

An insight into the antifungal pipeline: selected new molecules and beyond

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Abstract | Invasive fungal infections are increasing in incidence and are associated with substantial mortality. Improved diagnostics and the availability of new antifungals have revolutionized the field of medical mycology in the past decades. This Review focuses on recent developments in the antifungal pipeline, concentrating on promising candidates such as new azoles, polyenes and echinocandins, as well as agents such as nikkomycin Z and the sordarins. Developments in vaccines and antibody-based immunotherapy are also discussed. Few therapeutic products are currently in active development, and progression of therapeutic agents with fungus-specific mechanisms of action is of key importance.

Invasive fungal infections have transitioned from a rare curiosity to an everyday problem for the practising physician. Invasive candidiasis is the third to fourth most common bloodstream infection in surveys in the United States. Similar trends have been reported in several regions throughout the world, although the incidence by country can vary dramatically^{1–4}. Rates of invasive aspergillosis and mucormycosis continue to increase in parallel with the growth of immunocompromised patient populations^{5–9}. Invasive fungal infections are associated with substantial morbidity and mortality, despite optimal antifungal therapy, and carry a high financial burden. Improved diagnostics, new epidemiological studies and the availability of new antifungals have revolutionized the field of medical mycology in the past few decades.

Although systemic antifungals have been available since the 1950s, the early antifungal pipeline was characterized by compounds with limited efficacy owing to toxicity, such as nystatin and amphotericin B¹⁰, and by the lack of substantial developments for decades. The 1980s saw the development of the triazoles, which revolutionized medical mycology as these compounds were available both in intravenous and oral formulations, and were effective against fungal pathogens that were refractory to the polyenes (FIG. 1). The 1990s were characterized by further triazole development and by the reformulation of amphotericin B with lipid compounds. These lipid-based preparations were considerably less toxic and allowed the true efficacy of amphotericin B to be explored, expanding the pharmacokinetic and pharmacodynamic limits of the agents¹⁰. The new millennium brought the echinocandins to the market, which are the

newest class of antifungals. They are characterized by their inhibition of the synthesis of (1,3)- β -D-glucan (a key component of many fungal cell walls) and are therefore the first class of antifungal agents that act against a specific component of the fungal organisms and not mammalian cells^{11–13}. Their safety profile is remarkable, setting the bar for new antifungals that are under development. The mechanism of action of these agents, along with that of other antifungals currently in development, is illustrated in FIG. 2.

Antifungal research and development is challenging. Apart from the fungal cell wall, fungi are metabolically similar to mammalian cells and therefore offer few pathogen-specific targets. Clinical development is hampered by challenges in timely and definitive diagnosis of the less common or more resistant fungi. Despite these issues, some novel products are progressing in development. This Review focuses on the most recent developments in the antifungal pipeline, concentrating on the most promising candidates that are nearing or currently in clinical development.

New and small molecules

Azoles. The imidazole and triazole antifungal class has been the most successful in terms of numbers of different agents that have entered clinical use. However, most of the antifungal imidazoles are formulated only for topical use, usually because of toxicity or bioavailability problems that limit their potential as systemic agents. Agents that are licensed for clinical use in invasive fungal disease are all triazoles: fluconazole, itraconazole, posaconazole and voriconazole.

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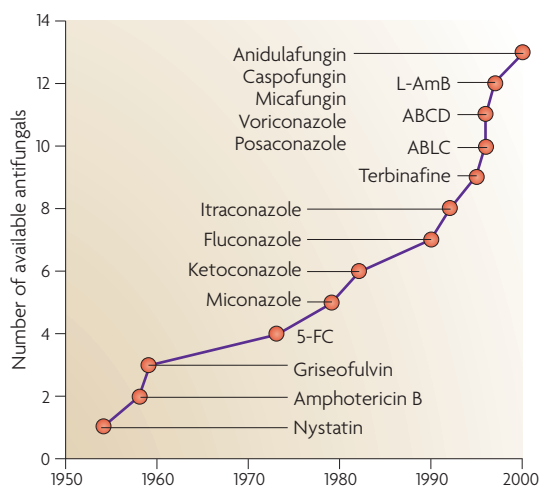


Figure 1 | **Timeline of systemic antifungals.** The graph shows the name, number and rate of development of available systemic antifungal agents from the 1950s to the 2000s. ABCD, amphotericin B colloidal dispersion; ABLCL, amphotericin B lipid complex; 5-FC, flucytosine; L-AmB, liposomal amphotericin B.

Posaconazole and voriconazole represent specific advances in the understanding of structure–activity relationships for antifungal azoles, as evidenced by their progression over the past 30 years (FIG. 3). A triazole-based pharmacophore has replaced the earlier imidazole pharmacophore in systemically active azoles because the triazole group enhances the specificity for fungal cytochrome P450 (Erg11) targets and slows metabolism of the agents *in vivo*¹⁴. In a fluconazole-based structural type, the extra methyl group at carbon atom number 3 enhances hydrophobic interactions at the Erg11 active site and extends the antifungal spectrum¹⁵. A 2,4-difluorophenyl substituent rather than a dichlorophenyl at carbon atom number 2 has become standard for triazole agents. Replacement of the 1,3-dioxolane moiety in ketoconazole and itraconazole with a furan ring, as in posaconazole, alters and enhances activity, as extensively demonstrated in mouse models of fungal disease¹⁶.

Triazole agents in the development pipeline benefit from these optimized structural features. Albaconazole (UR-9825) (FIG. 3) is currently under development by Stiefel under licence from its discoverers, Uriach. The orally active agent has shown efficacy in animal models of infections caused by *Aspergillus*, *Candida*, *Cryptococcus* and *Scedosporium* spp.¹⁷. However, its most recent positioning in a Phase II trial in vulvovaginal candidiasis suggests it may no longer be under development for invasive fungal disease. An intravenous formulation of albaconazole does not seem to be available. Ravuconazole (BMS-207147) (FIG. 3), which was originally discovered by Eisai, underwent considerable development by Bristol–Myers Squibb as an oral broad-spectrum triazole for invasive mycoses. However, it no longer seems to be under active development and an intravenous formulation has not been developed. The ravuconazole isomer isavuconazole (BAL-4815) (FIG. 3) is

undergoing development in the form of the orally active, water-soluble prodrug BAL-8557, which is amenable to intravenous formulation¹⁸. Isavuconazole is currently in Phase III clinical trials.

Many new antifungal triazole molecules are disclosed in primary research papers and patents, but only the compounds listed above meet the definition of the antifungal pipeline used in this article — namely, promising candidates that are nearing or currently in clinical development. It is too early to know whether any of the triazoles mentioned will show genuine improvements over existing, licensed triazoles. New triazoles could offer advantages by extending the spectrum of activity to include rare but difficult-to-treat invasive mycoses (infections caused by *Fusarium* spp., *Scedosporium* spp. or the Zygomycota) or by improving the drug–drug interaction profile. In addition, new triazoles might have a pharmacokinetic profile that would substantially reduce dosing frequency and/or a more favourable adverse effects profile. However, none of the triazoles in the pipeline has yet clearly shown such advantages, mainly because of the paucity of clinical data.

Polyenes. The broad-spectrum polyene antibiotic amphotericin B became the early mainstay for the treatment of many types of invasive fungal infection. Amphotericin B binds strongly to ergosterol, the principal sterol in fungal membranes. Aggregates of amphotericin B–ergosterol complexes form membrane pores, leading to leakage of intracellular constituents¹⁹. The differential binding affinity of amphotericin B for ergosterol over cholesterol, the mammalian membrane sterol, accounts for its selective toxicity. However, amphotericin B can affect mammalian cells, and nephrotoxicity is a common sequela of clinical usage²⁰. The introduction of lipid-complexed amphotericin B formulations considerably reduced the incidence of renal toxicity from amphotericin B. Two lipid products — a liposomal formulation and a lipid complex — are now commonly used to treat a wide range of life-threatening mycoses.

Although numerous new polyenes, modifications of existing polyenes and novel polyene formulations have been reported, only two meet the criteria for inclusion in this Review. A cochleate formulation of amphotericin B has shown efficacy in experimental models of candidiasis and aspergillosis^{21–23}. This formulation entraps the amphotericin B molecules in a large, stable, spirally rolled lipid bilayer. The formulation promises oral bioavailability of amphotericin B, but to date the only pharmacokinetic study involves intravenous injection in mice²⁴. The current development status is unclear: the cochleate formulation is a product of BioDelivery Sciences International, who currently list the product as being in preclinical development. A liposomal formulation of the tetraene nystatin has undergone extensive testing preclinically and in clinical trials²⁵. However, the results of Phase III clinical trials for treatment of fever in neutropenia and in cryptococcal meningitis have not been reported in detail or published in the peer-reviewed literature. The developmental status of liposomal nystatin is therefore unknown.

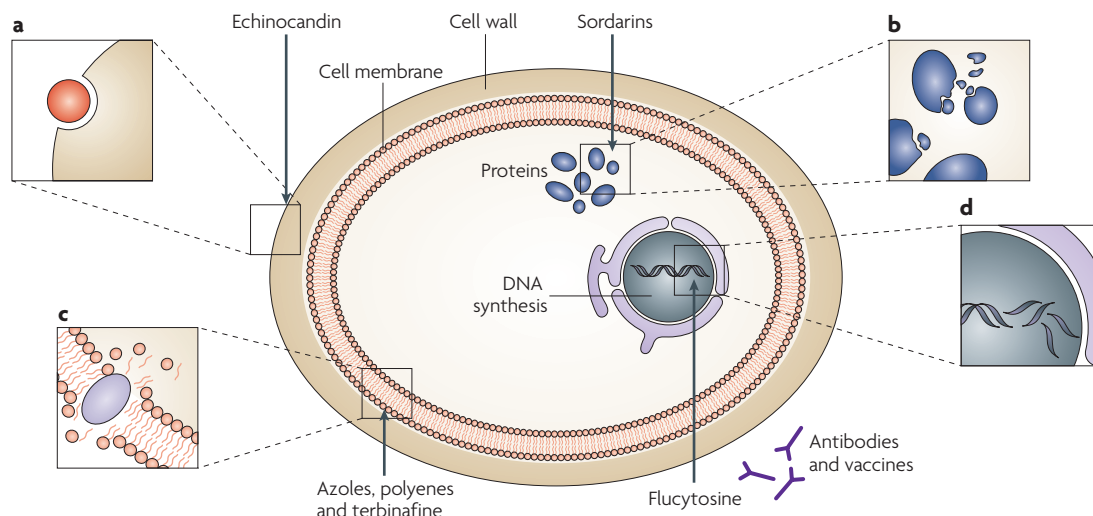


Figure 2 | Mechanisms of action of selected antifungals. An illustration of the mechanisms of action of currently available antifungals as well as selected antifungals under development. **a** | Echinocandins and nikkomycin Z inhibit the formation of the fungal cell wall. **b** | Sordarins interfere with protein assembly. **c** | Azoles, polyenes and terbinafine disrupt the fungal cell membrane. **d** | Flucytosine interferes with DNA synthesis. Antibodies and vaccines prevent fungal infection or block and/or destroy the fungal cells.

Sordarins. Sordarins are a class of semi-synthetic natural products that were shown to have antifungal activity in the early 1970s²⁶. They generated considerable interest when several types of novel sordarin derivatives were developed preclinically in the 1990s by Glaxo–Wellcome and Merck²⁷. The compounds were found to inhibit a novel target for antifungal agents: elongation factor 2 in protein biosynthesis^{28,29}. A number of molecules based on the sordarin pharmacophore were shown to have therapeutic efficacy in various animal models of fungal disease²⁷ but no obvious candidate for clinical development has yet emerged, and the frequency of publications on sordarins has greatly reduced since 2002. The most recent company to show interest in sordarin antifungal agents is Sankyo, whose sordarin R-135853 has shown *in vivo* efficacy in various experimental models of candidiasis³⁰. FR290581, a sordarin derivative that is under development, has shown excellent antifungal activity against *Candida albicans*³¹.

Echinocandins. The echinocandin class is new enough that researchers are still carrying out basic *in vivo* and *in vitro* modelling, along with Phase III and Phase IV clinical trials on the three echinocandins in the market: caspofungin, anidulafungin and micafungin. Development of new agents in this class is slow, and there is only one candidate in early preclinical development: aminocandin (IP960/HMR3270). This agent has shown good *in vivo* and *in vitro* activity against *Candida* spp. and filamentous fungi^{32–34}.

Nanoparticle preparations. Nanoparticle formulation technology has revolutionized drug delivery and release. As some antifungals have historically been hampered by toxicity, availability and solubility issues, nanoparticle formulations have been developed and are

currently under preclinical investigation. These include various nanoparticle formulations of amphotericin B³⁵ that have shown good *in vitro* and *in vivo* activity with decreased toxicity, even by lipid-formulation standards. Nanoparticle formulations of itraconazole have also been developed and are currently under investigation^{36,37}. Other uses of nanoparticle technology in mycology include the formulations of common antiseptics, such as silver, for topical use³⁸.

Nikkomycin Z. Nikkomycin Z was first identified by Bayer Pharmaceutical Company in the 1970s. However, its development has only recently been renewed. The mode of action of nikkomycin Z is to competitively inhibit chitin synthases and thereby interfere with fungal cell wall construction³⁹. As mammalian hosts do not possess this target, nikkomycin Z is potentially pathogen selective. This is supported by data from preclinical toxicology studies.

Nikkomycin Z given orally to mice after receiving an otherwise lethal infection with *Coccidioides* spp. by the pulmonary route resulted in complete survival. Furthermore, 2 days after infection, pulmonary fungal burdens were reduced by 6-log⁴⁰. These and other observations led a small pharmaceutical company (Shaman Pharmaceuticals) to license nikkomycin Z for development. Shaman filed an investigational new drug (IND) application with the US Food and Drug Administration (FDA), and manufactured sufficient nikkomycin Z suitable for human use to complete a single-dose Phase I study, which confirmed the drug's safety⁴¹. Although Shaman Pharmaceuticals closed down, the sponsorship of the IND and approximately 2.5 kg nikkomycin Z that had not been used in the initial clinical safety trial were acquired by a foundation associated with the California State University at

Bakersfield. In May 2005, the foundation transferred those holdings to the Valley Fever Center for Excellence at the University of Arizona.

The existing supply of nikkomycin Z was found to be suitable for continued clinical use and was used to conduct a Phase I multi-dose safety trial, with subjects receiving 250 mg twice daily to 750 mg three times daily for 14 days. All subjects have completed drug administration, and preliminary evaluation has not identified any safety concerns. Full safety analysis and pharmacokinetics should be available in 2010. Other work that is currently underway by the University of Arizona and a small start-up company (Valley Fever Solutions) is directed at more fully defining the pharmacokinetics and pharmacodynamics in experimental murine infections. This will assist in clinical trial design and the development of a new, less expensive manufacturing process for use in future clinical trials. These studies have been supported by awards from the US National Institutes of Health (NIH), the FDA Office of Orphan Drug Development and the JT Tai and Company Foundation. A US\$3 million small-business grant has recently been awarded by the NIH to support further drug manufacturing and a Phase II clinical trial in patients with early coccidioidal pneumonia.

Antibodies

The rationale for developing antibody therapy derives from the limited effectiveness of antifungal drugs in curing certain fungal diseases⁴². Most individuals who suffer from systemic mycosis have impaired immunity that makes them susceptible to fungal diseases. In the absence of effective immunity, it is difficult for drug therapy to eradicate the infection. Given that antibody therapy has been used for over a century and has a long history of efficacy against infectious diseases, the antibody option seems logical with the caveat that such therapies are expensive and face hurdles in development⁴³.

Historically, humoral immunity was thought to have little or no role in host defence against fungi⁴⁴. The basis of this assumption was the difficulty in demonstrating antibody efficacy in passive-protection experiments and the lack of association between serum antibody and immunity to disease⁴⁴. However, when investigators began to evaluate individual monoclonal antibodies (mAbs) to fungal antigens, it became clear that certain antibodies conferred protection. Protective mAbs have now been described to *Cryptococcus neoformans*^{45–47}, *C. albicans*^{48,49}, *Histoplasma capsulatum*⁵⁰, *Aspergillus fumigatus*⁵¹, *Paracoccidioides brasiliensis*⁵² and *Sporothrix shenkii*⁵³. Studies with mAbs also revealed the existence of both protective and non-protective antibodies to fungal pathogens, which provided a possible explanation for the inconclusive results in passive-antibody experiments with immune sera. The existence of protective and non-protective antibodies suggests that immune serum preparations could be difficult reagents to develop for therapeutic use unless the immune response can be focused on the generation of protective antibodies. Consequently, it is likely that antibody-based antifungal therapeutics will use mAbs or their derivatives. Consistent with this trend, a mAb that is specific for

*C. neoformans*⁵⁴ and a mAb fragment that is specific for a heat shock protein of *C. albicans*⁵⁵ have been evaluated clinically in recent years. Furthermore, several studies have shown that adjunctive antibody therapy can enhance the efficacy of antifungal therapy in animal models of infection, providing a rationale for the use of combination therapy^{50,56–58}. The availability of protective mAbs, combined with the continuing difficulty in treating fungal diseases with conventional antifungal therapy, has heightened interest in the use of adjunctive antibody therapy.

Targets for protective antibodies in medically important fungi.

Given that fungal surfaces are highly complex, there are numerous antigens that can elicit antibody responses, and protective antibodies have been described that target polysaccharide, protein, lipid and melanin antigens. For example, in the case of *C. neoformans*, antibodies to the polysaccharide capsule^{46,47,59}, cell wall lipids⁶⁰ and melanin⁶¹ confer protection in animal models. For *C. albicans*, antibodies to cell wall polysaccharides⁴⁸ and cell surface proteins are protective⁶². Analysis of protective antibodies has established that there are numerous targets in each fungal pathogen against which antibody therapy can be developed. Although most protective antibodies have been pathogen-specific, there are three examples of mAbs that are protective across species, raising the possibility that broad spectrum antibody therapy could be developed for fungal diseases. First, an anti-idiotypic antibody to an antibody that binds killer toxin is active against various fungi that express the killer toxin receptor⁶³. Second, an antibody fragment to heat shock protein, efungumab, is active against several *Candida* spp.⁶⁴ and *C. neoformans*⁶⁵. Third, antibodies to laminarin are protective against *C. albicans*, *C. neoformans* and *A. fumigatus*⁶⁶. Furthermore, it is likely that only a small fraction of antigens that elicit protective antibodies have been identified. Target identification remains an empirical, laborious process that involves the generation of mAbs and their subsequent testing in animal models to identify those that can modify the course of infection. The fact that there are numerous targets for antibody-mediated immunity implies that cocktails of protective mAbs could be designed for more effective therapy.

Mechanisms of antibody action against fungi.

The classical mechanisms of antibody action are opsonization, complement activation, toxin neutralization and antibody-dependent cellular cytotoxicity. Opsonization and complement activation can enhance host defences by promoting fungal cell killing by phagocytic cells and cellular recruitment, respectively. Recent studies suggest additional mechanisms by which therapeutic antibodies can help host defence. For *C. neoformans*, antibody-mediated protection is associated with changes in the inflammatory response that are thought to reduce host-mediated damage⁶⁷. Furthermore, it is increasingly apparent that some antibodies to fungal antigens have direct antimicrobial properties. For *C. albicans*, certain antibodies to cell wall antigens affect germ tube formation and morphogenesis⁴⁹. These antibodies could

Anti-idiotypic antibody

An antibody that is directed against the antigen-specific binding site of an immunoglobulin or a T-cell receptor and therefore may compete with antigen for binding.

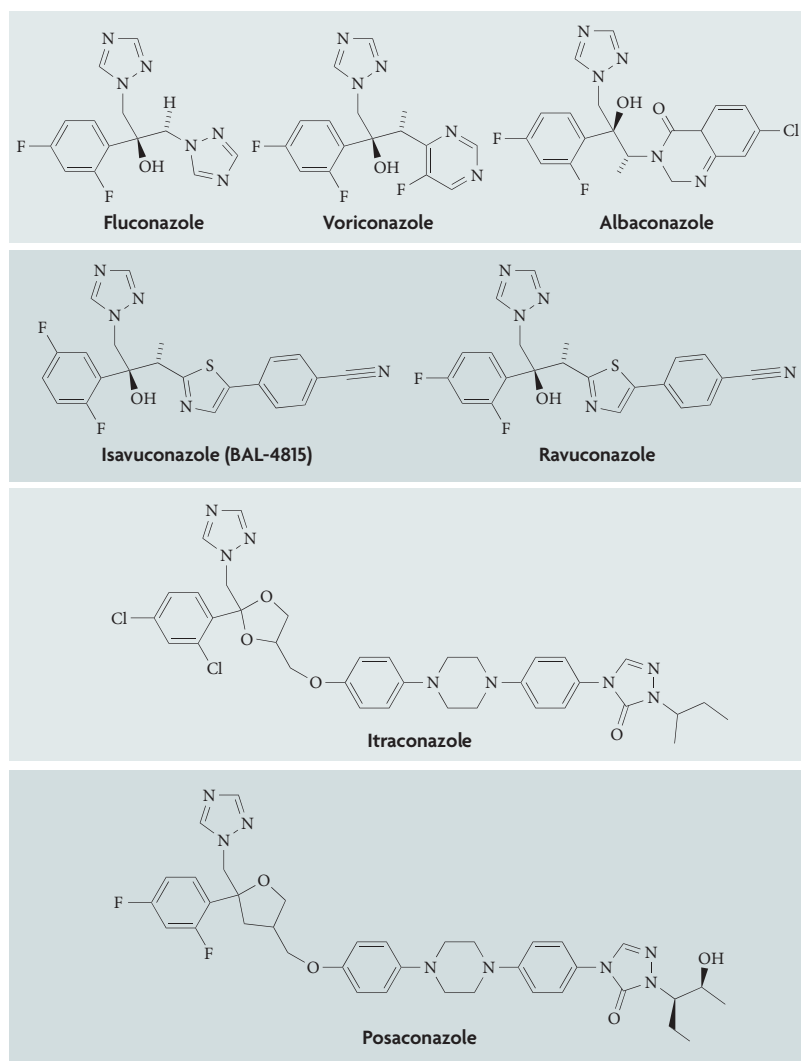


Figure 3 | Structures of triazoles. The chemical structures of selected triazoles. Fluconazole, voriconazole, itraconazole and posaconazole are currently available, albaconazole (UR-9825) and isavuconazole (BAL-4815) are under development, whereas ravuconazole is no longer under active development.

contribute to host defence by interfering with yeast to hyphal cell transition. Similarly, antibodies to melanin have been shown to mediate fungistatic effects on fungal cells⁶¹. Antibody-mediated immunity can therefore contribute to host defence by enhancing the effectiveness of innate immunity through opsonization and complement activation, and by direct antimicrobial effects on fungal cells. The mechanism of action is an important consideration when designing combination therapy with antifungal agents. For example, if the antibody acts by potentiating the efficacy of innate immune effector cells, antifungal agents that stimulate the immune system, such as amphotericin B, could produce synergistic effects. Conversely, if the antibody has a direct antimicrobial role, it is important to consider the effect of antifungal therapy on antigen expression, as drug effects that damage the cell wall and/or reduce antigen expression could lead to synergistic or antagonistic effects.

Hyphal cell
A complex filamentous structure typically formed by moulds.

Clinical experience with antibody therapy. In the early 1960s, three patients with cryptococcal meningitis were treated with rabbit immune sera, and treatment was accompanied by reduction in serum antigen levels⁶⁸. More recently, a mouse immunoglobulin G1 (IgG1) antibody to capsular glucuronoxylomannan in *C. neoformans* was evaluated in a Phase I–II trial in patients with AIDS-related cryptococcal meningitis⁵⁴. This study showed that mAb administration was safe and resulted in transient reductions in serum antigen when administered at high doses. However, intravenous administration of the murine IgG1 did not result in substantial penetration of immunoglobulin into the cerebrospinal fluid despite the presence of chronic meningitis. Further clinical development of this mAb is currently on hold owing to a lack of industrial support.

Recent work (discussed below) has suggested that more effective therapeutics could be developed by incorporation of radionuclides into the immunoglobulin molecule⁶⁹. The other major effort to develop antibody-based therapy for a fungal disease involves efungumab, a recombinant antibody fragment to the heat shock protein of *C. albicans*, which has progressed through a Phase III clinical trial⁵⁵. Although this trial suggested a potential advantage of the combination of efungumab with liposomal amphotericin B relative to liposomal amphotericin B alone, low response rates in the amphotericin B arm have caused controversy. However, if this result is validated by subsequent studies, it will provide a strong impetus for the development and use of adjunctive antibody therapy against selected fungal diseases. Efungumab was acquired by Novartis but European licensing has been delayed. At the time of writing it remains in the company's development pipeline.

New options for antibody-mediated antifungal therapy. Although the overwhelming majority of preclinical and clinical experience has been with 'naked' antibodies, it is possible to enhance the antimicrobial activity of specific antibodies by linking them to radionuclides. The concept of radio-immunotherapy emerged from research in oncology, in which radiolabelled antibodies are already in clinical use for the treatment of tumours and the identification of metastatic lesions. The proof of principle that radio-immunotherapy could be effective against an experimental infectious diseases was shown using mAbs to *C. neoformans* and *H. capsulatum*^{69–71}. Radio-immunotherapy was used to treat experimental cryptococcal infection in mice and had minimal side effects^{69–71}. In theory, all that is required for radio-immunotherapy is the availability of an antibody with specificity for the pathogenic microbe and nuclides for conjugation. Hence, this option dispenses with the need to identify protective antibodies and has the potential to allow rapid development of antibody therapeutics.

Advantages, hurdles and promises of antibody-based therapies. Antibody-based therapies have a long record of established efficacy and safety against infectious diseases. Antibodies are natural products of the immune system that can enhance the antimicrobial function of

other immune system components. There are no conceptual limitations for the development of antibody-based therapy against fungal pathogens. With sufficient efforts, antibody therapeutics could be developed against each of the major fungal pathogens. They are therefore an attractive therapeutic option for fungal diseases because they can enhance host immune function in individuals in whom impaired immunity is directly responsible for susceptibility to mycotic infections.

However, the development of antibody-based therapies remains challenging. Given that antifungal drugs are available, the clinical evaluation of any antibody-based therapy has to involve their use as adjunctive therapy, and establishing efficacy would require showing that combination therapy is superior to conventional therapy. For fungal diseases for which existing therapy provides a reasonable benefit, demonstrating the added value of antibody-based therapy could require large clinical trials with their associated logistics and expenses. Immunoglobulins are perishable reagents that require expensive processes for manufacture, purification and storage. Conversely, antibody-based therapies provide new and versatile options for developing new antifungal therapeutics, and it is hoped that immunoglobulins will become part of the antifungal armamentarium in future.

Vaccines

The scientific appeal of preventing fungal infections by vaccination is most obvious for endemic dimorphic fungi (*Blastomyces dermatitidis*, *Coccidioides* spp., *H. capsulatum* and *P. brasiliensis*) as exposures to these pathogens usually results in long-term immunity. For other fungal pathogens that cause invasive infections in the setting of immunosuppression and other deficiencies in normal host defences (*Candida* spp., *Aspergillus* spp. and *C. neoformans*), it might be possible to use intact immunological responses to prevent disease. Recent approaches to use humoral immunity to provide resistance are described in detail in the previous section. Here we focus on two programmes — one against candidiasis and the other against coccidioidomycosis — that have developed preventative vaccine candidates for potential clinical trials. More general reviews can be found in several recent publications^{72–76}.

A vaccine to prevent candidaemia. Sepsis due to *Candida* spp. is a worldwide problem. In the United States, *Candida* spp. are the fourth most common cause of nosocomial bloodstream infections, and approximately half of these are due to a single species, *C. albicans*. There are numerous factors that predispose an individual to candidal infection⁷⁷ but none of them is likely to interfere with immunological sensitization, making an effective vaccine feasible.

Vaccination of mice with the *Candida* spp. adhesin agglutinin-like ALS1 protein (*Als1*) and *Als3* results in protection from lethal candidiasis⁷⁸. Expression of the *ALS1* gene in *S. cerevisiae* was shown to mediate fungal adherence to endothelial cells⁷⁹. Further analysis by site-directed mutagenesis supported a structural model of the recombinant *Als1* in which the amino-terminal region

of 256 amino acids (amino acids 29–285) was associated with endothelial attachment, a middle region of tandem repeats of which at least 5 were needed to support adhesion, and a glycosylphosphatidylinositol anchor⁸⁰. A vaccine composed of an *S. cerevisiae*-expressed recombinant protein derived from *Als1* (amino acids 17–432) and Freund's adjuvant, when administered peritoneally to mice, resulted in modest but statistically significant prolongation of survival following a lethal infection with *C. albicans*⁸¹. Further studies with a similar N-terminal recombinant protein (amino acids 19–433), administered as a subcutaneous vaccine, produced greater prolongation of survival and reduction of fungal burden in both immunocompetent and neutropenic mice⁷⁸. Additional studies showed that this vaccine could also confer protection against infections produced by three different strains of *C. albicans*⁸². Comparable protection has been obtained with the homologous N-terminal fragment (amino acids 17–432) from *Als3*, which also conferred greater reduction of colony-forming units from mucosal invasion than the *Als1* vaccine⁸³. Based on these studies of *Als1* and *Als3*, patent applications have been filed covering the *Als* gene family as vaccines for *Candida* spp. With higher doses of *Als1* and *Als3*, Freund's adjuvant could be substituted with alum without compromising protection⁸⁴. This finding provided a potential pathway for a formulation that is suitable for clinical trials.

Although not aimed at candidaemia, a Phase I clinical trial has been announced by Pevion Biotech for a vaccine based on an inactive form of the *C. albicans* virulence enzyme candidapepsin 2 (also known as Sap2) for the prevention of chronic or recurrent vaginitis (A. Cassone, personal communication and Poster 195A, 10th ASM Candida and Candidosis meeting, Miami, USA, 2010).

A vaccine to prevent coccidioidomycosis. Approximately 150,000 people in the United States are infected with *Coccidioides* spp. each year, mostly in California and Arizona. Of these infections, 50,000 lead to clinical illnesses that warrant medical attention. Most illnesses manifest as pneumonia and often last for weeks or months. In the endemic regions, coccidioidal infections account for nearly one-third of all community-acquired pneumonias⁸⁵. A small percentage of infections become chronic and disabling, and require years of or life-long antifungal medication and medical care⁸⁶. Approximately 100 deaths per year are attributed to coccidioidomycosis (Arizona Department of Health Services and REF.87), and recent increases in disease activity further accentuate the impact of this disease⁸⁸.

Because so many people develop a durable immunity following infection, the complete prevention of disease through vaccination has been a goal for numerous investigators over the past half century^{89–92}. Most research during this time centred upon a whole-cell formalin-killed vaccine prepared from mature spherules, the parasitic phase of *Coccidioides* spp. However, experimental protection required repeated doses of approximately 1 mg per dose, which produced a marked inflammatory reaction at the injection site^{93,94}. Moreover, a clinical trial of this vaccine to prevent clinical illness failed to

Dimorphic fungi

Fungi that can exist as both the mould or yeast forms, mainly depending on environmental conditions.

demonstrate significant protection⁹⁵. Subsequent work has therefore focused primarily upon recombinant antigen vaccines.

For the past decade, several research groups have worked collaboratively on the Valley Fever Vaccine Project, with support from many California-based sources and administered by the California State University at Bakersfield Foundation⁹⁶. Two of the most promising recombinant antigens identified to date are a proline-rich cell wall protein identified as antigen 2/PRA⁹⁷ (Ag2/PRA) and Coccidioides-specific antigen (CSA)⁹⁸. Ag2/PRA is heavily glycosylated on the carboxy-terminal half of the molecule but virtually all of the protective response to vaccination seems to reside in the N-terminal 106 amino acids (Ag2/PRA₁₋₁₀₆)⁹⁹. Recently, Ag2/PRA₁₋₁₀₆ and CSA were found to produce greater protection as a vaccine when used in combination than when used separately¹⁰⁰. Prolongation of survival for over 8 weeks after vaccination with the combination of Ag2/PRA₁₋₁₀₆ and CSA proteins approached 100% in mice receiving intranasal infection with 50–153 spores, which was otherwise lethal in nearly all animals within 2–3 weeks. Of additional interest, expression of the two antigens as a single chimeric fusion protein vaccine (Ag2/PRA₁₋₁₀₆-CSA) provided equivalent protection to a vaccine in which the two separate proteins were administered as a mixture. This observation has a practical implication for vaccine development as production of a single recombinant protein would probably be less expensive than producing two antigens separately. Based upon these and other results, the Ag2/PRA₁₋₁₀₆-CSA vaccine antigen candidate was awarded a patent in 2006.

Translating discovery into clinical trials

The Als candidal vaccine candidate has been licensed to NovaDigm Therapeutics. Currently, development is focused on using the recombinant N terminus of Als3p (rAls3p-N), formulated in alum as an adjuvant. The rAls3p-N vaccine is entering good manufacturing practice (GMP) evaluation, with subsequent preclinical toxicity studies planned. A Phase I clinical trial protocol has been developed and presented to the FDA in a PREIND meeting in preparation for future clinical studies, which are predicted to begin within the next year.

The pan-fungal laminarin-CRM 197 vaccine^{66,73} has been licensed to Novartis, with a GMP formulation of this vaccine ready to start clinical development (A. Cassone, personal communication).

By contrast, moving the coccidioidomycosis vaccine forward has been slowed by technical issues relating to formulation. These include scaling up the manufacturing process, protein solubility, and adjuvant availability and selection¹⁰¹. The development programme is further challenged by the small market for a coccidioidal vaccine. A cost-benefit analysis for a vaccine to prevent coccidioidomycosis showed a possible net benefit once the vaccine was available for clinical use¹⁰². However, the model did not take into account development costs. Costs to date administered through the California State University at Bakersfield for the Valley Fever Vaccine Project are more than \$15 million. However, manufacturing the current coccidioidal vaccine candidate in a formulation that is suitable for clinical trials is likely to require 2–3 times that amount in additional funding. In Arizona, the 2007 hospital costs alone for managing patients with coccidioidomycosis were \$86 million¹⁰³, underscoring the clear public health value of developing a vaccine to prevent this disease. The challenge now is to find ways to allocate the required funds to complete development and realize the public health benefit.

Conclusion

We have reviewed selected new small molecules with antifungal properties under development, as well as important advances in immunotherapy and prevention. There is an increasing need for antifungals due to the growth of susceptible populations, limitations of the activity spectrum or tolerability of current antifungals, and the development of antifungal resistance. However, the challenges of discovery and development for antifungal agents have made the pipeline less robust than that of other therapeutic areas such as bacteriology or virology. Prophylactic vaccines are intriguing but only offer protection against selected organisms. Few therapeutic products are currently in active development, and progression of therapeutic agents with fungus-specific mechanisms of action is of the highest priority.

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Competing interests statement

The authors declare **competing financial interests**: see web version for details.

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