for GVHD. Posaconazole was used as therapy for suspected invasive aspergillosis in one patient and for confirmed invasive aspergillosis in another, and as prophylaxis in the other two patients. During treatment, three patients developed pulmonary mucormycosis and one developed rhino-orbito-cerebral mucormycosis. When mucormycosis was diagnosed, posaconazole had been used for 11 days, 40 days, 8 weeks, and several weeks, respectively. Three *Rhizopus microsporus* isolates and one *R oryzae* isolate were recovered, and the posaconazole MICs were 2·0, 3·0, 3·0, and 0·5 μg/mL. All four patients died despite treatment.

Breakthrough infections in patients receiving long-term prophylactic therapy might be caused by other posaconazole-susceptible organisms, especially *Candida* spp.[83] The new triazole isavuconazole, which is currently being tested in clinical trials, is available in intravenous and oral formulations and has activity against moulds, including Mucormycotina.[84]

Echinocandins

Echinocandins are deemed inactive against Glomeromycota.[85] The incidence of breakthrough mucormycosis ranges from 0% to 0·2% during echinocandin prophylaxis and is 0% during echinocandin treatment.[86] Nevertheless, studies have demonstrated that *R oryzae* possesses a target enzyme for echinocandins, and these agents might have a therapeutic role against mucormycosis when combined with polyenes. Low-dose, but not high-dose, caspofungin improved survival in mice with diabetes, ketoacidosis, and *R oryzae* infection.[87] Additionally, combination regimens of amphotericin B phospholipid complex and caspofungin, and of liposomal amphotericin B and micafungin or anidulafungin improved survival of mice with disseminated mucormycosis compared with each antifungal agent alone, despite a lack of improvement in fungal clearance.[88,89]

In a retrospective study of 41 cases of biopsy-proven rhino-orbital-cerebral mucormycosis, patients treated with combination amphotericin B phospholipid complex and caspofungin had better response rates than those treated with amphotericin B phospholipid complex alone (100% *vs* 45%, p=0·02), as well as better Kaplan-Meier survival times (p=0·02).[48] Whether the observed survival benefits result from the effect of combination therapy on improved polyene delivery to the cell membrane after disruption of β-glucan in the cell wall, or are due to immunomodulation of the host response remains to be determined.[48,89]

Non-conventional therapeutic agents

Given the poor outcomes of mucormycosis, several agents with potential activity against Glomeromycota have been evaluated (table 2).

Synergy with immunosuppresive agents in vitro

A development with potential clinical relevance is that several immunosuppressive agents now in use demonstrate in-vitro activity against Glomeromycota. Tacrolimus and ciclosporin inhibit calcineurin, and sirolimus and everolimus inhibit mTOR, which leads to immunosuppressive effects.[109] These agents also have antifungal activities because fungi such as *Cryptococcus neoformans*, *Candida albicans*, and *Aspergillus fumigatus* have homologues of calcineurin and mTOR that play vital parts in fungal growth and pathogenesis.[28,91,92,110,111] Additionally, synergistic interactions against *Candida* spp, *C neoformans*, and *Aspergillus* spp have been observed between antifungal agents and calcineurin inhibitors or sirolimus.[110,112,113] Although the immunosuppressive effects of these immunosuppressants outweigh their antifungal activities, receipt of a calcineurin inhibitor was independently associated with lower mortality in solid-organ transplant recipients with cryptococcosis.[114]

The MICs of tacrolimus and sirolimus in Glomeromycota vary dependent on the species tested. With tacrolimus MICs of 0·5 μg/mL or lower have been reported for *R oryzae*, *Mucor circinelloides*, and *Absidia corymbifera*, and with sirolimus MICs of 0·25 μg/mL or lower have been reported for *R microspores* and *M circinelloides*.[91] Another study demonstrated antifungal activity of tacrolimus and ciclosporin against some clinical strains of *Rhinopholus pusillus*, *R microspores*, and *Mucor* spp (MICs 1–2 mg/L).[91] Furthermore, an independent association between tacrolimus therapy and a reduced risk of mucormycosis in solid-organ transplant recipients has been proposed to be due to the antifungal attributes of tacrolimus.[27]

Given the limited therapeutic options for and high mortality in patients with mucormycosis, knowledge of interactions between antifungal agents and immunosuppressants against Glomeromycota could improve outcomes. Susceptibility testing of tacrolimus in combination with posaconazole for 26 clinical isolates of Glomeromycota from solid-organ transplant recipients showed synergistic interactions between tacrolimus and posaconazole in 21 (81%).[93] Another report also demonstrated consistent synergy between posaconazole and ciclosporin or tacrolimus against *Cunninghamella bertholletiae*, *A corymbifera*, and *A elegans*.[92] Additionally, a study reported synergy between ciclospoin and various azoles (posaconazole, itraconazole, and ravuconazole).[91] Synergy was also seen with tacrolimus for itraconazole and ravuconazole, but the frequencies were notably lower; no synergy was observed between posaconazole and tacrolimus.[91]

Data on interactions between posaconazole and sirolimus have been contradictory, with one study reporting synergy[91] and another antagonism;[92] different readings of MICs for endpoints were thought to have led to this difference. In addition to posaconazole,

	Mechanisms of antifungal action	Synergy with other agents	Clinical outcome
Immunosuppressive agents			
Calcineurin inhibitors	Inhibition of calcineurin homologues[90]	Amphotericin B,[91] posaconazole,[91–93]	Tacrolimus protective[27]
mTOR	NA	Amphotericin B,[91] posaconazole,[91]	NA
Iron chelators*	Iron starvation[94]	Liposomal amphotericin B[94]	Cure of disease when used as salvage therapy[95,96]
Colistin	Disruptive activity on Mucorales cytoplasmic and vacuolar membranes[97]	Amphotericin B[97]	NA
Statins	Modification of inflammatory cascades at multiple levels,[98] inhibition of protein prenylation[99]	Voriconazole[100]	NA
Granulocyte transfusion	Prevention of germination of spores and the killing of hyphal forms of the fungus[1]	GM-CSF, interferon γ[101]	Clinical efficacy seen for interventional and prophylactic use[102,103]
Cytokines			
G-CSF	Augmentation of phagocytosis, oxidative burst, and antifungal activity of PMNLs[104]	Granulocytes[101]	Successful response as adjunctive therapy[102,105,106]
GM-CSF	Augmentation of PMNL-induced hyphal damage,[101] phagocytosis, and oxidative burst[104]	Liposomal amphotericin B[107]	..
Interferon γ	Augmentation of PMNL-induced hyphal damage[101] induces a T-helper-1 response[104]	Granulocytes[101]	..
Hyperbaric oxygen	Increase in production of oxygen-based free radicals and indirect antimicrobial effects; contribution to tissue healing[104]	NA	Possible survival benefit in patients with diabetes[108]

NA=data not available. GM-CSF=granulocyte-macrophage colony-stimulating factor. G-CSF=granulocyte colony-stimulating factor. PMNL=polymorphonuclear leucocyte. *Iron chelator pertains to deferasirox, not deferoxamine.

***Table 2*: Mechanisms of antifungal action, synergistic relations, and clinical outcomes for non-conventional antifungal drugs in the treatment of mucormycosis**

amphotericin B has interacted synergistically with ciclosporin, sirolimus, and tacrolimus against 90%, 70%, and 30%, respectively, of ten clinical isolates of Glomeromycota, whereas caspofungin has exhibited synergy with tacrolimus against only two (8%) of 26 isolates.[91,93] Antagonistic interactions have been observed only rarely between these immunosuppressive and antifungal agents.[91–93]

Iron chelating agents

Since iron is critical for the growth and pathogenesis of Glomeromycota, iron chelators have the potential to treat mucormycosis.[115] Deferasirox, an approved oral iron chelator, has fungicidal effects and interacts synergistically with liposomal amphotericin B against Glomeromycota.[94] The fungicidal activity of deferasirox resulted in iron starvation; the addition of iron completely reversed the fungicidal effects. Additionally, in mice with diabetes, ketoacidosis, and mucormycosis, combination therapy with liposomal amphotericin B and deferasirox significantly improved survival and reduced brain fungal burden, compared with placebo and either drug alone.[94]

Clinical outcomes have been reported for 11 patients with mucormycosis treated with deferasirox (15–20 mg/kg daily, except for one patient treated with 5 mg/kg daily owing to coexisting hepatic disease) in combination with other antifungal agents.[95,96,116] Deferasirox was added to the initial antifungal therapy in five patients without disease progression: three were deemed to be cured of the infection and in two signs and symptoms resolved or improved and they were started on maintenance therapy. The remaining six patients received deferasirox as salvage therapy after receiving regimens not containing this drug. No effect was seen in one patient, improved radiographic response but no change in clinical response was reported in another, and cure was reported in four. A double-blind, randomised, placebo-controlled, phase 2 study of safety and efficacy is being done of combined liposomal amphotericin B and deferasirox for mucormycosis.[117]

Colistin

With the emergence of bacteria resistant to currently available antibiotics, colistin, which contains polymyxin E, has emerged as a valuable option for treating infections caused by multidrug-resistant, Gram-negative bacteria. Colistin interacts with lipopolysaccharide and kills these bacteria through disruption of their cell membrane.[118] Polymyxin B has antifungal properties against the yeast *Saccharomyces cerevisiae* via its effect on the cell membrane and, therefore, whether colistin has similar properties has been studied.[97,119] Colistin exerted fugicidal activity in vitro against *R oryzae* by damaging cytoplasmic and vacuolar membranes, which led to the leakage of intracellular contents. Additionally, prophylactic intranasal colistimethate substantially lowered mortality and pulmonary fungal burden in immunosuppressed mice with pulmonary infection, whereas therapeutic intraperitoneal colistimethate had no effect.[97] Whether colistin is useful in the treatment of mucormycosis deserves further investigation.

Statins

In addition to lipid-lowering attributes, statins have beneficial effects on sepsis or infection by modification of the inflammatory cascades of hosts at multiple levels,

and by action against clinically important opportunistic pathogens.[98,120,121] These drugs have fungicidal activity against Glomeromycota and act synergistically with other antifungal agents, such as voriconazole. [100,122,123] Additionally, statins can induce an apoptosis-like cell death of *Mucor racemosus* through inhibition of protein prenylation.[99] They modulate signal transduction and cytokine transcriptional pathways of the key host defences, and exerting a mediating effect on fungal infections independent of their antifungal activity[98,124] and, therefore, statins might offer benefits to patients with mucormycosis. Since statins have high MICs for Glomeromycota, their efficacy might be greatest when used in conjunction with antifungal agents.

Granulocyte transfusion and cytokines

Mononuclear and polymorphonuclear phagocytes are responsible for the innate immune responses to mucormycosis. Neutropenia seems to be associated with mucormycosis in patients with haemataological disease (OR 3·0, 95% CI 1·06–8·51; p=0·05); this feature is seen in 71–100% of patients.[10,41] Additionally, recovery from neutropenia significantly correlates with an improvement in mucormycosis[41] and, therefore, granulocyte transfusion could improve outcomes. After exposure to *R oryzae*, neutrophils exhibit reduced hyphal damages associated with impaired superoxide anion release compared with that seen after exposure to *A fumigatus*.[14] Nevertheless, in combination with granulocyte-macrophage colony-stimulating factor (GM-CSF) and interferon γ, the antifungal activities of neutrophils are notably increased.[101] Furthermore, despite some variations in different Glomeromycota species, lipid formulations of amphotericin B interact with human polymorphonulcear leucocytes synergistically by predominantly inducing augmented hyphal damage.[125]

Cytokines play a critical part in activation and recruitment of granulocyte and mononuclear phagocytes.[126] Efficacy of granulocyte colony-stimulating factor (G-CSF), GM-CSF, and interferon γ as adjunctive agents for invasive mycosis have been evaluated. In addition to the aforementioned improvement of fungicidal activity of polymorphonulcear leucocytes, G-CSF and GM-CSF increase phagocytosis and oxidative burst, and interferon γ induces T-helper-1 immunological response against infection.[104] Moreover, combined GM-CSF and liposomal amphotericin B have notably prolonged survival and reduced fungal burden of the brain tissue in a murine model of systemic mucormycosis compared with liposomal amphotericin B alone. Interferon γ, alone or in combination with liposomal amphotericin B, however, did not yield better results than liposomal amphotericin B alone.[107]

Partial and complete responses have been reported in patients with mucormycosis receiving G-CSF, GM-CSF, and interferon γ as adjunctive agents for invasive mucormycosis.[102,105,106] In a review of mucormycosis patients who received granulocyte transfusion or G-CSF, two (29%) of seven and 15 (83%) of 18, respectively, survived.[20] The findings were, however, limited by publication bias and small numbers of cases. Additionally, in a prospective, non-randomised study granulocyte transfusions were used in patients with haematological malignancies and long-term neutropenia to control life-threatening infections, including one episode of mucormycosis (intervention group), and to prevent recurrence of severe fungal infection, including five episodes of mucormycosis after HSCT or during intensive chemotherapy (preventive group).[103] Disease control was achieved in 36 (82%) of 44 patients in the intervention group and no recurrence was observed in the preventive group.

Hyperbaric oxygen

Hyperbaric oxygen has a direct antifungal effect in vitro through increased production of oxygen-based free radicals.[104] This approach also yields several indirect antimicrobial effects and contributes to tissue healing.[104] These attributes indicate that benefits might be derived from its adjunctive use in the treatment of mucormycosis, but clinical data are limited.[104,127,128] In a review of 28 reported cases of mucormycosis, adjunctive hyperbaric oxygen seemed beneficial in patients with diabetes (94% survival).[108]

Surgery

Angioinvasion, thrombosis, and tissue necrosis associated with mucormycosis lead to poor penetration of antifungal agents at the sites of infection and compromise their efficacy.[129] Consequently, debridement of necrotic tissues plays a crucial part in the treatment of mucormycosis.[130] Surgery is independently associated with successful treatment and survival, particularly in rhino-orbital-cerebral and pulmonary mucormycosis, although patients undergoing surgery might have less severe disease or fewer comorbidities than those not undergoing surgery.[28,43,45,131,132] In rhino-orbital-cerebral disease, aggressive early and repeated surgical excision of the infected craniofacial tissues is the cornerstone of successful treatment.[133] Orbital exenteration might be life-saving after fungal invasion.[133] A so-called conservative-aggressive approach, in which the limits of surgical excision are guided by intraoperative use of frozen sections, has, however, led to favourable outcomes in rhino-orbital-cerebral mucormycosis and uninvolved orbital tissue may be spared.[48] In one study of surgery for pulmonary mucormycosis, mortality was significantly lowered from 55% to 27% (p<0·05).[132] Adequate surgical debridement is also deemed essential for the successful management of colonic and cutaneous mucormycosis.[25,134]

Risk factors for poor outcomes

Although an expanding range of antifungal agents has improved outcomes in mucormycosis, mortality remains

high, particularly in patients with disseminated (96%) or gastrointestinal (85%) disease, and in HSCT recipients (91%).[20] Disseminated disease, renal failure, and infection with *Cunninghamella* spp are independently associated with death, whereas type 1 diabetes and absence of underlying disorders reduce the risk of death.[20] In patients with haematological malignancies, active malignancy, and monocytopenia at the time of diagnosis of mucormycosis, the outlook is poor, whereas patients receiving posaconazole-based salvage therapy that leads to neutrophil recovery have a favourable outlook.[44] Additionally, delayed initiation of polyene therapy is associated with poor outcomes.[44] In solid-organ transplant recipients, renal failure and disseminated disease were independently predictive of treatment failure, whereas surgical resection was associated with treatment success.[28]

Preventive strategies

In patients with GVHD who are receiving immunosuppressive therapy, and in patients with neutropenia due to chemotherapy for acute myelogenous leukaemia and myelodysplastic syndrome, prophylaxis with posaconazole substantially decreases the incidence of invasive fungal infections, particularly invasive aspergillosis.[78,79] In two studies mortality associated with invasive fungal infections was lowered,[78,79] although overall mortality was only lowered in one.[78] These findings, along with the extended spectrum and good safety profile of posaconazole, suggest this drug is a rational option for prophylaxis in patients with GVHD.[135,136]

Advances in non-culture-based diagnostic methods and chest CT have led to antifungal drugs being proposed for prevention of invasive mycoses. No effective diagnostic laboratory markers for mucormycosis are, however, currently available and presentation of disease is frequently non-specific. This approach, therefore, is unlikely to be implemented imminently. Nevertheless, empirical use of antifungal agents with activity against mucormycosis is reasonable in patients with defined risk factors for these moulds.[23] A drawback of this approach is that non-selective use of posaconazole could increase the risk of fungi becoming resistant to posaconazole[10,136] and, therefore, routine use remains controversial.

Conclusions

Mucormycosis is an important opportunistic fungal infection, particularly in patients with haematological disorders, malignancy, and diabetes mellitus, and in those undergoing transplantation. Whether the rising rates in certain populations are related to evolving characteristics of patients or to antimicrobial selection pressure from prescription practices for current antifungal agents deserves further investigation. Liposomal amphotericin B is the preferred therapeutic agent for mucormycosis. Given the limited clinical experience with posaconazole as first-line therapy, this drug should currently be used as second-line or salvage therapy. The combination of polyene with an echinocandin is attractive and its use warrants assessment in well-designed clinical studies. Early diagnosis, reversal of predisposing factors, prompt employment of appropriate antifungal therapy, and surgical debridement are critical for good outcomes. Harnessing the potential of novel synergistic agents, such as immunosuppressants, iron chelators, colistin, statins, and GM-CSF, in combined regimens might improve outcomes. Furthermore, adjunctive therapy with immunomodulatory treatments, such as statins, granulocyte transfusion, and cytokines, has the potential to fine-tune and improve host response to mucormycosis.

Search strategy and selection criteria

Data were identified by searches of PubMed for articles published from 1950 to 2010, with the terms "zygomycetes", "mucormycosis", "Mucorales" and "breakthrough", "voriconazole", "posaconazole", "echinocandins", "caspofungin", "micafungin", "anidulafungin", "hematological malignancy", "transplant", "polyene", "amphotericin B deoxycholate", "liposomal amphotericin B", "amphotericin B lipid complex", "calcineurin inhibitors", "tacrolimus", "cyclosporine", "mammalian target of rapamycin", "sirolimus", "everolimus", "iron chelator", "deferasirox", "colistin", "statins", or "granulocyte transfusion", "granulocyte-colony stimulating factor", "granulocyte macrophage-colony stimulating factor", "interferon-γ", "hyperbaric oxygen", or "surgery". Full-text articles and abstracts were retrieved. References from relevant articles were searched manually. Articles were also identified through searches of the authors' own extensive files on these topics. Only articles published in English were reviewed.

Contributors

H-YS reviewed the literature and participated in writing the paper. NS outlined the Review, reviewed the literature, and participated in writing the paper.

Conflicts of interest

NS has received support from Pfizer. H-YS declares that she has no conflicts of interest.

References

1 Kontoyiannis DP, Lewis RE. Agents of mucormycosis and entomophthoramycosis. In: Mandell GL, Bennett JE, Dolin R, eds. Principles and practice of infectious diseases, 7th edn. Philadelphia, PA: Churchill Livingstone/Elsevier, 2009.

2 Hibbett DS, Binder M, Bischoff JF, et al. A higher-level phylogenetic classification of the Fungi. *Mycol Res* 2007; **111:** 509–47.

3 Bennett JE. Introduction to mycoses. In: Mandell GL, Bennett JE, Dolin R, eds. Principles and practice of infectious diseases, 7th edn. Philadelphia, PA: Churchill Livingstone/Elsevier, 2009.

4 Ribes JA, Vanover-Sams CL, Baker DJ. Zygomycetes in human disease. *Clin Microbiol Rev* 2000; **13:** 236–301.

5 Kontoyiannis DP, Marr KA, Park BJ, et al. Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001-2006: overview of the Transplant-Associated Infection Surveillance Network (TRANSNET) Database. *Clin Infect Dis* 2010; **50:** 1091–100.

6 Pappas PG, Alexander BD, Andes DR, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). *Clin Infect Dis* 2010; **50:** 1101–11.

7 Chakrabarti A, Das A, Sharma A, et al. Ten years' experience in zygomycosis at a tertiary care centre in India. *J Infect* 2001; **42:** 261–66.

8 Chakrabarti A, Das A, Mandal J, et al. The rising trend of invasive zygomycosis in patients with uncontrolled diabetes mellitus. *Med Mycol* 2006; **44:** 335–42.

9 Chakrabarti A, Chatterjee SS, Das A, et al. Invasive zygomycosis in India: experience in a tertiary care hospital. *Postgrad Med J* 2009; **85:** 573–81.

10 Kontoyiannis DP, Lionakis MS, Lewis RE, et al. Zygomycosis in a tertiary-care cancer center in the era of aspergillus-active antifungal therapy: a case-control observational study of 27 recent cases. *J Infect Dis* 2005; **191:** 1350–60.

11 Bitar D, Van Cauteren D, Lanternier F, et al. Increasing incidence of zygomycosis (mucormycosis), France, 1997–2006. *Emerg Infect Dis* 2009; **15:** 1395–401.

12 Husain S, Alexander BD, Munoz P, et al. Opportunistic mycelial fungal infections in organ transplant recipients: emerging importance of non-aspergillus mycelial fungi. *Clin Infect Dis* 2003; **37:** 221–29.

13 Stelzmueller I, Lass-Floerl C, Geltner C, et al. Zygomycosis and other rare filamentous fungal infections in solid organ transplant recipients. *Transpl Int* 2008; **21:** 534–46.

14 Chamilos G, Lewis RE, Lamaris G, Walsh TJ, Kontoyiannis DP. Zygomycetes hyphae trigger an early, robust proinflammatory response in human polymorphonuclear neutrophils through toll-like receptor 2 induction but display relative resistance to oxidative damage. *Antimicrob Agents Chemother* 2008; **52:** 722–24.

15 Diwakar A, Dewan RK, Chowdhary A, Randhawa HS, Khanna G, Gaur SN. Zygomycosis—a case report and overview of the disease in India. *Mycoses* 2007; **50:** 247–54.

16 Garcia-Covarrubias L, Bartlett R, Barratt DM, Wassermann RJ. Rhino-orbitocerebral mucormycosis attributable to *Apophysomyces elegans* in an immunocompetent individual: case report and review of the literature. *J Trauma* 2001; **50:** 353–57.

17 Liang KP, Tleyjeh IM, Wilson WR, Roberts GD, Temesgen Z. Rhino-orbitocerebral mucormycosis caused by *Apophysomyces elegans*. *J Clin Microbiol* 2006; **44:** 892–98.

18 Meis JF, Chakrabarti A. Changing epidemiology of an emerging infection: zygomycosis. *Clin Microbiol Infect* 2009; **15** (suppl 5)**:** 10–14.

19 Antoniadou A. Outbreaks of zygomycosis in hospitals. *Clin Microbiol Infect* 2009; **15** (suppl 5)**:** 55–59.

20 Roden MM, Zaoutis TE, Buchanan WL, et al. Epidemiology and outcome of zygomycosis: a review of 929 reported cases. *Clin Infect Dis* 2005; **41:** 634–53.

21 Almyroudis NG, Sutton DA, Linden P, Rinaldi MG, Fung J, Kusne S. Zygomycosis in solid organ transplant recipients in a tertiary transplant center and review of the literature. *Am J Transplant* 2006; **6:** 2365–74.

22 Sun HY, Aguado JM, Bonatti H, et al. Pulmonary zygomycosis in solid organ transplant recipients in the current era. *Am J Transplant* 2009; **9:** 2166–71.

23 Chamilos G, Marom EM, Lewis RE, Lionakis MS, Kontoyiannis DP. Predictors of pulmonary zygomycosis versus invasive pulmonary aspergillosis in patients with cancer. *Clin Infect Dis* 2005; **41:** 60–66.

24 Wahba H, Truong MT, Lei X, Kontoyiannis DP, Marom EM. Reversed halo sign in invasive pulmonary fungal infections. *Clin Infect Dis* 2008; **46:** 1733–37.

25 Lo OS, Law WL. Ileocolonic mucormycosis in adult immunocompromised patients: a surgeon's perspective. *World J Gastroenterol* 2010; **16:** 1165–70.

26 Kontoyiannis DP, Lewis RE. Invasive zygomycosis: update on pathogenesis, clinical manifestations, and management. *Infect Dis Clin North Am* 2006; **20:** 581–607.

27 Kalpatti R, Peters B, Kane I, et al. Safety and efficacy of high dose intravenous desferrioxamine for reduction of iron overload in sickle cell disease. *Pediatr Blood Cancer* 2010; **55:** 1338–42.

28 Singh N, Aguado JM, Bonatti H, et al. Zygomycosis in solid organ transplant recipients: a prospective, matched case-control study to assess risks for disease and outcome. *J Infect Dis* 2009; **200:** 1002–11.

29 Trifilio SM, Bennett CL, Yarnold PR, et al. Breakthrough zygomycosis after voriconazole administration among patients with hematologic malignancies who receive hematopoietic stem-cell transplants or intensive chemotherapy. *Bone Marrow Transplant* 2007; **39:** 425–29.

30 Marty FM, Cosimi LA, Baden LR. Breakthrough zygomycosis after voriconazole treatment in recipients of hematopoietic stem-cell transplants. *N Engl J Med* 2004; **350:** 950–52.

31 Siwek GT, Dodgson KJ, de Magalhaes-Silverman M, et al. Invasive zygomycosis in hematopoietic stem cell transplant recipients receiving voriconazole prophylaxis. *Clin Infect Dis* 2004; **39:** 584–87.

32 Imhof A, Balajee SA, Fredricks DN, Englund JA, Marr KA. Breakthrough fungal infections in stem cell transplant recipients receiving voriconazole. *Clin Infect Dis* 2004; **39:** 743–46.

33 Ruping MJ, Heinz WJ, Kindo AJ, et al. Forty-one recent cases of invasive zygomycosis from a global clinical registry. *J Antimicrob Chemother* 2010; **65:** 296–302.

34 Martin T, Sharma M, Damon L, et al. Voriconazole is safe and effective as prophylaxis for early and late fungal infections following allogeneic hematopoietic stem cell transplantation. *Transpl Infect Dis* 2009; **12:** 45–50.

35 Torres A, Serrano J, Rojas R, et al. Voriconazole as primary antifungal prophylaxis in patients with neutropenia after hematopoietic stem cell transplantation or chemotherapy for acute myeloid leukemia. *Eur J Haematol* 2010; **84:** 271–73.

36 Gergis U, Markey K, Greene J, et al. Voriconazole provides effective prophylaxis for invasive fungal infection in patients receiving glucocorticoid therapy for GVHD. *Bone Marrow Transplant* 2009; **45:** 662–67.

37 Trifilio S, Singhal S, Williams S, et al. Breakthrough fungal infections after allogeneic hematopoietic stem cell transplantation in patients on prophylactic voriconazole. *Bone Marrow Transplant* 2007; **40:** 451–56.

38 Walsh TJ, Pappas P, Winston DJ, et al. Voriconazole compared with liposomal amphotericin B for empirical antifungal therapy in patients with neutropenia and persistent fever. *N Engl J Med* 2002; **346:** 225–34.

39 Vigouroux S, Morin O, Moreau P, et al. Zygomycosis after prolonged use of voriconazole in immunocompromised patients with hematologic disease: attention required. *Clin Infect Dis* 2005; **40:** e35–37.

40 Pagano L, Gleissner B, Fianchi L. Breakthrough zygomycosis and voriconazole. *J Infect Dis* 2005; **192:** 1496–97.

41 Pagano L, Valentini CG, Fianchi L, Caira M. The role of neutrophils in the development and outcome of zygomycosis in haematological patients. *Clin Microbiol Infect* 2009; **15** (suppl 5)**:** 33–36.

42 Lamaris GA, Ben-Ami R, Lewis RE, Chamilos G, Samonis G, Kontoyiannis DP. Increased virulence of Zygomycetes organisms following exposure to voriconazole: a study involving fly and murine models of zygomycosis. *J Infect Dis* 2009; **199:** 1399–406.

43 Spellberg B, Walsh TJ, Kontoyiannis DP, Edwards J Jr, Ibrahim AS. Recent advances in the management of mucormycosis: from bench to bedside. *Clin Infect Dis* 2009; **48:** 1743–51.

44 Chamilos G, Lewis RE, Kontoyiannis DP. Delaying amphotericin B-based frontline therapy significantly increases mortality among patients with hematologic malignancy who have zygomycosis. *Clin Infect Dis* 2008; **47:** 503–09.

45 Sun HY, Forrest G, Gupta KL, et al. Rhino-orbital-cerebral zygomycosis in solid organ transplant recipients. *Transplantation* 2010; **90:** 85–92.

46 Kara IO, Tasova Y, Uguz A, Sahin B. Mucormycosis-associated fungal infections in patients with haematologic malignancies. *Int J Clin Pract* 2009; **63:** 134–39.

47 Shoham S, Magill SS, Merz WG, et al. Primary treatment of zygomycosis with liposomal amphotericin B: analysis of 28 cases. *Med Mycol* 2009; **48:** 511–17.

48 Reed C, Bryant R, Ibrahim AS, et al. Combination polyene-caspofungin treatment of rhino-orbital-cerebral mucormycosis. *Clin Infect Dis* 2008; **47:** 364–71.

49 Cordonnier C, Bresnik M, Ebrahimi R. Liposomal amphotericin B (AmBisome) efficacy in confirmed invasive aspergillosis and other filamentous fungal infections in immunocompromised hosts: a pooled analysis. *Mycoses* 2007; **50:** 205–09.

50 Greenberg RN, Mullane K, van Burik JA, et al. Posaconazole as salvage therapy for zygomycosis. *Antimicrob Agents Chemother* 2006; **50:** 126–33.

51 van Burik JA, Hare RS, Solomon HF, Corrado ML, Kontoyiannis DP. Posaconazole is effective as salvage therapy in zygomycosis: a retrospective summary of 91 cases. *Clin Infect Dis* 2006; **42:** e61–65.

52 Pagano L, Offidani M, Fianchi L, et al. Mucormycosis in hematologic patients. *Haematologica* 2004; **89:** 207–14.

53 Gleissner B, Schilling A, Anagnostopolous I, Siehl I, Thiel E. Improved outcome of zygomycosis in patients with hematological diseases? *Leuk Lymphoma* 2004; **45:** 1351–60.

54 Herbrecht R, Letscher-Bru V, Bowden RA, et al. Treatment of 21 cases of invasive mucormycosis with amphotericin B colloidal dispersion. *Eur J Clin Microbiol Infect Dis* 2001; **20:** 460–66.

55 Walsh TJ, Hiemenz JW, Seibel NL, et al. Amphotericin B lipid complex for invasive fungal infections: analysis of safety and efficacy in 556 cases. *Clin Infect Dis* 1998; **26:** 1383–96.

56 Oppenheim BA, Herbrecht R, Kusne S. The safety and efficacy of amphotericin B colloidal dispersion in the treatment of invasive mycoses. *Clin Infect Dis* 1995; **21:** 1145–53.

57 Clemons KV, Sobel RA, Williams PL, Pappagianis D, Stevens DA. Efficacy of intravenous liposomal amphotericin B (AmBisome) against coccidioidal meningitis in rabbits. *Antimicrob Agents Chemother* 2002; **46:** 2420–26.

58 Takemoto K, Yamamoto Y, Ueda Y. Influence of the progression of cryptococcal meningitis on brain penetration and efficacy of AmBisome in a murine model. *Chemotherapy* 2006; **52:** 271–78.

59 Albert MM, Stahl-Carroll L, Luther MF, Graybill JR. Comparison of liposomal amphotericin B to amphotericin B for treatment of murine cryptococcal meningitis. *J Mycol Med* 1995; **5:** 1–6.

60 Groll AH, Giri N, Petraitis V, et al. Comparative efficacy and distribution of lipid formulations of amphotericin B in experimental *Candida albicans* infection of the central nervous system. *J Infect Dis* 2000; **182:** 274–82.

61 Sun HY, Alexander BD, Lortholary O, et al. Lipid formulations of amphotericin B significantly improve outcome in solid organ transplant recipients with central nervous system cryptococcosis. *Clin Infect Dis* 2009; **49:** 1721–28.

62 Ibrahim AS, Avanessian V, Spellberg B, Edwards JE Jr. Liposomal amphotericin B, and not amphotericin B deoxycholate, improves survival of diabetic mice infected with *Rhizopus oryzae*. *Antimicrob Agents Chemother* 2003; **47:** 3343–44.

63 Ibrahim AS, Gebremariam T, Husseiny MI, et al. Comparison of lipid amphotericin B preparations in treating murine zygomycosis. *Antimicrob Agents Chemother* 2008; **52:** 1573–76.

64 Lewis RE, Liao G, Hou J, Chamilos G, Prince RA, Kontoyiannis DP. Comparative analysis of amphotericin B lipid complex and liposomal amphotericin B kinetics of lung accumulation and fungal clearance in a murine model of acute invasive pulmonary aspergillosis. *Antimicrob Agents Chemother* 2007; **51:** 1253–58.

65 Lewis RE, Albert ND, Liao G, Hou J, Prince RA, Kontoyiannis DP. Comparative pharmacodynamics of amphotericin B lipid complex and liposomal amphotericin B in a murine model of pulmonary mucormycosis. *Antimicrob Agents Chemother* 2010; **54:** 1298–304.

66 Casadevall A, Pirofski LA. The damage-response framework of microbial pathogenesis. *Nat Rev Microbiol* 2003; **1:** 17–24.

67 Ben-Ami R, Lewis RE, Kontoyiannis DP. Immunocompromised hosts: immunopharmacology of modern antifungals. *Clin Infect Dis* 2008; **47:** 226–35.

68 Lass-Florl C, Singh N, Knezovic I. Lipid-based amphotericin B formulations in opportunistic infections. *Clin Adv Hematol Oncol* 2010; **8:** 1–8.

69 Saliba F, Dupont B. Renal impairment and amphotericin B formulations in patients with invasive fungal infections. *Med Mycol* 2008; **46:** 97–112.

70 Wingard JR, Kubilis P, Lee L, et al. Clinical significance of nephrotoxicity in patients treated with amphotericin B for suspected or proven aspergillosis. *Clin Infect Dis* 1999; **29:** 1402–07.

71 Wingard JR, White MH, Anaissie E, Raffalli J, Goodman J, Arrieta A. A randomized, double-blind comparative trial evaluating the safety of liposomal amphotericin B versus amphotericin B lipid complex in the empirical treatment of febrile neutropenia. L Amph/ABLC Collaborative Study Group. *Clin Infect Dis* 2000; **31:** 1155–63.

72 Alastruey-Izquierdo A, Castelli MV, Cuesta I, et al. In vitro activity of antifungals against Zygomycetes. *Clin Microbiol Infect* 2009; **15** (suppl 5)**:** 71–76.

73 Cornely OA, Vehreschild JJ, Ruping MJ. Current experience in treating invasive zygomycosis with posaconazole. *Clin Microbiol Infect* 2009; **15** (suppl 5)**:** 77–81.

74 Dannaoui E, Meis JF, Loebenberg D, Verweij PE. Activity of posaconazole in treatment of experimental disseminated zygomycosis. *Antimicrob Agents Chemother* 2003; **47:** 3647–50.

75 Sun QN, Najvar LK, Bocanegra R, Loebenberg D, Graybill JR. In vivo activity of posaconazole against *Mucor* spp. in an immunosuppressed-mouse model. *Antimicrob Agents Chemother* 2002; **46:** 2310–12.

76 Rodriguez MM, Serena C, Marine M, Pastor FJ, Guarro J. Posaconazole combined with amphotericin B, an effective therapy for a murine disseminated infection caused by *Rhizopus oryzae*. *Antimicrob Agents Chemother* 2008; **52:** 3786–88.

77 Ibrahim AS, Gebremariam T, Schwartz JA, Edwards JE Jr, Spellberg B. Posaconazole mono- or combination therapy for treatment of murine zygomycosis. *Antimicrob Agents Chemother* 2009; **53:** 772–75.

78 Cornely OA, Maertens J, Winston DJ, et al. Posaconazole vs. fluconazole or itraconazole prophylaxis in patients with neutropenia. *N Engl J Med* 2007; **356:** 348–59.

79 Ullmann AJ, Lipton JH, Vesole DH, et al. Posaconazole or fluconazole for prophylaxis in severe graft-versus-host disease. *N Engl J Med* 2007; **356:** 335–47.

80 Mousset S, Bug G, Heinz WJ, Tintelnot K, Rickerts V. Breakthrough zygomycosis on posaconazole prophylaxis after allogeneic stem cell transplantation. *Transpl Infect Dis* 2009; **12:** 261–64.

81 Schlemmer F, Lagrange-Xelot M, Lacroix C, de La Tour R, Socie G, Molina JM. Breakthrough rhizopus infection on posaconazole prophylaxis following allogeneic stem cell transplantation. *Bone Marrow Transplant* 2008; **42:** 551–52.

82 Lekakis LJ, Lawson A, Prante J, et al. Fatal rhizopus pneumonia in allogeneic stem cell transplant patients despite posaconazole prophylaxis: two cases and review of the literature. *Biol Blood Marrow Transplant* 2009; **15:** 991–95.

83 Winston DJ, Bartoni K, Territo MC, Schiller GJ. Efficacy, safety, and breakthrough infections associated with standard long-term posaconazole antifungal prophylaxis in allogeneic stem-cell transplant recipients. *Biol Blood Marrow Transplant* 2010; published online May 10. DOI:10.1016/j.bbmt.2010.04.017.

84 Thompson GR 3rd, Wiederhold NP. Isavuconazole: a comprehensive review of spectrum of activity of a new triazole. *Mycopathologia* 2010; **170:** 291–313.

85 Denning DW. Echinocandin antifungal drugs. *Lancet* 2003; **362:** 1142–51.

86 Sun HY, Singh N. Characterisation of breakthrough invasive mycoses in echinocandin recipients: an evidence-based review. *Int J Antimicrob Agents* 2010; **35:** 211–18.

87 Ibrahim AS, Bowman JC, Avanessian 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 a high dose during murine disseminated zygomycosis. *Antimicrob Agents Chemother* 2005; **49:** 721–27.

88 Spellberg B, Fu Y, Edwards JE Jr, Ibrahim AS. Combination therapy with amphotericin B lipid complex and caspofungin acetate of disseminated zygomycosis in diabetic ketoacidotic mice. *Antimicrob Agents Chemother* 2005; **49:** 830–32.

89 Ibrahim AS, Gebremariam T, Fu Y, Edwards JE Jr, Spellberg B. Combination echinocandin-polyene treatment of murine mucormycosis. *Antimicrob Agents Chemother* 2008; **52:** 1556–58.

90 Steinbach WJ, Cramer RA Jr, Perfect BZ, et al. Calcineurin inhibition or mutation enhances cell wall inhibitors against *Aspergillus fumigatus*. *Antimicrob Agents Chemother* 2007; **51:** 2979–81.

91 Dannaoui E, Schwarz P, Lortholary O. In vitro interactions between antifungals and immunosuppressive drugs against zygomycetes. *Antimicrob Agents Chemother* 2009; **53:** 3549–51.

92 Narreddy S, Manavathu E, Chandrasekar PH, Alangaden GJ, Revankar SG. In vitro interaction of posaconazole with calcineurin inhibitors and sirolimus against zygomycetes. *J Antimicrob Chemother* 2010; **65:** 701–03.

93 Best LA, Singh N, Ben-Ami R, et al. In vitro interactions of tacrolimus with posaconazole or caspofungin against zygomycetes. 47th Annual Meeting of the Infectious Diseases Society of America; Philadelphia, PA; Oct 29–Nov 1, 2009 (abstr M-1046).

94 Ibrahim AS, Gebermariam T, Fu Y, et al. The iron chelator deferasirox protects mice from mucormycosis through iron starvation. *J Clin Invest* 2007; **117:** 2649–57.

95 Spellberg B, Andes D, Perez M, et al. Safety and outcomes of open-label deferasirox iron chelation therapy for mucormycosis. *Antimicrob Agents Chemother* 2009; **53:** 3122–25.

96 Reed C, Ibrahim A, Edwards JE Jr, Walot I, Spellberg B. Deferasirox, an iron-chelating agent, as salvage therapy for rhinocerebral mucormycosis. *Antimicrob Agents Chemother* 2006; **50:** 3968–69.

97 Ben-Ami R, Lewis RE, Tarrand J, Leventakos K, Kontoyiannis DP. Antifungal activity of colistin against mucorales species in vitro and in a murine model of *Rhizopus oryzae* pulmonary infection. *Antimicrob Agents Chemother* 2010; **54:** 484–90.

98 Terblanche M, Almog Y, Rosenson RS, Smith TS, Hackam DG. Statins and sepsis: multiple modifications at multiple levels. *Lancet Infect Dis* 2007; **7:** 358–68.

99 Roze LV, Linz JE. Lovastatin triggers an apoptosis-like cell death process in the fungus *Mucor racemosus*. *Fungal Genet Biol* 1998; **25:** 119–33.

100 Chamilos G, Lewis RE, Kontoyiannis DP. Lovastatin has significant activity against zygomycetes and interacts synergistically with voriconazole. *Antimicrob Agents Chemother* 2006; **50:** 96–103.

101 Gil-Lamaignere C, Simitsopoulou M, Roilides E, Maloukou A, Winn RM, Walsh TJ. Interferon- gamma and granulocyte-macrophage colony-stimulating factor augment the activity of polymorphonuclear leukocytes against medically important zygomycetes. *J Infect Dis* 2005; **191:** 1180–87.

102 Gonzalez CE, Couriel DR, Walsh TJ. Disseminated zygomycosis in a neutropenic patient: successful treatment with amphotericin B lipid complex and granulocyte colony-stimulating factor. *Clin Infect Dis* 1997; **24:** 192–96.

103 Mousset S, Hermann S, Klein SA, et al. Prophylactic and interventional granulocyte transfusions in patients with haematological malignancies and life-threatening infections during neutropenia. *Ann Hematol* 2005; **84:** 734–41.

104 Tragiannidis A, Groll AH. Hyperbaric oxygen therapy and other adjunctive treatments for zygomycosis. *Clin Microbiol Infect* 2009; **15** (suppl 5)**:** 82–86.

105 Garcia-Diaz JB, Palau L, Pankey GA. Resolution of rhinocerebral zygomycosis associated with adjuvant administration of granulocyte-macrophage colony-stimulating factor. *Clin Infect Dis* 2001; **32:** e145–50.

106 Abzug MJ, Walsh TJ. Interferon-gamma and colony-stimulating factors as adjuvant therapy for refractory fungal infections in children. *Pediatr Infect Dis J* 2004; **23:** 769–73.

107 Rodriguez MM, Calvo E, Marine M, Pastor FJ, Fernandez-Ballart J, Guarro J. Efficacy of liposomal amphotericin B combined with gamma interferon or granulocyte-macrophage colony-stimulating factor for treatment of systemic zygomycosis in mice. *Antimicrob Agents Chemother* 2009; **53:** 3569–71.

108 John BV, Chamilos G, Kontoyiannis DP. Hyperbaric oxygen as an adjunctive treatment for zygomycosis. *Clin Microbiol Infect* 2005; **11:** 515–17.

109 Halloran PF. Immunosuppressive drugs for kidney transplantation. *N Engl J Med* 2004; **351:** 2715–29.

110 Singh N, Heitman J. Antifungal attributes of immunosuppressive agents: new paradigms in management and elucidating the pathophysiologic basis of opportunistic mycoses in organ transplant recipients. *Transplantation* 2004; **77:** 795–800.

111 Steinbach WJ, Reedy JL, Cramer RA Jr, Perfect JR, Heitman J. Harnessing calcineurin as a novel anti-infective agent against invasive fungal infections. *Nat Rev Microbiol* 2007; **5:** 418–30.

112 Kontoyiannis DP, Lewis RE, Alexander BD, et al. Calcineurin inhibitor agents interact synergistically with antifungal agents in vitro against *Cryptococcus neoformans* isolates: correlation with outcome in solid organ transplant recipients with cryptococcosis. *Antimicrob Agents Chemother* 2008; **52:** 735–38.

113 Kontoyiannis DP, Lewis RE, Osherov N, Albert ND, May GS. Combination of caspofungin with inhibitors of the calcineurin pathway attenuates growth in vitro in *Aspergillus* species. *J Antimicrob Chemother* 2003; **51:** 313–16.

114 Singh N, Alexander BD, Lortholary O, et al. *Cryptococcus neoformans* in organ transplant recipients: impact of calcineurin-inhibitor agents on mortality. *J Infect Dis* 2007; **195:** 756–64.

115 Ibrahim AS, Spellberg B, Edwards J Jr. Iron acquisition: a novel perspective on mucormycosis pathogenesis and treatment. *Curr Opin Infect Dis* 2008; **21:** 620–25.

116 Soummer A, Mathonnet A, Scatton O, et al. Failure of deferasirox, an iron chelator agent, combined with antifungals in a case of severe zygomycosis. *Antimicrob Agents Chemother* 2008; **52:** 1585–86.

117 Anon. The Deferasirox-AmBisome Therapy for Mucormycosis (DEFEAT Mucor) Study. Jan 5, 2007. http://clinicaltrials.gov/ct2/show/NCT00419770 (accessed Jan 31, 2011).

118 Falagas ME, Kasiakou SK. Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. *Clin Infect Dis* 2005; **40:** 1333–41.

119 Schwartz SN, Medoff G, Kobayashi GS, Kwan CN, Schlessinger D. Antifungal properties of polymyxin B and its potentiation of tetracycline as an antifungal agent. *Antimicrob Agents Chemother* 1972; **2:** 36–40.

120 Tleyjeh IM, Kashour T, Hakim FA, et al. Statins for the prevention and treatment of infections: a systematic review and meta-analysis. *Arch Intern Med* 2009; **169:** 1658–67.

121 Sun HY, Singh N. Antimicrobial and immunomodulatory attributes of statins: relevance in solid-organ transplant recipients. *Clin Infect Dis* 2009; **48:** 745–55.

122 Lukacs G, Papp T, Nyilasi I, Nagy E, Vagvolgyi C. Differentiation of *Rhizomucor* species on the basis of their different sensitivities to lovastatin. *J Clin Microbiol* 2004; **42:** 5400–02.

123 Galgoczy L, Papp T, Lukacs G, Leiter E, Pocsi I, Vagvolgyi C. Interactions between statins and *Penicillium chrysogenum* antifungal protein (PAF) to inhibit the germination of sporangiospores of different sensitive zygomycetes. *FEMS Microbiol Lett* 2007; **270:** 109–15.

124 Broady R, Levings MK. Graft-versus-host disease: suppression by statins. *Nat Med* 2008; **14:** 1155–56.

125 Simitsopoulou M, Roilides E, Maloukou A, Gil-Lamaignere C, Walsh TJ. Interaction of amphotericin B lipid formulations and triazoles with human polymorphonuclear leucocytes for antifungal activity against zygomycetes. *Mycoses* 2008; **51:** 147–54.

126 Safdar A. Antifungal immunity and adjuvant cytokine immune enhancement in cancer patients with invasive fungal infections. *Clin Microbiol Infect* 2007; **13:** 1–4.

127 Segal E, Menhusen MJ, Shawn S. Hyperbaric oxygen in the treatment of invasive fungal infections: a single-center experience. *Isr Med Assoc J* 2007; **9:** 355–57.

128 Bitterman H. Hyperbaric oxygen for invasive fungal infections. *Isr Med Assoc J* 2007; **9:** 387–88.

129 Spellberg B, Edwards J Jr, Ibrahim A. Novel perspectives on mucormycosis: pathophysiology, presentation, and management. *Clin Microbiol Rev* 2005; **18:** 556–69.

130 Ibrahim AS, Spellberg B, Avanessian V, Fu Y, Edwards JE Jr. *Rhizopus oryzae* adheres to, is phagocytosed by, and damages endothelial cells in vitro. *Infect Immun* 2005; **73:** 778–83.

131 Kontoyiannis DP, Wessel VC, Bodey GP, Rolston KV. Zygomycosis in the 1990s in a tertiary-care cancer center. *Clin Infect Dis* 2000; **30:** 851–56.

132 Lee FY, Mossad SB, Adal KA. Pulmonary mucormycosis: the last 30 years. *Arch Intern Med* 1999; **159:** 1301–09.

133 Rapidis AD. Orbitomaxillary mucormycosis (zygomycosis) and the surgical approach to treatment: perspectives from a maxillofacial surgeon. *Clin Microbiol Infect* 2009; **15** (suppl 5)**:** 98–102.

134 Skiada A, Petrikkos G. Cutaneous zygomycosis. *Clin Microbiol Infect* 2009; **15** (suppl 5)**:** 41–45.

135 Ruping MJ, Vehreschild JJ, Cornely OA. Patients at high risk of invasive fungal infections: when and how to treat. *Drugs* 2008; **68:** 1941–62.

136 Ruping MJ, Vehreschild JJ, Cornely OA. Antifungal treatment strategies in high risk patients. *Mycoses* 2008; **51** (suppl 2)**:** 46–51.

137 Leventakos K, Lewis RE, Kontoyiannis DP. Fungal infections in leukemia patients: how do we prevent and treat them? *Clin Infect Dis* 2010; **50:** 405–15.