



PERSPECTIVES

INFECTIOUS DISEASE

How to bolster the antifungal pipeline

Few drugs are coming to market, but opportunities for drug development exist

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About 1.2 billion people worldwide are estimated to suffer from a fungal disease (1, 2). Most are infections of the skin or mucosa, which respond readily to therapy, but a substantial minority is invasive or chronic and difficult to diagnose and treat. An estimated 1.5 to 2 million people die of a fungal infection each year, surpassing those killed by either malaria or tuberculosis (3). Most of this mortality is caused by species belonging to four genera of fungi: *Aspergillus*, *Candida*, *Cryptococcus*, and *Pneumocystis*. Although great strides were made in the 1990s, drug development has largely stalled since then.



INFECTIOUS DISEASE SERIES

Opportunities exist for accelerating development, particularly in fungal asthma, and to treat chronic and invasive aspergillosis.

Antifungal therapy has become progressively more effective since second-generation azoles, echinocandins, and lipid formulations of amphotericin B were introduced from the 1990s onward (3). These compounds act by inhibiting ergosterol and β -D-1,3 glucan synthesis and perturbing the cell membrane (see the figure). Voriconazole is now the agent of choice for invasive aspergillosis, allowing patients to survive leukemia and transplantation who would otherwise have died (4, 5).

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Photomicrograph of *Aspergillus fumigatus*. This fungus causes life-threatening invasive and chronic aspergillosis as well as driving severe asthma. Although drug treatments exist, mortality remains high.

Nevertheless, ~30 to 50% of invasive aspergillosis patients still die, for reasons that include late diagnosis, infection of sites such as the brain that are not effectively treated with drugs, and drug resistance. The mortality from candidemia, a fungal infection mainly treated with echinocandins and fluconazole, also remains high at ~50%. Since 2006, no new classes of antifungals have been approved. This is problematic because current agents are not sufficiently active, cannot be given orally, carry drug- or class-specific toxicities, or have major drug interactions.

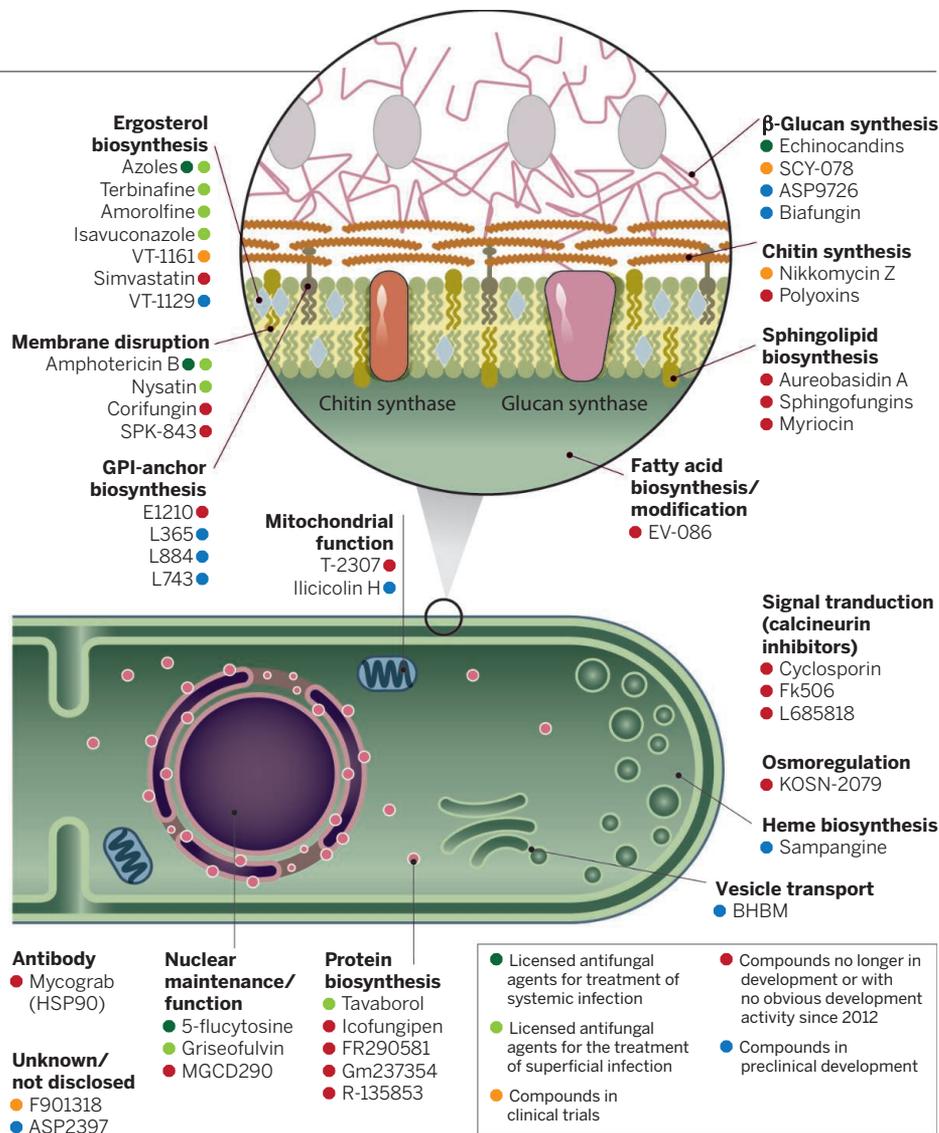
VORICONAZOLE—A CASE STUDY. The development of voriconazole illustrates many of the challenges in developing novel antifungal treatments, particularly for invasive aspergillosis. Scientists at Pfizer synthesized over 1200 azole analogs before selecting voriconazole, which kills over 50% of *Aspergillus* strains. Both intravenous and oral formulations were developed (most antifungals are either intravenous or oral).

In phase 1 studies, blood levels of voriconazole varied widely among subjects. Increased abnormalities in liver function tests were seen at high blood levels. Furthermore, a peculiar visual adverse event of flickering lights or zigzag lines occurred in some subjects shortly after receiving higher doses. This last side effect had a silver lining: It showed that voriconazole entered the central nervous system. It thus correctly anticipated much-improved outcomes with voriconazole for cerebral aspergillosis, previously a uniformly fatal disease.

Phase 2 studies in Europe began in 1993 and laid the foundation for two phase 3 (parallel randomized controlled registration) studies (6). In the phase 3 studies, patients with low or undetectable voriconazole blood concentrations failed therapy, whereas those with very high levels experienced toxicity, mostly resulting in death. Thus, therapeutic drug monitoring is advised in every patient receiving this drug (7).

The phase 3 studies compared intravenous voriconazole with intravenous amphotericin B, followed by lipid amphotericin B or itraconazole (8). Over the course of 3 years from July 1997, 379 patients with invasive aspergillosis from 19 countries and 95 clinical referral centers were enrolled. In the study, voriconazole was shown to be superior to amphotericin B; several later case series confirmed it to lower mortality by 15 to 20% compared to non-azole drugs. Since voriconazole was licensed in 2002, it has been prescribed to millions of patients worldwide.

Despite voriconazole's undoubted benefits, it remains a challenging agent to use clinically, with major variations in exposure and metabolism, notable drug interactions,



Antifungal drugs. With the exception of 5-flucytosine (the use of which is limited because of its narrow spectrum of activity and rapid development of resistance), all agents licensed for treating systemic fungal infection target cellular integrity. The pipeline of antifungal development is sparse, but it is promising that two compounds currently in clinical development are active against novel targets (nikkomycin Z, which targets chitin synthesis, and F901318, the novel target of which remains undisclosed) and that a number of preclinical agents target functions other than cellular integrity. GPI, glycosylphosphatidylinositol.

and appreciable toxicity in many patients. Thirty to 50% of patients with invasive aspergillosis still die despite treatment.

DRUG RESISTANCE IN FUNGI. Many fungi are intrinsically resistant to certain antifungals; notably, *Candida krusei* (to fluconazole), *Aspergillus terreus* (to amphotericin B), *Cryptococcus* spp. (to the echinocandins), and *Scedosporium* spp. (to all current antifungals). About 20 years ago, azole-sensitive *Candida albicans* dominated infections, with other *Candida* species rarely seen. After over two decades of systemic azole usage, this picture has changed. *C. glabrata* is particularly problematic: It is the second-most-commonly isolated *Candida* species in the European Union (EU) (>10%) and United States (>20%) and has high rates of

resistance to fluconazole and voriconazole, as well as (more recently) to echinocandins. Intrinsically resistant mold infections are also being observed more frequently, such as zygomycetes and *Fusarium* spp. Azole- and echinocandin-resistant *C. glabrata* can only be treated with intravenous amphotericin B, which is often toxic and does not penetrate into urine, making some infections untreatable. Zygomycetes only respond to posaconazole and amphotericin B, and there are no drugs for *Scedosporium* (9).

The emergence of azole resistance is a growing problem, particularly in the Netherlands, where azole-resistant *Aspergillus fumigatus* is now commonplace. Unlike bacteria, fungi are not known to transfer resistance genes between them, nor is patient-to-patient transmission common. The

prognosis for individuals infected with a resistant *Aspergillus* strain is poor, with nearly 90% mortality against an expected mortality of ~50% for invasive aspergillosis.

Most resistant *A. fumigatus* strains that have been isolated have a specific mutation in the target of azole action, Cyp51A. Multi-azole-resistant isolates carrying the same mutation have been found in Belgium, Denmark, Germany, the United Kingdom, China, India, Tanzania, Kuwait, and Iran. Agricul-

“In recent years there have been a number of failures in antifungal development...”

tural and other commercial uses of azole fungicides are the likely, but not yet proven, culprit for the emergence of these resistant strains (10). Restriction of azole fungicide use has been proposed but is challenging for multiple reasons, notably, the lack of alternative fungicides for many key crops. Withdrawal of azole agricultural agents would reduce the annual wheat crop value by an estimated €4.6 billion by 2020 in the EU alone (11). Nonetheless, restriction of azole spraying to essential crops (i.e., not fence posts, plasterboard, cut flowers, etc.) may be a sensible first step to curb the problem. Once a novel human antifungal class is developed, fungicides of similar chemical structure should not be approvable, to minimize resistance development.

ASPARSE DISCOVERY PIPELINE. In recent years there have been a number of failures in antifungal development, notably the antibody against HSP90 (heat shock protein 90) Mycograb and the histone deacetylase inhibitor MGCD290, both of which were insufficiently active in patients. Searches of current literature, conference reports, and drug company pipeline reports suggest that only four compounds are in active clinical development for the treatment of systemic disease, with a further two agents expected to enter clinical development in 2015 (12). A few other compounds are in preclinical development, many with modes of action that differ from the currently marketed agents (see the figure); whether they will progress into clinical development remains uncertain.

Difficulties in identifying new broad-spectrum compounds and a chronic lack of investment in novel antifungal agents are both responsible for the limited drug development pipeline. Most major pharmaceutical companies are not investing in antifungals, preferring to focus on other, apparently more

lucrative areas. EU (New Drugs 4 Bad Bugs) and U.S. (BARDA Broad Spectrum Antimicrobials) initiatives have stimulated investment in the development of antibiotics, but no similar initiatives exist for the development of antifungals. Attempts to address the lack of investment include the U.S. Food and Drug Administration's (FDA's) Generating Antibiotics Incentives Now (GAIN) Act, which allows a 5-year extension of market exclusivity for anti-infectives and specifically names *Aspergillus*, *Candida*, and *Cryptococcus* species as qualifying diseases.

In some ways, the challenges to the development of antifungals are more pronounced than those faced by antibacterial development. Because fungi, like mammals, are eukaryotes, many proteins that are potential targets for therapy are also found in humans, with substantial drug toxicity risk. However, there are some advantages to working in eukaryotes, particularly those with a diploid life stage. Chemically induced haploinsufficiency, a functional genomics technology, has been used in *C. albicans* and *Saccharomyces cerevisiae* to identify the mechanism of action of novel antifungal agents, yielding many new drug targets (13).

DEVELOPMENT OPPORTUNITIES. Awareness of the spectrum of fungal diseases continues to grow. For example, *Aspergillus* may have an important and treatable amplifier effect in cystic fibrosis (CF), asthma, and chronic obstructive pulmonary disease (COPD) (14). Severe asthma with fungal sensitization responds to oral antifungal therapy, and with 350,000 asthma deaths annually (1), approved and novel antifungals could play an important part in reducing deaths. Treatment of chronic pulmonary aspergillosis (including aspergilloma) probably reduces death rates, and certainly reduces morbidity (15). No drug development candidates exist yet for these indications, but alternatives to the azoles are urgently needed—partly because of inadequate response rates, partly because of adverse events, and definitively because of resistance.

Cryptococcal meningitis is another important development target. It responds poorly to fluconazole, with >60% mortality at 10 weeks in sub-Saharan Africa; the combination of amphotericin B and flucytosine is better, but not widely available and difficult to administer safely. A new potent agent for cryptococcal meningitis has the potential to save many lives.

OUTLOOK. Despite some effective drug treatments, mortality from fungal infections remains high, and new drugs are urgently needed. A major barrier to improved understanding of fungal diseases

and the development of faster diagnostics and novel therapies is the general lack of capacity in fungal pathogen research (16). Greatly improved diagnostics mean that narrower-spectrum antifungals can now confidently be developed, making the early stages of drug development more straightforward. Alternative development pathways, relying heavily on pharmacodynamic modeling and focused clinical studies, have been proposed for antibacterial agents and should be applied to antifungals (17). Galactomannan *Aspergillus* antigen testing has been recognized as a surrogate marker for clinical studies (18), facilitating clinical development. The FDA has helped by granting new antifungals “orphan status,” lowering clinical trial barriers. As understanding of key risk factors for fungal disease improves, stratification of patient groups will be possible, permitting more targeted clinical trials and ultimately leading to improved treatment regimens for patients. ■

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