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Fighting the rising tide of antifungal resistance: a global challenge

The evolution of antifungal resistance is undermining the armamentarium of antifungal therapeutics and threatens novel agents coming through the pipeline. F2G, an antifungal-focused biopharma company, is addressing the need for medicines to treat fungal infections, which is hard but not insurmountable.

The threat posed to human health by the evolution of antibiotic-resistant bacterial strains is a widely acknowledged global health problem. By comparison, the risks surrounding the emergence of antifungal resistance, and its impact on human health, are much less appreciated, and current approaches to address it are insufficient to tackle this growing problem. F2G is a biotech company that specializes in developing therapies to treat invasive fungal infections through a novel class of antifungal agents called the orotomides.

There are at least 5 million fungal species found in nature, 300 of which can cause disease in humans, with a small subset of 20–25 fungal species being responsible for the majority of observed infections. Invasive fungal diseases (IFDs), while not as common as bacterial infections, still occur in significant numbers of people. Globally, up to 150 million people experience an IFD every year, leading to around 1.5 million deaths. For healthy adults, invasive fungal infections are relatively unlikely to occur. However, fungal infections are a serious concern for immunocompromised individuals, and in this patient population, infection with certain fungal species has a mortality risk of at least 50%—some fungal infections are resistant to all available antifungals.

Many drugs, such as monoclonal antibodies and especially chemotherapeutics, can affect immune system function and compromise immune surveillance, which has led to a growing population of immunocompromised patients who are susceptible to serious and potentially life-threatening fungal infections: even if a cancer patient responds well to a course of cancer therapy, they may tragically succumb to an IFD.

Among the most clinically significant fungal pathogens are: *Aspergillus* species, which cause sinopulmonary and disseminated diseases in patients with hematologic cancers or who are receiving stem cell transplants, as well as patients critically ill with influenza and COVID-19; *Candida* species, which are a frequent cause of deep-seated and bloodstream infections among hospitalized, immunocompromised patients; and *Cryptococcus neoformans*, a major cause of meningoencephalitis in immunocompromised patients, especially those who are HIV-positive.

In addition to acute invasive infections, fungal pathogens also cause a variety of chronic infections. These include respiratory diseases such as chronic pulmonary aspergillosis, fungal sinusitis, and allergic bronchial aspergillosis, as well as dermatophyte infections that are prevalent in low- to middle-income countries. In addition, *Candida* species cause a variety of mucosal and superficial skin infections that affect many people.

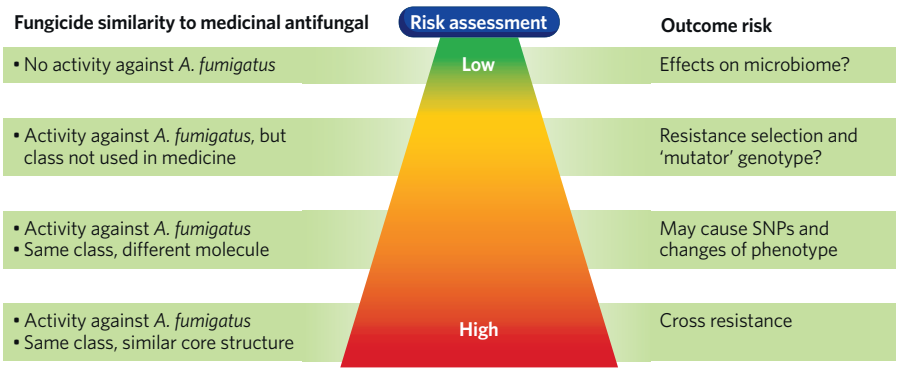

Fig. 1 | Dual use of antifungals in agriculture and the clinic and associated risks for cross-resistance. SNP, single nucleotide polymorphism.

Until recent years, the armamentarium of antifungal agents for management of IFDs derived from just three major chemical classes: the azoles (including di- and triazoles), echinocandins and polyenes. Drugs from these three classes are often used in conjunction with the pyrimidine flucytosine and terbinafine, an allylamine compound.

Today, the repertoire of antifungals is expanding. In 2021, ibrexafungerp, a first-in-class triterpenoid, received United States (US) Food and Drug Administration (FDA) approval primarily for use in treating certain chronic *Candida* infections. Another new class of antifungals, the orotomides, were discovered and developed by F2G in the past decade. The first drug from the class of orotomides is currently in clinical-stage development with other compounds such as fosmanogepix and new formulations of polyenes are in varying stages of development.

Antifungals: a brief history

Antifungal development is inherently difficult, in part because humans and fungi share similar eukaryotic cellular machinery, and so identifying targets that are selectively found in fungi poses challenges. Historically, 80% of leads identified in the literature have proved to be false positives. Yet despite these difficulties, a limited number of targets have been effectively utilised in antifungals.

Amphotericin B, which came into use in 1958 and dominated fungal therapy until the 1980s, has the widest spectrum of available antifungals. In its original deoxycholate formulation its use is associated with frequent and sometimes severe side effects, particularly nephrotoxicity and infusion reactions, though lipid formulations have developed with lower toxicity and remain important for IFDs such as mucormycosis. In 1981, the first azole antifungal, the diazole ketoconazole, was introduced to the market; then, in the early 1990s the first-generation triazoles, fluconazole and itraconazole, became available.

The emergence of the triazoles marked a big step forward for antifungal therapy because they can be formulated for oral delivery and have favourable safety profiles. These properties make them suitable both for prophylactic applications (preventing IFDs in patients receiving treatment that leaves them immunocompromised, such a chemotherapy and stem-cell or solid-organ transplantation), as well as the outpatient treatment of IFDs. Indeed, the availability of the triazoles enabled the more widespread use of highly immunosuppressive therapeutic regimens, which otherwise would have been too risky given the threat of IFDs.

The early 2000s saw the development of the second-generation triazole, voriconazole, which, due to its efficacy and comparatively low toxicity, displaced amphotericin B as the drug of choice for the treatment of invasive aspergillosis and other filamentous fungal infections. Voriconazole remains on the World Health Organisation's (WHO) list of essential medicines.

The third major class of antifungals to be developed, in the 2000s, were the echinocandins, which have low oral bioavailability and so must be administered intravenously, making them unsuited to outpatient care. The echinocandins have, however, become one of the first-line treatments for invasive *Candida* infections.

In 2015, scientists from F2G reported the discovery of the orotomides, a reversible inhibitor of the enzyme dihydroorotate dehydrogenase (DHODH), which ordinarily catalyses the conversion of dihydroorotate to orotate, a key component of pyrimidine used to build nucleic acids. As such, the orotomides block pyrimidine synthesis, affect the fungal cell wall and result in cell lysis. F2G's lead orotomide candidate, olorofim, has been shown to be effective in vitro against phylogenetically clustered species of molds that cause infections in humans, including *Aspergillus*, *Fusarium*, difficult-to-treat *Scedosporium/Lomentospora* and *Penicillium* spp., as well as endemic fungi including *Coccidioides immitis* (it is not effective against yeasts or the Mucorales). In 2016, the European Medicines Agency Committee for Orphan Medicinal Products granted olorofim orphan drug status for the treatment of scedosporiosis, and subsequently for the treatment of invasive aspergillosis; olorofim has also received Orphan Drug Designation by the FDA for *Aspergillus*, *Scedosporium* and *Cocci* spp.

Antifungal resistance: a growing problem

In recent decades the problem of antibiotic resistance has been a primary focus for many organizations, with less attention directed towards antifungal resistance. The tide is turning, however. In October 2022, the WHO released its first-ever list of 19 fungal 'priority pathogens' that pose the greatest threat to public health—a list that included azole-resistant *Aspergillus fumigatus*.

A leading cause of antifungal resistance in molds is the use of fungicides in agriculture, a factor highlighted by the WHO report as the major driver of azole-resistant *A. fumigatus*. Although *A. fumigatus* does not infect plants, other *Aspergillus* species do. *Aspergillus* spp. and other phytopathogenic fungi account for the majority of microbial crop damage, which amounts to billions of dollars' worth of crop losses annually.

The fungicides used to protect crops are often structurally related to medicinal antifungals, and so agricultural fungicides have the potential to drive the evolution of resistance to antifungals developed to treat human diseases. In the case of *A. fumigatus*, this is exactly what seems to have happened.

A. fumigatus is found widely in the environment, thriving on decaying plant matter such as compost heaps created from the detritus of crop and flower growing. As such, *A. fumigatus* is inevitably exposed to triazole-based fungicides such as tebuconazole that create a selective pressure driving the evolution of resistant strains. Tebuconazole-resistant *A. fumigatus* has also been found in residential gardens, and can spread far and wide through airborne dispersal—finally finding its way in to the clinic.

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Surveillance studies in countries from Europe, Asia and the Americas have detected azole-resistant *A. fumigatus* in clinical isolates from 1.4% to 11.4%, which are associated with 25% excess 90-day mortality in patients with invasive aspergillosis. As azoles are the first-line and only oral treatment for *A. fumigatus*, azole-resistance represents a clinically significant challenge.

New classes of drugs with new mechanisms of action, like the orotomides, offer a way round the resistance that has emerged, and continues to develop, against earlier classes of antifungals. Olorofim, F2G's orotomide currently in clinical development, has shown promising in vitro and in vivo activity against azole-resistant *A. fumigatus* and other difficult-to-treat fungal infections.

At the same time, there is reason for concern over the possibility that orotomide resistance could evolve among *Aspergillus* and other fungal species even as drugs from this class enter the clinic. The US Environmental Protection Agency has recently authorized the fungicide ipflufenoquin, which shares the same mechanism of action as the orotomides: inhibition of DHODH. Just as azole-based fungicides have driven the evolution of azole-resistant *A. fumigatus*, the widespread use of ipflufenoquin or other fungicides that share the DHODH-inhibiting mechanisms of action could fuel the evolution of orotomide resistance—a risk that has been assessed as moderate to high by the Fungicide Resistance Action Committee.

This risk assessment is supported by in vitro data that show an overall similarity between olorofim and ipflufenoquin in their growth-inhibitory effects on *Aspergillus* spp., suggesting that ipflufenoquin use could promote cross-resistance to olorofim. A crucial open question is whether the selective pressure created by ipflufenoquin can select for mutations in the *PyrE* gene that would confer resistance to olorofim and other orotomides. If this turns out to be the case, environmental monitoring programs to explore whether resistance selection in *Aspergillus* species and especially *A. fumigatus* occurs in the field would be highly warranted.

And this is not just an issue for olorofim or the orotomides more generally; the same problem may affect other antifungals with novel mechanisms of action that are in development. For example, the medical antifungal fosmanogepix, which targets the fungal protein Gwt1, is currently in clinical development—but at the same time, pyridine fungicides such as aminopyrifen that target the same protein are in development, and present similar potential risks as the azole fungicides have for the medicinal triazoles and ipflufenoquin poses for the orotomides. In general, the chemically closer an agricultural fungicide is to a medicinal antifungal, the greater the risk of human health-relevant resistance to evolve (Fig. 1).

Combating the global challenge of antifungal resistance requires adopting what the WHO calls 'One Health': an integrated approach to balancing and optimizing the health of humans, animals and the wider environment, through the coordinated efforts of public health bodies, veterinary experts, environmental specialists, and regulatory agencies. In the case of fungal resistance, the conversation also needs to include the commercial producers of fungicides, as well as their end users and pharmaceutical companies.

Moving forward, public health agencies need to incorporate the monitoring and reporting of antifungal resistance in the same way that antibiotic resistance is closely followed—not just in humans but in animal and environmental samples, as the One Health approach mandates. Increased surveillance, in turn, requires research into molecular markers of antifungal resistance for improved diagnostics, and globally accessible genomic databases of antifungal resistance.

Developing new antifungals is extremely challenging, but as the examples of ibrexafungerp and orolofim show it is not a hopeless task. Yet there is no room for complacency: future breakthroughs are not guaranteed, and the medical utility of the antifungals that have either just entered clinical use, or are on the verge of doing so, must be preserved.

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