38.1 OVERVIEW OF ANTIFUNGAL THERAPY

In 1939 the war against fungal diseases began in earnest with the discovery of griseofulvin, a metabolite of *Penicillium griseofulvum* [1]. It was the first truly effective antifungal agent and remains the sole licensed orally active drug for childhood dermatophyte infections. Griseofulvin inhibits fungal mitosis and, while it has good activity against dermatophyte fungi, it has no other clinically useful antifungal activity. The shared eukaryotic origin of fungal and human cells had presented a problem for rational drug development since the common metabolic pathways and cellular organelles increase the risk of dose-limiting toxicity (see Figure 38.1). Previous attempts at therapy, using potassium iodide and the sulphonamides, therefore had limited efficacy.

A decade later, the first of the polyene drugs, nystatin, was discovered, followed in 1955 by amphotericin. Amphotericin B is synthesized by an aerobic actinomycete, *Streptomyces nodosus*. The drug was discovered during a survey of soil samples carried out by a pharmaceutical company looking for antibiotic-producing *Streptomyces* strains. The strain producing amphotericin B was first recovered from the Orinoco River valley in Venezuela. These drugs established the modern era in antifungal therapy and remain widely used today. In addition to the polyenes, two other drug classes dominate systemic antifungal therapy: the triazoles and the echinocandins. Other drugs, such as flucytosine and terbinafine have a role in specific diseases or superficial infections, respectively. A general overview of the spectrum of activity of the currently used antifungal drugs is shown in Table 38.1.

38.1.1 Polenes

The polyene antifungals that are in current clinical use are nystatin, natamycin, and amphotericin B. They are fungicidal, and their mode of action is disruption of the fungal cell membrane by binding to ergosterol, the main sterol in the membrane. This results in pore formation and leakage of cellular cations and anions, leading to fungal cell death [2].
Although the presence of ergosterol in the fungal cell membrane confers some selectivity to the action of polyenes, there are notable toxicities associated with their systemic use. Nystatin and natamycin are currently only used topically and, although initial studies with a liposomal formulation of nystatin (Nyotran) demonstrated good efficacy, they were halted due to infusion-related toxicity. Amphotericin B is used systemically, but as it is...
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not absorbed orally, it must be administered intravenously (IV). It has a broad spectrum of activity against yeasts and molds (see Figure 38.2) and has been widely used over several decades to treat a range of diseases, including invasive aspergillosis, cryptococcosis, candidosis, the endemic mycoses, and leishmaniasis [2].

In vivo amphotericin B is largely protein bound, with high concentrations in the liver and spleen, and lower concentrations in the lung and kidney. It is eliminated via the urine (21%) and bile (43%), but there is little metabolic breakdown and most of the drug is excreted unchanged over a period of several weeks. The half-life is initially 10–24 hours but after prolonged use may be as long as 15 days [3].

Toxicity associated with amphotericin B includes infusion-related nausea, vomiting, and rigors; chronic toxicity is primarily renal [4]. This toxicity may lead to discontinuation of therapy; in response, lipid formulations were developed and are characterized by reduced toxicity [5]. There are two such formulations currently available in the United Kingdom: Ambisome, which uses liposomes to package the amphotericin, and Abelcet, which uses amphotericin in lipid complexes. These lipid preparations of amphotericin B are also only very slowly eliminated, with only 9% excreted at the end of 7 days [6]. Despite the significantly increased cost, the improved tolerability associated with lipid formulations of amphotericin B has led to a major shift away from the use of amphotericin B deoxycholate except in some resource-poor settings [7].

Drug interactions associated with amphotericin B generally reflect its nephrotoxicity, being most pronounced when concomitant medications undergo renal excretion. Amphotericin B enhances renal toxicity of ciclosporin, aminoglycosides, and some cytotoxic agents such as cisplatin, and it requires vigilant monitoring of renal function if coadministered [2]. Lipid formulations of amphotericin B require no dose adjustment for renal insufficiency and generally none for hepatic insufficiency, although specific recommendations vary with different lipid forms.

### 38.1.2 Triazoles

The first description of antifungal properties in azole compounds was in 1944 when researchers studying biotin deficiency in animals noted that benzimidazole, which is structurally similar to biotin, had activity against yeasts [8]. This finding was not followed up at the time, but later groups routinely screened other azoles for antifungal activity. Early agents to be developed in the group, including clotrimazole, ketoconazole, and miconazole, are generally now confined to topical use because of unfavorable safety profiles.

Further refinements and chemical modification of these compounds resulted in the triazole group, which is characterized by a five-member azole ring. Triazoles such as

<table>
<thead>
<tr>
<th>Yeasts</th>
<th>Dimorphic fungi</th>
<th>Molds</th>
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<tbody>
<tr>
<td>Unicellular organisms, which usually reproduce by budding</td>
<td>Exist as either yeast or mold forms and the change in form is usually related to temperature</td>
<td>Multicellular organisms, with hyphae, and they often produce specific spore-producing structures</td>
</tr>
<tr>
<td>Microscopic appearance (x 400 magnification)</td>
<td>Microscopic and cultural appearance depends on temperature: usually mold at 27°C and yeast or yeast-like at 37°C</td>
<td>Microscopic appearance (x 400 magnification)</td>
</tr>
<tr>
<td>Culture appearance</td>
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<td>Culture appearance</td>
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<tr>
<th>Examples</th>
<th>Examples</th>
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<tbody>
<tr>
<td>Candida</td>
<td>Histoplasma</td>
<td>Aspergillus</td>
</tr>
<tr>
<td>Cryptococcus</td>
<td>Coccidioides</td>
<td>Fusarium</td>
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<tr>
<td>Malassezia</td>
<td>Blastomyces</td>
<td>Scedosporium</td>
</tr>
<tr>
<td>Trichosporon</td>
<td>Paracoccidioides</td>
<td>Mucoraceous molds</td>
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<td></td>
<td></td>
<td>Dermatophytes</td>
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</tbody>
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**FIGURE 38.2** Different fungal morphologies with culture and microscopic appearance.
fluconazole, itraconazole, voriconazole, and posaconazole are widely used for prophylaxis and treatment of invasive fungal disease (IFD) [9]. The azoles share a common mode of action: inhibition of the fungal cytochrome P450 enzyme (CYP450) lanosterol 14-α-demethylase, which facilitates conversion of lanosterol to ergosterol, a component of the fungal, but not the human, cell membrane. Disruption of ergosterol biosynthesis causes damage to the fungal cell membrane, accumulation of methylsterols, and either cell lysis leading to fungal cell death or inhibition of fungal cell growth, depending on theazole and fungal species [9].

All classes of azoles are substrates and inhibitors of CYP450 enzymes, but the extent to which they are metabolized by or inhibit the various CYP isoforms varies between drugs [10–12]. Individual triazoles exhibit different properties, spectra of activity, and therapeutic indications, and these are discussed next. Their pharmacokinetic properties are summarized in Table 38.2.

38.1.2.1 Fluconazole
The first triazole antifungal to be marketed appeared in 1990. This was fluconazole, a fluorinated bistriazole (i.e., with two triazole rings). Available in capsule and oral solution, and as an intravenous preparation, it has activity against Cryptococcus, most Candida species (except C. krusei and sometimes reduced activity against C. glabrata), and some dimorphic fungi. It is generally considered to be fungistatic. Because of its hydrophilic properties, it has excellent oral bioavailability (85–90%) and its absorption is unaffected by gastric pH [9,13]. As fluconazole is largely excreted via the kidneys, dose adjustment is required in patients with reduced creatinine clearance, and it should be used with caution in patients with hepatic insufficiency, although the data are limited. Fluconazole has a favorable safety profile and minimal toxicity, although hepatotoxicity and prolongation of the QT interval in ECG have been noted [3]. Fluconazole is a substrate of CYP3A4 and inhibits CYP 2C9, 2C19, and, to a lesser extent, CYP3A4, an effect that may only occur at doses >200 mg/d [10]. It is also a substrate of P-glycoprotein (P-gp), but not an inhibitor [14]. Drug interactions mediated via the CYP450 enzymes are numerous and include those with alfentanil, ciclosporin A, and midazolam mediated via interactions with CYP3A4; those with amitriptyline, fluvastatin, and phenytoin are mediated via inhibition of CYP2C9 [15].

38.1.2.2 Itraconazole
Itraconazole is a lipophilic triazole, available as capsules, oral solution, and, in some countries, a concentrate for parenteral administration. It has a broad spectrum of activity,
including Candida, Aspergillus, dimorphic fungi, and some melanized fungi, but not Fusarium, Scedosporium, or the mucoraceous molds [16]. Its activity may be either fungistatic or fungicidal depending on the fungal species [17]. Oral bioavailability of itraconazole varies with formulation. Absorption of the capsules is dependent on gastric pH, with increased absorption at acidic pH, while the solution is best absorbed during fasting [9]. Itraconazole has nonlinear pharmacokinetics and because of variable absorption, drug concentrations show significant variability in individuals [18].

In patients with renal insufficiency, use of the IV formulation is not recommended if creatinine clearance is less than 30 ml/min, as the cyclodextrin vehicle is cleared by glomerular filtration. In hepatic insufficiency, there is an increase in the elimination half-life and dose modification may be required [18].

Adverse events associated with itraconazole use include nausea and vomiting (particularly for the oral solution), hepatotoxicity, rash, and headache [18]. There is some evidence that the occurrence of these toxicities correlates with the itraconazole concentration in serum.

Itraconazole is a substrate and potent inhibitor of CYP 3A4, with many metabolites also showing potent CYP3A4 inhibitory activity [19]. It is also an inhibitor of CYP 2C9 and P-gp [15]. These interactions form the basis for many drug interactions seen with itraconazole. Interactions mediated via CYP3A4 include those with fluticasone, lovastatin, phenytoin, rifampin, terfenadine, triazolam, and vincristine; those mediated via P-gp include vincristine, cimetidine, and digoxin [15].

38.1.2.3 Voriconazole

Voriconazole is a second-generation triazole based chemically on fluconazole but with a much broader spectrum of activity. It is active against Candida (including most of the fluconazole-resistant species), Cryptococcus, and many species of Aspergillus; it has some activity against Fusarium [16]. Voriconazole activity may be either fungicidal or fungistatic depending on the fungal species [17]. It is the treatment of choice for invasive aspergillosis and is used to treat infections due to Scedosporium or Fusarium. Formulations of voriconazole include tablets, an oral suspension, and an IV solution.

The suspension has good oral bioavailability, especially during fasting, but this is reduced in the presence of high-fat meals [20]. The pharmacokinetics of voriconazole are nonlinear in adults, meaning that relatively small changes in dosing can result in unpredictable changes in drug exposure—being either much larger or smaller than expected.

Voriconazole is extensively metabolized by the CYP 450 enzymes—mainly CYP2C19 but also CYP2C9 and CYP3A4 [21]. It is eliminated as inactive metabolites predominantly via feces (80%), with about 20% excreted in the urine [22].

The vehicle used for IV voriconazole (sulphobutylether beta cyclodextrin sodium, or SBECO) is cleared by the kidneys and hence can accumulate in mild–moderate renal dysfunction; in these patients, the oral formulation is preferable. In mild–moderate hepatic insufficiency, the standard loading dose should be used and then half the maintenance dose, but there are no data for use in severe hepatic insufficiency.

Toxicity associated with voriconazole includes visual disturbances, rash, and hepatotoxicity. Visual disturbances often decrease with continued use and may manifest as photophobia, color changes, blurred vision, or flashes of light; they seem to correlate with high drug concentrations [23]. Cutaneous adverse events are often associated with sun exposure and usually resolve after discontinuation of the drug, although an increased risk of squamous cell carcinoma has been reported with voriconazole use in solid-organ transplant recipients [24]. Hepatotoxicity includes elevated bilirubin, alkaline phosphatase, and transaminases, and correlates with drug concentrations [25].

Voriconazole is both a substrate and an inhibitor for CYP2C19, 2C9, and 3A4, and is also an inhibitor of 2B6 [26]. These properties underpin its multiple interactions with drugs such as sirolimus, alfentanil, cyclosporine, diclofenac, methadone, efavirenz, and midazolam [15].

38.1.2.4 Posaconazole

Posaconazole is the latest triazole to come to market. It was developed from itraconazole and is available only as an oral suspension, although a new tablet formulation is currently under development [27]. Posaconazole has the broadest spectrum of activity in vitro of all the azoles, with good activity against Candida, Cryptococcus, Aspergillus, many dimorphic or melanized fungi, and several of the mucoraceous molds [16]. It is fungicidal against Aspergillus, Cryptococcus, and Candida [28]. Oral bioavailability is very dependent on gastric pH, with absorption maximized in the presence of fatty foods [29]. As with itraconazole, absorption is improved by concomitant administration of acidic drinks, but it is saturated at a dose of 800 mg/d and improved by dividing the dose. Drug concentrations achieved in serum vary between patient groups and are known to be reduced in the presence of mucositis and diarrhea, or with the use of proton pump inhibitors (PPIs) or H₂ antagonists [30].

Most of the metabolism of posaconazole is by glucuronidation, with only a limited amount via oxidative CYP450-mediated transformation [31]. Posaconazole has a long half-life and is mainly eliminated unchanged in the feces (66%), with some excreted via the urine, mainly as metabolites (14%) [32]. It can be used in renal insufficiency with no dose modification, and limited data suggest that it should be used with caution in hepatic insufficiency. Toxicity associated with posaconazole use is generally mild, with headache, gastrointestinal upset and rash reported, although
at the moment there are no data to suggest that these are related to the drug’s concentration. Although posaconazole is minimally metabolized by the CYP450 system, it is an inhibitor of CYP3A4 and P-gp, resulting in some drug interactions, notably with rifabutin, phenytoin, efavirenz, and fosamprenavir [15].

38.1.3 Echinocandins

The echinocandin antifungals, the first new class of antifungals for 15 years, are synthetically modified lipopeptides with three currently licensed: caspofungin, micafungin, and anidulafungin. The lead compound for anidulafungin was discovered in 1974, and all three drugs are derived from fermentation products of fungi [33]. Echinocandins are noncompetitive inhibitors of β-1,3-glucan synthase, an enzyme critical to the synthesis of β-1,3-glucan, which is a major component of the fungal cell wall. The absence of β-1,3-glucan from the cell wall causes loss of rigidity and integrity with resultant lysis. Echinocandins target the FK56 gene, which encodes a component of the β-1,3-glucan synthase. Since mammalian cells do not possess a cell wall or β-1,3-glucan synthase, the action of echinocandins is specific to fungi, probably explaining their excellent safety profile. Parenteral administration is obligatory, as their large lipophilic molecular structures have very low oral bioavailability (<10%) [34]. The three echinocandins all have a similar spectrum of activity, a reflection of their mode of action, and they are active against Candida and Aspergillus, but not against Cryptococcus and mucoraceous molds which have less β-1,3-glucan in their cell walls [33,34]. The echinocandins are fungicidal for Candida and fungistatic for Aspergillus [34]. The pharmacokinetic properties of the echinocandins are summarized in Table 38.3.

Use of the echinocandins is generally well tolerated, although histamine release, some liver toxicity, and headache have all been reported. Caspofungin use is relatively commonly associated with fever and local irritation at the site of administration, while rashes can occur infrequently with all three drugs [35].

The echinocandins are poor substrates for the CYP450 enzymes, are not substrates for P-glycoprotein, and have few drug interactions. Caspofungin is an inhibitor of CYP3A4

<table>
<thead>
<tr>
<th>TABLE 38.3 Pharmacokinetic Properties of the Echinocandins</th>
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<tbody>
<tr>
<td><strong>Caspofungin</strong></td>
</tr>
<tr>
<td>Route of administration</td>
</tr>
<tr>
<td>Protein binding (%)</td>
</tr>
<tr>
<td>Metabolism</td>
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<tr>
<td>Tissue distribution</td>
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<tr>
<td>Half-life for β phase (hour)</td>
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<tr>
<td>Adult dosing regimen</td>
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<tr>
<td>Route of elimination</td>
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<tr>
<td>Dose adjustment in renal insufficiency</td>
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<tr>
<td>Dose adjustment in hepatic insufficiency</td>
</tr>
<tr>
<td>Source: Data from Bellman [3], Johnson and Mohr [33], and Wagner et al. [34].</td>
</tr>
</tbody>
</table>
While ciclosporin A and tacrolimus do interact with it, there is no requirement for dose adjustment although liver function and tacrolimus levels respectively should be closely monitored.

Coadministration of powerful hepatic enzyme inducers (e.g., rifampicin, efavirenz, phenytoin, carbamazepine) require an increase in the daily dose of caspofungin to 70 mg/d [35]. The mechanism of this is unknown, but it is perhaps surprising given that caspofungin is not metabolized by the CYP enzymes. It may be a nonspecific effect or could perhaps be due to induction of the N-acetyltransferases that are involved to some extent in caspofungin metabolism.

Micafungin administration with sirolimus or nifedipine may increase exposure to those drugs and so dosage adjustments may be required if toxicity occurs. Anidulafungin has as yet no known clinically relevant drug interactions [33].

### 38.1.4 Flucytosine

Flucytosine is a fluorinated pyrimidine that was originally developed as an agent to treat leukemia. It has two modes of action: inhibition of protein synthesis and inhibition of DNA synthesis. The drug penetrates the fungal cell wall via cytosine permease and, once in the cell, is deaminated to form 5-fluorouracil. This is incorporated into fungal RNA instead of uracil and hence disrupts protein synthesis. DNA synthesis inhibition occurs via conversion of 5-fluorouracil to 5-fluorodeoxyuridylic acid, a noncompetitive inhibitor of thymidylate synthase [37]. Flucytosine has fungistatic activity against Candida and Cryptococcus, and its main current indication is for the treatment of cryptococcal meningitis, in combination with amphotericin B, with which it shows synergy, [38] or the azoles [37]. Flucytosine is available in oral and IV preparations, although the oral preparation is not available in all countries.

Flucytosine has good oral bioavailability, penetrates well into body fluids because of its low protein binding, and achieves good concentrations in cerebrospinal fluid, bone, spleen, liver, heart, and lung. It is mainly eliminated via the kidneys, with almost all of the dose eliminated by glomerular filtration, mainly as unchanged drug. The half-life in patients with normal renal function is 3–4 hours, but can be up to 85 hours in patients with severe renal failure. Renal insufficiency necessitates dose alterations, as does renal replacement therapy, which removes a significant proportion of the drug [37,38]; however, there are no recommendations for use in hepatic insufficiency.

Use of flucytosine is associated with gastrointestinal, hepatic, and hematological toxicities. These side effects may reflect the conversion of flucytosine to 5-fluorouracil [39] and correlate with the levels of flucytosine in serum, with levels above 100 mg/L associated with leukopenia and thrombocytopenia. Hematological toxicity is more common in patients with advanced HIV infection [38]. Therapeutic drug monitoring is required to ensure appropriate dosing and to prevent toxicity.

A particular problem when flucytosine is used as a single agent is the rapid development of resistance [40], so combined use with amphotericin B (or its lipid formulations) is now recommended. Flucytosine has limited drug interactions, but is contraindicated with antiviral nucleoside drugs such as ganciclovir. It may interact with phenytoin and cytarabine, although the evidence for the latter is sparse [38].

#### 38.1.5 Terbinafine

Terbinafine is a member of the allylamine group of antifungals. It inhibits ergosterol biosynthesis by inhibiting squalene epoxidase, a catalyst of the conversion of squalene to squalene epoxide. This resulting accumulation of squalene within the fungal cell and depletion of ergosterol in the fungal cell membrane causes cell death [41]. Terbinafine is highly selective for fungal squalene epoxidase and has no effect on human sterol synthesis [42]. It has in vitro activity against a wide range of organisms, including dermatophytes, several melanized molds, Aspergillus, Cryptococcus, and several dimorphic fungi, although it is mainly used to treat dermatophytosis [43]. It is available as oral and topical preparations.

Terbinafine has good oral bioavailability (70–80%), and absorption is not affected by food. All allylamines are highly lipophilic and hence terbinafine achieves high concentrations in the stratum corneum, sebum, and nail. It is extensively metabolized in the liver, and 15 metabolites have been identified which are subsequently excreted in urine (80%) and feces (20%) [44]. At least 7 CYP enzymes are involved in terbinafine metabolism, of which 1A2, 2C9, and 3A4 are the most important [45]. Elimination takes place slowly, with an initial half-life of 22 hours and another at 90 hours, probably reflecting slow release from adipose tissue [44]. Use of terbinafine is not recommended in patients with active or chronic liver disease or with creatinine clearance <50 ml/min. It has been associated with gastrointestinal effects, skin eruptions, alteration or loss of taste, and hepatobiliary disturbances [46].

Initially, it was thought that terbinafine did not have any inhibitory effect on the CYP enzymes [47], but it is now known to be a potent competitive inhibitor of CYP2D6, although it has been suggested that one of its metabolites, rather than the parent drug, causes this [48]. Because CYP2D6 constitutes only 1.5% of the total liver CYP, there are minimal interactions via this route, except with venlafaxine [49] and amitriptyline [50]. Cimetidine decreases clearance of terbinafine, while fluconazole coadministration causes increased exposure to terbinafine via inhibition of CYP2C9 and 3A4.
38.2 PUBLIC HEALTH IMPACT OF FUNGAL DISEASES

The public health impact of fungal diseases is significant. The impact of superficial versus systemic disease is important for different reasons. Superficial fungal infections are extremely common: various studies estimated that 20–40% of adults in developed countries have some form of fungal foot disease [51], there is an increasing incidence of tinea capitis in children [52], and superficial candidosis is the most commonly seen manifestation of candidosis [53]. From a public health viewpoint, superficial infections are important because those caused by dermatophytes are infectious and are readily transmitted via communal facilities, such as sports centers or within the family home. While skin infections are not associated with any significant morbidity, the breaches in skin integrity they cause may become a portal of entry for other organisms that may cause more serious infections such as cellulitis. Dermatophytosis may result in alopecia or permanent scarring from scalp infections and difficulty walking in elderly patients with grossly thickened or clawed toenails due to fungal nail infection.

Additionally, because of the sheer numbers of patients affected, the potential cost of medical consultations, diagnosis, and treatment for fungal foot disease alone is huge. Figures from the Prescription Cost Analysis for England for 2012 show that prescription costs for terbinafine and griseofulvin, the main drugs used to treat dermatophytosis, totaled £3.8 and £1.2 million, respectively, highlighting the significant cost of treatment alone (www.hscic.gov.uk/catalogue/PUB10610).

For IFD, the public health impact is also significant, despite the small numbers of people affected. Patients in whom systemic diseases are likely to develop are those who are immunocompromised, either because of underlying disease (e.g., leukemia, AIDS) or because of treatments for their disease (e.g., cytotoxic agents, immunosuppressives, organ or haematopoietic stem cell transplantation (HSCT)). Multiple studies in many countries have shown that the incidence of IFD is increasing, partly because more patients are surviving their underlying diseases and partly because more aggressive treatments are being used, resulting in many profoundly immunosuppressed, at-risk individuals [54].

Systemic diseases are relatively infrequent when compared to the prevalence in the community of superficial fungal infections. However, the cost associated with each episode of IFD is disproportionately high. This is because IFDs are associated with increased length of hospital stay and prolonged courses of antifungal drugs. A recent American study estimated that a patient with a systemic fungal infection stays in the hospital an extra 7 days and costs an extra $29,000 compared to a patient with the same underlying disease but no fungal infection [55]. Overall, this represents an additional cost of $1.89 billion, demonstrating that the cost associated with IFD has a significant public health impact.

The recent emergence of *Aspergillus fumigatus* with resistance to the azoles has been documented. Although there is a low level of resistance in many countries throughout Europe, the levels in Holland and the United Kingdom are higher. In Holland, the widespread use of azole-based compounds in agriculture has been implicated as the cause [56], but in the United Kingdom most of the resistance has occurred in patients treated with azoles, usually for months or years, for chronic aspergillosis [57]. The development of resistance to an important class of drugs, which often confers cross-resistance between members of the class, is of concern and may impact our ability to treat these infections in the future.

38.3 THE NEED FOR PHARMACOGENOMICS AND STRATIFIED MEDICINE

Treatment of fungal disease has been increasingly successful over the last 10–20 years, partly due to the advent of better antifungal drugs with broader spectra and less toxicity; and partly due to improvements in diagnosis of IFD, enabling earlier interventions. However, the morbidity and mortality associated with some fungal diseases is still very high.

Various risk factors predispose to IFD, including malignancy (particularly hematological malignancy), the use of steroids or antimicrobial agents, surgery, burns, uncontrolled diabetes, AIDS, chemotherapy, immunosuppressive agents, and neutropenia. The risk of IFD can be crudely predicted based on the level of patient immunocompromise. Therefore, patients who have undergone allogeneic HSCT have a greater risk of fungal disease than do patients receiving autologous transplants, which partly relates to their degree of immune suppression [58]. However, even within groups known to be particularly “at risk,” susceptibility to fungal disease varies.

Approaches to prevention and diagnosis of fungal disease vary among institutions. Some use antifungal prophylaxis in high-risk patients in an attempt to avoid the disease. The triazole antifungals have been widely used in this setting, with fluconazole, itraconazole, and posaconazole all having a licence for this indication in the United Kingdom. Despite not having a licence for prophylaxis, voriconazole has also been used. The disadvantages associated with this approach include the cost of the drugs, the potential for drug interactions and toxicity, and the inability to achieve appropriate therapeutic levels in all patients. This can then require the use of therapeutic drug monitoring to ensure drug concentrations high enough but not toxic, which incurs a further cost [59]. Another disadvantage is that evidence
suggests that the use of mold-active prophylaxis reduces the sensitivity of the *Aspergillus* galactomannan (GM) ELISA, which is widely used in the diagnosis of invasive aspergillosis [60]. This may then lead to the use of empiric antifungal therapy because of a lack of confidence in the results of the diagnostic test.

Another approach is the routine screening of patients with PCR and GM ELISA—a so-called “enhanced diagnostic” approach. This relies on the high negative predictive value of the tests and, unless other features of funga l disease occur, it enables the patient to remain on prophylaxis rather than receive empiric antifungal therapy. One study showed no difference in morbidity or mortality when empiric therapy was withheld and a reduction in antifungal expenditure of £124,000 using the enhanced diagnostic approach [61].

Diagnosis of superficial fungal disease is relatively simple, with direct microscopy and culture of skin, hair, or nail samples allowing the recovery of the causative agent in most patients. The situation is more challenging in patients with suspected IFDs. Patients are likely to have serious underlying disease, and the development of new signs and/or symptoms, which are often nonspecific, may be difficult to pinpoint. Direct microscopy and culture may be useful if tissue is available, but is often slow and insensitive; the inability to take biopsies and deep tissue samples in patients with coagulopathies further limits the usefulness of this modality. Detection of antibodies to fungi is rarely helpful in patients who are immunocompromised.

The use of fungal antigen detection kits has become increasingly common, and several are available, including cryptococcal antigen, *Aspergillus* GM, *Candida* mannan, and the more general β-D-glucan detection kits. While the sensitivity and specificity of the cryptococcal antigen kit is very high, the corresponding figures for the GM kit vary with patient group. It is highest in the HSCT recipient and lower in solid organ transplant recipients. Other tests such as PCR have been useful for some diseases and in certain laboratories, but the lack of standardization and variable performance means that they are still not used routinely in many centers [62].

Whatever approach is used, it is recognized that the successful outcome of an IFD relies on the early institution of appropriate antifungal therapy and that successful outcomes correlate with early initiation of therapy [63].

Although there are now many efficacious antifungal drugs available, the prevention, diagnosis, and treatment of IFD still presents a major challenge. To date we still do not understand the subtle differences between patients that impact their risk of infection or their response to antifungal drugs. Developments in pharmacogenomics that better enable us to predict how antifungal drugs will behave in a given patient will help us move toward more tailored or personalized management in this area.

### 38.4 Genetic Variability Associated with Antifungal Drugs

The effect of genetic variability on antifungal drugs may occur at any point during their absorption, distribution, metabolism, or elimination from the body. In addition to genetic variability, there are many other factors that may impact the efficacy of antifungal drugs, including drug interactions and various physiological factors [59,64,65].

#### 38.4.1 Absorption

Antifungal drugs are administered either orally or intravenously, and absorption is affected by many factors but is most variable for drugs that are taken orally. With IV dosing, absorption is assumed to be complete and the total dose reaches the systemic circulation. When the drug is given orally, it must dissolve in the stomach (the extent of which may be affected by gastric pH and the presence and type of food) and may then be subjected to first-pass metabolism in the intestines and liver or transporter-mediated efflux back into the intestinal lumen. These processes are affected by the amount and activity of the drug transporters and metabolizing enzymes at those sites (see Figure 38.3). Biliary excretion at this initial stage may also reduce the net amount of absorption. Because most of the drug is absorbed from the intestines rather than the stomach, any factor that speeds gastric emptying is likely to enhance drug absorption.

Gastric pH is particularly important for the absorption of itraconazole and posaconazole, while it has little effect on the absorption of fluconazole, itraconazole, voriconazole, and terbinafine. Itraconazole and posaconazole are weak bases and hence do not dissolve well in an alkaline environment [66,67]. Gastric pH can vary by up to 10-fold between individuals [68], and men tend to have lower gastric pH than women [69]. Age is now no longer thought to affect pH. Specific patient groups also have alterations in gastric pH due to their disease: those with AIDS have increased gastric pH [70], while critically ill patients show reduced gastric pH [71].

The widespread practice of using PPIs or H2 antagonists to reduce the risk of gastric ulcers in hospitalized patients [72] will also have a significant impact on gastric pH. Indeed, it has been shown for posaconazole that concomitant PPIs or H2 antagonists cause a significant reduction in drug exposure in patients [30]; although this is not due to genetic variability, it is an important determinant of drug absorption. For itraconazole capsules (but not oral solution), factors that enhance gastric acidity improve absorption, such as administration with food; whereas factors that decrease gastric acidity (use of PPIs or fasting) reduce absorption [9]. Therefore, patients receiving itraconazole capsules may be affected by variations in gastric pH that...
result in serum concentrations of itraconazole that are insufficient to prevent or treat their disease.

The main efflux drug transporters in the intestines are P-gp, breast cancer resistance protein (BCRP), and multidrug resistance protein 2 (MRP2) [73]. These transporters are found at the luminal side of enterocytes and are important in limiting the uptake of drugs that are substrates for them. They are all members of the ATP-binding cassette transporters and use ATP to allow them to pump substrates against the concentration gradient. It was recently shown that there is a large amount of inter-individual variation in the quantity of each transporter in the intestines and liver [74] and this might contribute to the variability in drug absorption between different individuals. BCRP and P-gp are present in smaller amounts in the liver than in the gut, while MRP2 is present in smaller amounts in the gut.

All of these efflux transporters have genetic polymorphisms which result in clinically significant changes in substrate binding. P-gp has multiple genetic variants, caused by single-nucleotide polymorphisms (SNPs) that vary with ethnicity and cause clinically relevant differences in, for example, clearance of ciclosporin [75]. SNPs in BCRP and MRP2 also occur and can affect the extent of substrate binding [73].

Several of the orally administered antifungals are either substrates or inhibitors of these transporters (see Table 38.4). Itraconazole, fluconazole, and posaconazole are all P-gp substrates, with itraconazole having the strongest interaction with it; itraconazole and posaconazole are also P-gp inhibitors. Voriconazole binds to CaMdr1P, the yeast homolog of human P-gp, suggesting theoretically that it may be a substrate in humans as well, but as yet there are no data to support this contention [76]. Itraconazole is an inhibitor of BCRP. Based on the chemical similarity between itraconazole and posaconazole and the presence of a long side chain in posaconazole (which enhances inhibitory capacity), it is likely that it too is an inhibitor of BCRP [77]. There are little data on the interaction of terbinafine with these transporters, but it is unlikely to be an inhibitor of

**FIGURE 38.3** Major uptake and efflux transporters at different body sites in humans.

**Abbreviations:** BCRP: Breast Cancer Resistance Protein; BSEP: Bile Salt Export Pump; MATE: Multidrug And Toxin Extrusion type transporter; MRP: Multidrug Resistance Protein; NTCP: Na taurocholate cotransporting peptide; OAT: Organic Anion Transporter; OATP: Organic Anion Transport Protein; OCT: Organic Cation Transporter; OCTN: Novel Organic Cation Transporter; PEPT: Peptide Transporter; URAT: Urate Transporter
### Table 38.4: Interactions between Antifungals and Phase I and II Enzymes and Drug Transporters

<table>
<thead>
<tr>
<th>Antifungal</th>
<th>CYP450 enzymes</th>
<th>P-glycoprotein</th>
<th>BCRP</th>
<th>Other transporters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Substrate</td>
<td>Inhibitor</td>
<td>Substrate</td>
<td>Inhibitor</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>3A4 [10]</td>
<td>2C9 [30.3 µM] [12]; 2C19 [12.3 µM] [12]; 3A4 [13.1 µM] [11]; 1A2 [31]</td>
<td>++ [14]</td>
<td>− [11,14]</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>3A4 +++ [19]</td>
<td>3A4 [0.033 µM] [11]; 2C9 [&gt;10 µM] [12]; 2C19 [&gt;10 µM] [12]; 1A2 [31]</td>
<td>+++ [15]</td>
<td>2µM [14]</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>2C19 +++ 3A4 ++ 2C9 ++ [21]</td>
<td>2C9 [8.4 µM] [12]; 3.62 µM [26]; 2C19 [8.7 µM] [12]; [5.25 µM] [26]; 3A4 [10.5 µM] [12]; 2.9 µM [26]; 2B6 [1.7 µM] [26]</td>
<td>Possibly [76]</td>
<td>− [140]</td>
</tr>
<tr>
<td>Posaconazole</td>
<td>− [140]</td>
<td>3A4 [1.3 µM] [31]; 1A2 −; 2C9 −; 2C19 −; 2A6 −; 2D6 −</td>
<td>++ [78]</td>
<td>++ [78]</td>
</tr>
<tr>
<td>Caspofungin</td>
<td>− [120]</td>
<td>3A4 + [36]</td>
<td>ND</td>
<td>− [98]</td>
</tr>
<tr>
<td>Anidulafungin</td>
<td>− [114]</td>
<td>− [102]</td>
<td>Not 1A2, 2B6, 2C9, 2C19, 2D6, or 3A inhibitor [114]</td>
<td>ND</td>
</tr>
<tr>
<td>Flucytosine</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Terbinafine</td>
<td>1A2 +++ 2C9 +++ 3A4 +++ 2C8 + [45]</td>
<td>2D6 [0.2 µM] [45]; 3A4 − [47]</td>
<td>ND</td>
<td>Unlikely [10]</td>
</tr>
</tbody>
</table>

---

*IC₅₀ (µM) is shown in brackets where figures are available; these may vary between studies in which different probes have been used in vitro. *Relevant source in reference list.*

ND—no data available; − no inhibitory effect or not a substrate; + weak inhibitor or weak substrate; ++ moderate inhibitor or moderate substrate; +++ potent inhibitor or major substrate.
P-gp because of the lack of effect when it is coadministered with digoxin, a P-gp-dependent substrate [10].

The presence of SNPs in any of the efflux transporters potentially affects the absorption of orally administered antifungals if they cause altered binding due to those polymorphisms. The only drug for which data are available is posaconazole, where the effect of SNP’s in P-gp have been studied [78]. Homozygous expression of the T allele results in significantly less expression of P-gp than does expression of heterozygous or wild-type alleles. Volunteers (28 black and 28 Caucasian) were dosed with 400 mg of posaconazole twice daily after food for 8 days, after which concentrations of posaconazole and expression of SNPs in P-gp were investigated. Overall, 41 SNPs were found in the alleles, with 13 individuals being homozygous for the T allele and 43 being either heterozygous or wild-type. There was no difference in drug exposure for either group, and hence it seems that polymorphisms in P-gp have no effect on posaconazole absorption. However, as this study was performed in healthy volunteers, its applicability to patients is uncertain.

There are currently no other data for the other antifungals, but similar studies are of interest particularly for itraconazole, which is a potent substrate and inhibitor of P-gp.

### 38.4.2 Distribution

Once the drug has been absorbed after oral administration, it must reach the bloodstream. Drugs that are administered intravenously overcome the issues related to absorption, but they too must move from the bloodstream to the interstitial and intracellular fluid and ultimately to the site or sites of the fungal disease. Blood flow to the various organs is one of the main determinants of tissue delivery, with the well-perfused organs (e.g., liver, kidney, brain) receiving the highest concentrations initially, with the skin, muscles, and fat receiving the drug possibly several hours later. The extent to which the drug partitions between blood and tissue is determined by how much the drug is bound by serum proteins, as only an unbound drug can move across cell membranes. The main proteins that bind drugs in serum are albumin—the major carrier for acidic drugs—and α1 acid glycoprotein (AAG), which binds basic drugs. Lipoproteins also act as carriers for some drugs.

The concentrations that are achieved for different antifungals at different body sites vary considerably (see Tables 38.2 and 38.3). Some antifungals are highly protein bound (e.g., itraconazole, posaconazole), while others have much lower protein binding (e.g., fluconazole, flucytosine). Itraconazole and posaconazole are mainly bound to albumin, although itraconazole does also bind, to a lesser extent, to AAG. Although much less protein bound, fluconazole preferentially binds to AAG [79]. Amphotericin B is bound by lipoproteins, albumin, and AAG [80,81], while terbinafine is strongly bound to serum proteins and the binding is even across the lipoproteins and albumin [44]. All echinocandins are extensively protein bound, mainly to albumin, but the extent of binding of caspofungin varies between healthy volunteers and patients [34].

Many serum proteins are now known to have genetic variants, with human serum albumin having 67 genetic variants [82] and AAG having 2 [83]. These variants have altered substrate binding and because of the extensive protein binding of many antifungals, this may affect their distribution. If protein binding of the antifungal were reduced, this could allow greater concentrations to move into the cells more quickly; conversely, if the binding were increased, this could reduce the rate at which the drug dissociates and moves into the tissues. At the moment, however, this is entirely speculative and there are no data about how antifungals are affected by serum protein genetic variants.

Drugs are taken into cells by either passive diffusion or active transport. As well as the extent of its protein binding, a drug’s ability to cross the cell membrane and hence enter the cell is determined by factors such as its size, degree of ionization, and lipid solubility of its ionized and nonionized forms. Passive diffusion into the cell usually occurs because the drug is soluble in the lipid bilayer and its movement occurs down a concentration gradient. Hydrophilic drugs penetrate cell membranes via the aqueous channels. In contrast, active transport requires the expenditure of energy and usually moves a drug against the concentration gradient, is saturable, and may be subject to competitive inhibition. Uptake transporters include those that transport anionic drugs (organic anionic transporters, or OATs) and organic anionic transporting polypeptides (OATPs), as well as those that transport cationic drugs (organic cationic transporters, or OCTs) and peptide transporters (PEPTs). As with efflux transporters, genetic polymorphism occurs for the uptake transporters resulting in alterations in substrate binding [84].

The distribution of drugs around the body and entry into specific tissues is determined partly by the uptake transporters. Antifungal drugs must be taken up into tissues to reach the site of the fungal disease, and in sufficient concentrations to allow them to either inhibit or kill the fungi. If the concentration achieved locally is not sufficient, then although the fungus may appear to be susceptible to the agent in vitro, the drug will not be efficacious in vivo, resulting in clinical failure.

Amphotericin B achieves high concentrations in the liver and spleen, and moderate concentrations in the lung and kidney. The data on the involvement of drug transporters in the uptake of this drug into tissues are very limited. Amphotericin B potentiates the effects of various cytotoxic drugs. This may be due to increased cellular permeability or to P-gp inhibition [85]. P-gp is involved in the efflux of drugs from many types of cancer cells, which causes resistance to cytotoxic agents; however, if
amphotericin B inhibits P-gp, this may enhance both the level and the effects of cytotoxic agents in cancer cells. In contrast, it was shown that the coadministration of amphotericin B and cyclosporin in rats caused an induction of multidrug resistance gene 1 (MDR1) expression and higher levels of P-gp, suggesting that amphotericin B may induce P-gp rather than inhibit it [86]. Studies in vitro showed that amphotericin B has no activity as a P-gp inhibitor. For this reason, the extent to which amphotericin B interacts with P-gp is still not clear, and hence its role in the distribution of this drug is also unknown. There are no other data on which drug transporters amphotericin B interacts with, so few conclusions can be drawn about the likely effect of drug transporter polymorphisms on amphotericin B distribution.

Fluconazole has low protein binding and is hydrophilic; hence, it achieves good tissue penetration with high concentrations in cerebrospinal fluid (CSF), saliva, bronchial secretions, and brain [3]. It is not an inhibitor of OATP1B1 or OATP1B3, which are both involved in uptake into the liver. Fluconazole is probably taken up into cells and tissues by passive diffusion and so is unlikely to be affected by genetic variability in uptake transporters.

Itraconazole is found in the highest concentrations in the liver, spleen, kidney, and lung, and in very low concentrations in the eye and CSF. It is known to be a potent substrate and inhibitor of P-gp, which is likely to affect its tissue distribution. Absorption of itraconazole can be variable, and it may be that as a substrate of P-gp, some of the drug is pumped back into the lumen of the intestine by P-gp and hence reduces absorption. Similarly, P-gp acts within the brain to limit drug uptake into brain tissue. In a mouse model, itraconazole was shown to be effluxed from the brain by P-gp; in knockout mice that lacked P-gp, this efflux mechanism was removed, allowing accumulation of itraconazole within the brain [87]. Similarly, an inhibitor of P-gp allowed improved concentrations of itraconazole in a mouse model by overcoming the efflux due to P-gp [88]. The low concentrations of itraconazole seen in the brain and CSF suggest that this may be a possible mechanism responsible in humans, too. In the presence of genetic variants of P-gp, this has the potential to alter both the uptake of itraconazole from the intestine and its access into the brain. Itraconazole is also a substrate or inhibitor of several other drug transporters, including BCRP and MRP3, but at physiological concentrations it does not inhibit the bile salt extract pump (BSEP) or the hepatic uptake transporter Na+ taurocholate-cotransporting peptide (NTCP) [89]. Polymorphisms in BCRP or MDR3 could therefore also impact itraconazole distribution. Based on its interaction with atorvastatin [90] and fexofenadine [91], it has also been suggested that itraconazole may be an inhibitor of OATP1B1, which is important in uptake into the liver, although in vitro data do not support this idea [92,93]. Therefore, because of extensive interactions with drug transporters, there are multiple points at which genetic variability could impact the distribution of itraconazole in the body.

Voriconazole is found at high concentrations in the lung, spleen, liver, and kidney, with moderate concentrations in the brain, eye, and CSF [94,95]. The data on voriconazole’s interaction with drug transporters are currently very limited. Voriconazole does not inhibit P-gp or BCRP, and there is only circumstantial evidence that it may be a substrate for P-gp. Its penetration into brain and CSF tends to suggest that it is not a substrate of P-gp or it would be subject to efflux from the brain and CSF, as is seen for itraconazole. As more information emerges about interactions between voriconazole and drug transporters, it may be possible to determine whether there is potential for genetic variants to impact its distribution; at the moment, however, there is no obvious point where this might occur.

Little is known about the tissue distribution of posaconazole except that it does not achieve high concentrations in CSF, although it achieves concentrations above the MIC for most fungi in various compartments of the lung [96]. Posaconazole is a substrate for P-gp and hence P-gp-mediated efflux may explain some of the absorption problems seen with this antifungal and its low CSF concentration. Based on its structural similarity to itraconazole, it is probably an inhibitor of BCRP; if so, its inhibition of BCRP in the intestine might enhance its own uptake. Potentially, polymorphisms in either P-gp or BCRP might affect the distribution of posaconazole; at the moment, however, the evidence for this is limited.

Caspofungin is a large molecule and highly protein bound; nevertheless, it achieves high concentrations in the kidney and liver, moderate concentrations in the lung and spleen, but only low concentrations in the brain and eye. Its profile in plasma is determined by its rate of distribution into tissues rather than by its metabolism or elimination [97]. Uptake into the liver is a two-step process. The first part is a rapid reversible absorption to the cell surface, followed by a second slower phase with transport across the cell membrane [97,98]. OATP1B1 mediates uptake into hepatocytes, with no uptake by OATP1B3. Caspofungin has a relatively low affinity for OATP1B1 and is also a modest inhibitor of this transporter [98]. It also inhibits NTCP, OCT1, and OAT1 at 100 µM, although at physiological concentrations of 1–10 µM, this inhibition may not occur. Caspofungin has no inhibitory activity against P-gp [98].

The importance of OATP1 in the transport of caspofungin into the liver is illustrated by the interaction with rifampin, which inhibits OATP1 and causes a decrease in the trough levels of caspofungin by 14–31% after multiple rifampin doses [99]. The most important drug transporter for caspofungin is OATP1B1, and so it is only OATP1B1’s genetic variants that may affect caspofungin distribution. Multiple genetic polymorphisms have been described for OATP1B1, including some that occur with relatively high
frequency in several ethnic groups and cause reduced transport-
er activity. Therefore, if any of these polymorphisms occur in patients who are receiving caspofungin, they may potentially affect the drug’s uptake into the liver and hence its distribution.

Micafungin accumulates to high concentrations in the liver, lung, and kidney, and its uptake into the liver is well characterized and involves both passive and active transport. The hepatic uptake transporter NTCP is most important in micafungin uptake, with OATP playing a minimal role. This finding is unusual, as it was previously thought that NTCP was a specific transporter solely for bile salts. P-gp has no involvement in transport of micafungin [100]. NTCP shows genetic variability across different ethnic groups, resulting in reduced activity [101]; thus, individuals expressing variants in this transporter may have altered ability to take micafungin into the liver.

In clinical studies, anidulafungin reached high concentrations in the lung and liver, moderate concentrations in the spleen and kidney, and low concentrations in the brain, CSF, and eye. At concentrations achieved at steady-state (1–3µM), anidulafungin is not a substrate or inhibitor for OATP-B or OATP-C, although at higher concentrations (10µM) it causes inhibition of OATP1B3 [102]. The potential for anidulafungin distribution to be affected by genetic polymorphisms therefore appears to be very limited.

Flucytosine has very low protein binding and is very lipophilic. Because of these two factors, it can achieve good tissue concentrations in CSF, saliva, bronchial secretions, and brain [3]. There are currently no data on the interaction of flucytosine with any of the drug transporters, but because it appears to be taken up by passive diffusion into cells it is unlikely to be affected by genetic uptake transporter variability.

Terbinafine is primarily used for the treatment of superficial fungal infections and so its distribution in other tissues of the body has been little studied. The drug is lipophilic and achieves good concentrations in skin, hair, and nail. It is unlikely to be a P-gp inhibitor because of its lack of interaction with digoxin [10]. However, there are no other data on its interaction with drug transporters. Given this lack of information, it is not currently possible to determine whether polymorphisms in drug transporters may affect terbinafine distribution.

### 38.4.3 Metabolism

Many drugs are lipophilic, which allows them to transit biological membranes but hinders their removal from the body. These compounds must be metabolized to more hydrophilic molecules for excretion. Drug metabolism reactions are classified either as phase I or phase II. Phase I reactions include oxidation, reduction, and hydrolysis and generally result in inactive drugs. Phase II reactions conjugate the result of phase I reactions with a second molecule and result in metabolites with improved water solubility. Phase I reactions are carried out by superfamilies of related enzymes, including CYP450 enzymes and flavin monoxygenases (FMO).

CYP450 enzymes metabolize a wide range of endo-
genous and exogenous substrates, including steroids, fatty acids, vitamins, and a multitude of drugs. Substrate specificity for each enzyme is often very broad—for example, CYP3A4 is responsible for metabolizing about 50% of clinically used drugs, including many immunosuppres-
sives, statins, benzodiazepines, and other drugs. This lack of reaction specificity means that if coadministered drugs are metabolized by the same enzyme, there is substantial potential for drug interaction [103].

The expression of different CYP enzymes varies between tissues. The main CYPs expressed within the intestine are CYP3A, accounting for approximately 80% of total protein, and 2C9, which contributes ~14% [104]. In the liver, CYP3A4 contributes 29% of the total protein content; CYP2C (2C8, 2C9, 2C18, and 2C19), 18%; CYP 1A2, 13%; and other CYPs, smaller amounts [105]. CYP3A4 expression levels vary throughout the length of the intestines, with the highest levels in the duodenum and middle jejunum, declining in the distal jejunum and ileum [106]. As well as this variability in CYP enzyme distribution, enzyme activity can vary significantly between individuals, with up to 20-fold variability in the activity of CYP3A4 [107]. Because of this, the extent of metabolic activity on drugs can be markedly different between individuals and hence the concentrations achieved in the tissues, even though the individuals received the same initial dose.

Compounding this variability in expression and lack of enzyme specificity is the widespread occurrence of genetic variants within the different CYP alleles. Many of the alleles have SNPs that result in reduced, lack of, or occasionally increased metabolic activity. CYP3A4/5 currently has over 40 described allelic variants, 2C9 has more than 60, and 2C19 has more than 30 [108]. The prevalence of these alleles varies with ethnicity, as discussed in Chapter 16. For 2C9, about 80% of the Caucasian population express the wild-type allele (2C9*1A) and about 20% express the allele’s genetic variants [109]. CYP2C19 has several variants that result in a “poor metabolizer” phenotype, where individuals are not able to clear CYP2C19 substrates as well as those who have wild-type alleles and are either heterozygous or homozygous for the wild-type 2C19*1A allele. The incidence of 2C19 poor metabolizers is about 2–5% in Caucasians; in Asian populations this may be 15–20% [110]. The prevalence of polymorphic variants of 3A4 alleles is very low (about 1% in Caucasians), but it has been suggested that some of the activity ascribed to 3A4 is actually due to 3A5, which has a polymorphic allele frequency of 90% in Caucasians [111].
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The other main group of enzymes involved in phase I metabolism are the FMOs. These are involved in the oxygenation of compounds to make them more polar and hence easier to excrete. There are five isoforms of the enzyme, which vary in tissue distribution and activity, and have genetic variants with altered substrate activity [112].

Phase II reactions are carried out by various enzymes, including the glutathione-S-transferases (GSTs), UDP-glucuronosyltransferases (UGTs), and N-acetyltransferases (NAT). These reactions result in the conjugation of a substrate and usually ablate its activity. As with the phase I enzymes, genetic variants have been described that result in changes in the enzymes’ metabolic activity [113].

Some data exist on the effect of genetic polymorphisms on the metabolism of various antifungals. The magnitude of the effect mainly depends on the way in which the drug is metabolized. For drugs that either are not metabolized at all or undergo metabolism independent of the phase I and II enzymes, the potential for genetic variants affecting them is minimal. This includes anidulafungin, amphoterocin B, flucytosine, and fluconazole.

Anidulafungin is unusual among antifungal drugs in that it undergoes a slow nonenzymatic degradation mainly in the tissues, with no involvement of either the phase I or phase II enzymes. Initially, an inactive metabolite is formed that is thought to undergo further degradation by plasma peptidases [114]. Therefore, there are no points at which genetic variants in phase I or II enzymes can impact this process. Unlike anidulafungin, flucytosine and amphoterocin undergo virtually no metabolism and are almost entirely excreted unchanged [6], although very small quantities of metabolites have been described for flucytosine [115]. Because of this lack of metabolism, there is no way in which genetic polymorphisms can affect this process.

However, evidence from drug interactions suggests that amphoterocin B may have various effects on CYP450 enzymes. In animals, amphoterocin B caused reduced concentrations of hepatic CYP enzymes, and in patients with HIV a reduction in CYP enzymes was also reported [116]. The mechanism is unclear and may have been hepatocytic damage rather than a specific effect on CYP. In contrast, amphoterocin B was found to induce CYP3A2 and consequently first-pass metabolism of ciclosporin, which may contribute to decreased levels when amphoterocin B and ciclosporin are coadministered [86]. Nothing is known about its ability to interact with the genetic variants of these CYP enzymes.

Fluconazole is highly water soluble and so circumvents most of the hepatic metabolism needed by the other azoles. It is mainly excreted unchanged in urine, although about 10% is metabolized [117]. Fluconazole is a weak substrate for CYP3A4 [10] and a substrate [118] and inhibitor [119] of UGT2B7, both of which have been shown to have genetic variants with altered enzymic activity. However, as only 10% of fluconazole is metabolized, genetic polymorphisms in CYP3A4 or UGT2B7 are unlikely to affect this process significantly.

Caspofungin is not metabolized by phase I enzymes, but is broken down by peptide hydrolysis and N-acetylation. In the plasma, unchanged caspofungin is found mainly in the first 24 hours, but later M0, a linear peptide, is the main metabolite. In urine, in the initial 24 hours, the parent drug is mainly found, but later the hydrolytic metabolites M1 and M2 are the predominant form [120]. N-acetylation is carried out by NATs and numerous genetic polymorphisms affect their activity [113]. However, there is currently no data on which NAT is involved with caspofungin metabolism, and hence whether genetic variability has any impact on it.

Micafungin is metabolized to an M1 metabolite by arylsulfatase, which is then further metabolized by catechol-O-methyltransferase (COMT) to the M2 metabolite [121]. Micafungin’s side chain is also metabolized by CYP3A to produce a third metabolite, but this is only a minor metabolic pathway [122]. COMT is present in cells either as a soluble form in the cytoplasm or as a membrane-bound form in the rough endoplasmic reticulum. The soluble form is mainly expressed in peripheral tissues. Both arylsulfatase and COMT are polymorphic, which affects their enzymic activity [113]. Because both of the enzymes involved in micafungin metabolism have genetic variants with differing metabolic activity, the potential exists for this to affect an individual’s ability to metabolize micafungin; as yet there are no data as to whether this occurs.

Several antifungal drugs must undergo extensive metabolism before they can be excreted from the body; for them, the potential for genetic variants of the phase I and II enzymes to impact clearance is highest.

Terbinafine is metabolized to five main metabolites by four main metabolic pathways: N-demethylation, deamination, alkylic side chain oxidation, and dihydrodiol formation [45]. The CYP enzymes responsible for each of the metabolic pathways have been defined: N-demethylation is mediated by 2C9, 2C8, and 1A2; deamination, by 3A4; alkylic side chain oxidation, by 1A2, 2C8, 2C9, and 2C19; and dihydrodiol formation by 2C9 and 1A2. Overall, 2C9, 1A2, and 3A4 are the most important isozymes involved in metabolism. All of the CYP isoforms involved are genetically variable [108] and so polymorphisms could affect the metabolism of terbinafine. However, because metabolic activity is spread over a range of isoforms, the potential impact of each polymorphism is limited. As for most other antifungal drugs, there are currently no data on how terbinafine metabolism is affected by CYP polymorphisms.

Of the newer triazoles, posaconazole has the most limited interaction with the CYP enzymes, as it is not metabolized by this route. Indeed, posaconazole does not undergo extensive metabolism, being mainly excreted unchanged, and there are no active metabolites found in plasma [9,32]. The predominant metabolite that is formed is a posaconazole
glucuronide produced by UGT1A4 [123]. UGT1A4 is polymorphic, and some of the resultant enzymes have reduced catalytic activity [124]. This, then, is the only point at which genetic polymorphisms might affect posaconazole metabolism, but because most of the drug is excreted unchanged, their potential impact is limited.

Itraconazole is largely metabolized in the liver, with only 3–18% of the parent drug excreted in the feces. The main metabolic pathways include oxidation and N-dealkylation [125], resulting in at least 30 metabolites, of which hydroxy-itraconazole is microbiologically active and present in higher concentrations than the parent drug [19]. Metabolism is mainly carried out by CYP3A4, for which itraconazole is both a substrate and a potent inhibitor [19]. Initial metabolism results in production of hydroxy-itraconazole, which remains attached to the CYP3A4 enzyme complex which further catalyzes the conversion to keto-itraconazole and then N-desalkylitraconazole. In addition to itraconazole being a potent CYP3A4 inhibitor, its metabolites have a higher inhibitory capacity than the parent drug, which may explain why such potent CYP3A4 inhibition is seen with itraconazole [126]. Because CYP3A4 is the most abundant isofrom in the human intestines, it is estimated that first-pass metabolism may remove up to 50% of the itraconazole dose before it reaches the systemic circulation [18]. Although there are no data in humans, up to 76% of the oral dose and 21% of the IV dose in rats was metabolized by this mechanism, resulting in much lower drug exposure than predicted [127]. This suggests that first-pass metabolism may have significant impact on drug exposure in patients.

Multiple SNPs have been described in CYP3A4, but most of them are low frequency and usually heterozygous with a wild-type allele. However, CYP3A5 is highly polymorphic and, if it is involved in itraconazole metabolism, there is significant potential for genetic variants to cause an impact, both on first-pass and later metabolism; this might go some way to explaining the very variable levels of drug exposure seen in patients. At the moment, there are no data on how these polymorphisms might affect the onward metabolism of hydroxy-itraconazole—the first metabolite formed in the pathway. Because it too is metabolized by CYP3A4, it may be that it has similar potential to be as affected as the parent drug.

In addition, itraconazole is known to be a substrate for UGT1A4, another polymorphic enzyme [118]. The exact role that this enzyme plays in human itraconazole metabolism is not clear, but it too is polymorphic and so is another point at which itraconazole metabolism may be influenced by genetic variability. Although to date there have been no studies examining this area, the data indicating that polymorphisms may be important for itraconazole suggests that such studies may reveal more about itraconazole’s pharmacokinetics.

The last of the antifungal drugs to consider is voriconazole, for which there is the most evidence that genetic variability plays a clinically significant role in its metabolism. Voriconazole is extensively metabolized, with over 75–80% excreted via the urine and 20–25% via the feces [22] as multiple metabolites. The main circulating metabolite is voriconazole N-oxide, which is produced by CYP2C19 and CYP3A4, with 2C19 being high-affinity but low-capacity and 3A4 being a low-affinity but high-capacity. CYP2C9 also plays a minor role in voriconazole metabolism [21]. Another metabolite, 4-hydroxy voriconazole is produced by CYP3A4, and it has been suggested that the abundance of CYP3A4 over CYP2C19 in the liver means that this metabolic pathway is also significant [128]. In addition to CYP-mediated metabolism of voriconazole, which makes up 75% of the metabolic pathway, 25% of voriconazole metabolism is mediated by the FMO enzymes [129]. Although FMOs contribute to the production of voriconazole N-oxide, they are less important than CYP2C19. Voriconazole has recently been found to inhibit UGT2B [130], although the significance of this is unclear.

Patients receiving voriconazole have been shown to have significant variability in their pharmacokinetics, with trough plasma concentrations in one study of 122 patients ranging from <100–9700 mg/L despite the same dosing regimen [131]. CYP2C19 is the most important enzyme involved in voriconazole metabolism and, as discussed previously, its variants give rise to poor metabolizers, heterozygous wild-type metabolizers, and homozygous extensive metabolizers [110]. Voriconazole drug exposure in patients who are poor metabolizers is up to 5 times higher than in those who are homozygous extensive metabolizers [132]. Conversely, the rapid metabolizer phenotype leads to concentrations of voriconazole that are much lower than those seen in extensive or poor metabolizers [133]. Indeed, the variability in drug levels due to polymorphisms in CYP2C19 have been estimated to account for 49% of drug clearance variability. Thus, these polymorphisms are very important in voriconazole pharmacokinetics [134]. However, when multiple CYP genetic polymorphisms coexist, this does not lead to an additive effect on metabolism [135]. In addition to drug exposure variability caused by genetic variants of CYP2C19, there is also variability caused by FMO genetic variants [112], but the extent to which this impacts voriconazole metabolism is unknown.

Because it is known that CYP2C19 variability is important in voriconazole exposure, voriconazole was one of the first drugs for which pharmacogenomic data were included in the manufacturer’s FDA submission. It has also been suggested that genome analysis should be performed in the future for all patients receiving voriconazole, in addition to therapeutic drug monitoring to optimize exposure [136].

For voriconazole, more than for any other drug, understanding how genetic polymorphisms affect its metabolism have been pivotal in explaining the pharmacokinetic variability observed in patients who are treated with it.
38.4.4 Elimination

Elimination of drugs from the body is via the liver or kidneys. In the liver drugs are eliminated with bile via the feces, while in the kidneys they are eliminated via urine. Efflux transporters in the liver and kidneys are therefore critical to removal of drugs from the body. Transporters that are responsible for transit of substances across the canalicular membrane and into the bile include P-gp, BCRP, BSEP, MDR3, and multidrug and toxin extrusion type transporter 1 (MATE1) [137]. In the kidneys, excretion into urine is mediated via various transporters, including OCT1, OCTN2, MATE1, MATE2, P-gp, BCRP, MRP2, and MRP4 [138]. Transporters that are responsible for effluxing drugs into the intestinal lumen include P-gp, BCRP, and MRP2 [73], and these may mediate antifungal drug efflux directly into the feces.

Amphotericin B is predominantly eliminated via the bile, with 43% excreted unchanged, while 21% is excreted via the kidneys. Several drug transporters are important in transporting drugs across the canalicular membrane and into the bile, which suggests that amphotericin B must be a substrate of at least one of them. Limited evidence suggests that amphotericin B may be transported via P-gp [139], and this might represent a route for its elimination via both the liver and kidneys. P-gp polymorphisms could potentially impact elimination if indeed amphotericin B is a substrate of P-gp.

Fluconazole and flucytosine are largely excreted unchanged in the urine. Because of their hydrophilic nature and limited protein binding, they may diffuse into the urine passively. However, at the moment there are no data on whether drug transporters contribute to this process, and hence whether genetic variants influence the elimination of either drug.

Elimination of itraconazole is mainly via the feces as metabolites, with some excreted as either metabolites or unchanged in urine. Itraconazole is a substrate of P-gp and CYP3A4, and up to 50% of it may potentially be excreted directly into bile or feces via first-pass metabolism without absorption. Similarly, itraconazole that has been absorbed is likely to be eliminated via P-gp efflux into the intestines. The route of excretion of itraconazole’s main metabolite, hydroxy-itraconazole, is not well established and so there are no data as to whether it is eliminated via drug transporters or via passive diffusion. P-gp represents one point at which itraconazole elimination may be affected by genetic polymorphisms.

Voriconazole is mainly excreted as metabolites, with 80% in the urine and about 20% in the feces. It is not a substrate for BCRP, and there is only limited evidence that it is a substrate for P-gp [76,140]. Also, it is currently unknown whether any of voriconazole’s metabolites are substrates for drug transporters, but at the moment there is little to suggest that genetic variants impact its elimination.

Posaconazole is predominantly eliminated unchanged in the feces, with some metabolites in the urine. It is a substrate of P-gp, and the presence of large amounts of unchanged drug in the feces suggests that it may undergo some direct efflux from the systemic circulation into the intestines because of P-gp [32]. This may be partly why its absorption is limited. Thus, excretion of posaconazole may be affected by polymorphisms in P-gp, resulting in more or less being left in the circulation depending on the particular effect of the polymorphism.

Caspofungin and anidulafungin are eliminated mainly as metabolites: caspofungin equally in the urine and feces and anidulafungin mainly via the feces. Most of the metabolites for both drugs are peptides, so they are likely to be eliminated by transporters that excrete peptides from the body. It is unknown which transporters eliminate the metabolites from these drugs, and hence whether they have polymorphisms that may affect this process.

Micafungin is eliminated mostly in the bile via the feces. The rate-limiting step in its hepatic elimination is biliary excretion rather than hepatic uptake, and the main drug transporter involved in transport across the canalicular hepatocyte membrane is BSEP, with BCRP having little importance. MRP3 is responsible for transport of micafungin across sinusoidal hepatocyte membranes [100]. Both of these transporters have genetic polymorphisms, and hence there is the potential that the elimination of micafungin is affected by them.

Terbinafine is metabolized to five main metabolites which differ in their excretory route. In plasma, the main metabolite is carboxybutylterbinafine, which, along with the parent drug and three other metabolites, accounts for 80% of the total dose [141]. In urine the major metabolite is demethylcarboxybutylterbinafine, which with the parent drug and three other metabolites accounts for only 14% of the total dose over a 48-hour period. There are currently no data on the interaction of terbinafine with drug transporters, and hence no indication as to whether polymorphisms may affect its elimination.

38.5 CURRENT SUCCESSES IN STRATIFIED MEDICINE

The risk of developing IFD is determined by many factors, including the degree and duration of immunocompromise, the presence of other infections, and underlying disease. As detailed previously, there are various ways of managing patients, including prophylaxis, the enhanced diagnostic approach, and empiric antifungal therapy. Therefore, it has long been understood that not all patients are equally likely to develop infections and so there is a need to quantify each patient’s risk. In one study, this concept of risk stratification was formalized in neutropenic patients by dividing them into four categories: low-risk, intermediate low-risk,
intermediate high-risk, and high-risk [142]. Patients who had lymphoma, autologous HSCT, or childhood acute lymphoblastic leukemia were considered low-risk; those who had been neutropenic for more than 5 weeks, had graft versus host disease, received steroids at doses >2 mg/kg for more than two weeks, and various others were considered high-risk. Using these, the researchers found that the evidence supported prophylaxis in high-risk groups but not in the intermediate or low-risk groups. This risk stratification was evaluated and found to be valid as a way to define high-risk patients in the clinical setting [143].

Stratification has also been helpful in the interpretation of biomarkers. The Aspergillus GM ELISA is used as part of the diagnostic pathway for invasive aspergillosis, but has also been found to have prognostic value. The amount of GM in each sample is expressed as a ratio, with GM indices >0.5 considered positive. The GM index and its rate of decay are also predictive of mortality. If the GM index in the first week increases by 1 unit, the risk of mortality at 6 weeks increases by 25%, whereas each unit of fall decreases the risk of mortality at 6 weeks by 22% [144]. This ability to predict mortality based on the GM index provides another possibility for stratifying patients and in this way appropriately targeting management.

Another developing area in stratified medicine is our understanding of differences in immune recognition mechanisms between individuals that affects their predisposition to fungal disease, particularly invasive aspergillosis. Immune recognition of fungi involves multiple aspects of the immune system. Innate immunity, particularly phagocytic cells, is important in the uptake and clearance of fungi, using a range of ligand–receptor interactions. Triggering of the cytokine release that coordinates the interplay between innate and adaptive immune responses is also important in driving the host response toward either a Th1 pro-inflammatory and usually protective response, or toward a Th2 anti-inflammatory and often unfavorable response. Polymorphisms within many of the genes coding for the innate and adaptive immune responses is also important in determining the right dose, limiting toxicity but achieving efficacy—all informed by an understanding of a patient’s genetic profile—is becoming a more achievable goal. Ultimately this will not only provide personalized medicine for the patient, but also save resources in an era in which financial constraints increasingly influence patient care.

### 38.6 FUTURE PERSPECTIVES

Successful treatment of IFDs relies on patients receiving an antifungal that achieves sufficient drug exposure at the site of infection; therefore, improving the absorption and distribution of drugs is of paramount importance. As we understand more about the pharmacokinetics of antifungals, it is becoming clear that many interact with genetically polymorphic enzymes and/or transporters, which means that there is significant potential for these polymorphisms to affect a drug’s journey through the body. For some drugs, such as voriconazole, polymorphisms in the CYP2C19 enzyme cause most of the variability in drug exposure between patients. For other drugs, there is less evidence for this, but this is probably a result of the lack of relevant studies. A priority now should be to systematically examine the pharmacogenomic data that do exist for all antifungal drugs and conduct clinical studies, including appropriately chosen populations with relevant polymorphisms, to determine how much these polymorphisms impact clinical patient outcomes. Understanding this is pivotal to our ability to tailor the management of each patient and provide truly personalized medicine.

Once such studies have been conducted, the use of next-generation sequencing [146] will allow screening for polymorphisms as part of a patient’s diagnostic workup. For some patients who undergo intensive diagnostic investigations before procedures—for example, tissue typing before HSCT or solid organ transplantation—the addition of screening for specific polymorphisms involved in a patient’s susceptibility to either IFD or drug pharmacokinetics is an obvious additional step. The ability to use the right drug at the right dose, limiting toxicity but achieving efficacy—all informed by an understanding of a patient’s genetic profile—is becoming a more achievable goal. Ultimately this will not only provide personalized medicine for the patient, but also save resources in an era in which financial constraints increasingly influence patient care.

### REFERENCES


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