REVIEW

Has the era of individualised medicine arrived for antifungals? A review of antifungal pharmacogenomics

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Treatment or prophylaxis of invasive fungal infection in recipients of haemopoietic SCT (HSCT) may require management of coexistent malnutrition, organ dysfunction and GVHD, all of which create added potential for inter- and intra-patient variations in drug metabolism as well as drug interactions. Polymorphism is common in genes encoding pathway components of antifungal drug metabolism such as enzymes (cytochrome P450 (CYP450), glutathione S-transferase, N-acetyltransferase and uridine 5’-diphospho-glucuronosyltransferase), uptake transporters (organic cationic transporter, novel organic cationic transporter, organic anion transporter protein (OATP), organic anion transport (OAT), and peptide transporter) and efflux transporters (breast-cancer resistance protein, bile salt export pump (BSEP), multidrug and toxin extrusion type transporter, multidrug resistance protein (MRP), OAT, permeability glycoprotein (P-gp), and urate transporter). Specific polymorphisms may be generalised throughout a population or largely confined to ethnic groups. CYP450 enzymes, especially 2C9 and 2C19, exhibit extensive polymorphism and are central to the metabolism ofazole antifungals and their interactions with other drugs including calcineurin inhibitors, cytotoxics and benzodiazipines. Polymorphism may ultimately affect drug efficacy: CYP2C19 variation leads to a fivefold variation in voriconazole levels between individuals. Anticipated routine provision of pharmacogenomic data in the future for new drugs, together with accumulating knowledge about established agents, challenge physicians to assimilate and apply that information to drug prescribing. Increasing availability of pharmacogenomic data may strengthen demand for rapid turnaround therapeutic drug monitoring of antifungal agents in HSCT recipients.

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Keywords: echinocandin; pharmacogenomics; polyene; therapeutic drug monitoring; triazole

Introduction

Achievements such as the Human Genome Project provide sound foundations for the development of ‘individualised medicine’. Therapy tailored to individual genotype may enable prediction of efficacy and adverse events.¹ The Food and Drugs Administration clearly anticipates such developments and encourages the inclusion of pharmacogenomic data for new drugs² such as voriconazole, one of the first to include these data. Hence, it is timely to review current knowledge of pharmacogenomics of antifungal drugs and consider whether we might soon individually tailor therapy based on our understanding of genotypic variability in patients.

Absorption, distribution and metabolism of drugs

Table 1 presents an overview of factors affecting drug absorption, distribution and metabolism.

Gastric pH varies markedly within the population, with up to tenfold interindividual variation.³ The impact of gastric pH on absorption of antifungal drugs is most clinically relevant for itraconazole and posaconazole. Several antifungals are extensively protein bound, rendering them susceptible to variations in the levels of serum proteins, particularly albumin. Recipients of haemopoietic SCT (HSCT) are prone to malnutrition,⁴ with consequent reduction in serum proteins. At least in the case of itraconazole, this results in increased levels of unbound drug.⁵ Structural variation in plasma proteins, such as is seen in the genetic variants of albumin,⁶ seven may affect substrate binding,⁸ but no relevant data are yet available as to whether this affects antifungal drugs.

Most cellular uptake of drugs occurs by passive diffusion, but some drugs are actively imported, requiring specific transporters (see Table 2) and energy expenditure. Most of the transporters exhibit genetic variants, the relative frequency of which varies with ethnicity.⁹-¹¹ This may be reflected in differing transport characteristics and drug levels among ethnic groups.¹¹ This is relevant to antifungal therapy as several antifungals are substrates or inhibitors of these transporters. The effect of genetic variability of transporters on the cyclodextrin carrier used to solubilise some antifungal drugs is currently unknown.
interindividual variability of CYP450 enzyme activity is influenced by the frequent presence of allelic single-nucleotide polymorphisms (SNPs), some of which result in reduction or ablation of their metabolic activity. CYP2C19 has over 20 polymorphisms that result in truncated or inactive enzyme and one that causes enhanced activity. Other polymorphic cytochromes involved in antifungal drug metabolism include CYP2C9 and CYP3A4.

The cytochrome P450 (CYP450) enzyme system carries out phase I oxidative metabolism of a vast range of endogenous and exogenous substrates, including 75% of all drugs. Drugs metabolised by the same CYP enzymes often interact, and several websites detail the role of CYP enzymes in drug interactions (for example, http://bioinformatics.charité.de/supercyp/index.php?site=home, http://medicine.iupui.edu/clinpharm/ddis/ and http://www.drugbank.ca/). CYP expression varies with site and age (see Table 1) and, in addition, there may be up to 20-fold variability in CYP3A4 activity between individuals. Thus, the same drug given orally to two patients may be subject to different levels of first-pass metabolism and hence achieve very different levels in the circulation. A further complication is the overlapping substrate specificities of CYP3A4 and permeability glycoprotein (P-gp) 

### Table 2  Key uptake and efflux transporters

<table>
<thead>
<tr>
<th>Uptake transporters</th>
<th>Efflux transporters</th>
<th>Comments</th>
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<tbody>
<tr>
<td>OCT1&lt;sup&gt;135,156&lt;/sup&gt;</td>
<td>BCRP&lt;sup&gt;142,143&lt;/sup&gt;</td>
<td>Relative distribution of each transporter varies in intestines, kidney, liver and brain (references describing genetic variants are in bold). As several antifungals interact with these transporters in vitro, potentially they may interact in vivo.</td>
</tr>
<tr>
<td>OCT1&lt;sup&gt;137,138&lt;/sup&gt;</td>
<td>BSEP&lt;sup&gt;144,145&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>OATP&lt;sup&gt;139,140&lt;/sup&gt;</td>
<td>MATE&lt;sup&gt;146&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>OAT&lt;sup&gt;137,140&lt;/sup&gt;</td>
<td>MRP&lt;sup&gt;142,147&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>PEPT&lt;sup&gt;137,141&lt;/sup&gt;</td>
<td>OAT&lt;sup&gt;140,149&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>P-glycoprotein&lt;sup&gt;140,151&lt;/sup&gt;</td>
<td>URAT&lt;sup&gt;140,152&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: BCRP = breast cancer resistance protein; BSEP = bile salt export pump; MATE = Multidrug and toxin extrusion type transporter; MRP = multidrug resistance protein; OAT = organic anion transporter; OATP = organic anion transport protein; OCT = organic cation transporter; OCTN = novel organic cation transporter; PEPT = peptide transporter; URAT = urate transporter.

The prevalence of these polymorphisms varies in different populations and hence the likelihood of expressing a particular genotype/phenotype varies with ethnicity. In Caucasians, ~80% will have wild-type 2C9 alleles and ~20% the
commonly occurring variants, resulting in alterations in their substrate affinity.\textsuperscript{23} For 2C19, the incidence of the ‘poor metaboliser’ phenotype associated with inactive 2C19 protein production is 2–6\% in Caucasians, but 15–20\% in Asians.\textsuperscript{17}

Phase II metabolic processes, or conjugation reactions, are catalysed by a range of enzymes, including glutathione \(S\)-transferase, \(N\)-acetyltransferase and uridine 5'-diphosphogluconuronosyltransferase (UGT). These enzymes are genetically polymorphic,\textsuperscript{24–26} with some of the resultant enzymes having decreased activity.\textsuperscript{26,27} The CYP enzymes are central to the metabolism of azole antifungals, but some of the phase II enzymes have also been shown to be important for certain antifungal drugs.

Absorption, distribution, metabolism and elimination of antifungal drugs

Polyenes

Amphotericin B deoxycholate and the lipid formulations of amphotericin B. Amphotericin B deoxycholate is a polyene antifungal first licensed in the late 1950s, but now little used in its original formulation because of its toxicity. It binds to ergosterol in the fungal cell membrane, resulting in pore formation and leakage of cell constituents.\textsuperscript{28} It binds to ergosterol in the fungal cell membrane, resulting in pore formation and leakage of cell constituents.\textsuperscript{28} The therapeutic advantages provided by the broad spectrum of activity, including yeasts and moulds, are offset by infusion-related toxicity and nephrotoxicity. In contrast, lipid formulations of amphotericin B maintain similar efficacy and are generally associated with fewer toxicities, but are significantly more expensive.\textsuperscript{29} None of the amphotericin B preparations is absorbed orally and they must be given i.v. Amphotericin B is extensively protein bound in serum to lipoproteins via cholesterol,\textsuperscript{30} serum albumin and human \(\alpha1\)-acid glycoprotein,\textsuperscript{31} but liposomal amphotericin largely remains within the liposomes in serum and hence does not appear to be significantly protein bound (Table 3).\textsuperscript{31}

The metabolic pathway of amphotericin B and liposomal amphotericin is largely unknown,\textsuperscript{28,32} rendering assessment of the impact of genetic variation somewhat speculative. In the case of amphotericin B deoxycholate, most of the drug is excreted unchanged in urine or faeces with >90\% recovered within 7 days, suggesting that little metabolic breakdown occurs.\textsuperscript{33} In contrast, <10\% of liposomal amphotericin B is excreted unchanged within 7 days,\textsuperscript{33} possibly reflecting slow tissue release.\textsuperscript{34} No metabolites have been detected from either drug.

There are few data on the interaction of amphotericin B with the CYP system or drug transporters (see Table 4). Amphotericin B was associated with reduced levels of hepatic CYP3A4 in patients seropositive for HIV but this may have been because of nonspecific hepatocyte damage.\textsuperscript{35} There are currently no data on the interaction of liposomal amphotericin and the CYP enzymes or drug transporters.

Amphotericin B deoxycholate reduces oral bioavailability of ciclosporin, an effect observed clinically.\textsuperscript{36} This is mediated via increased expression of the multidrug resistance gene \(MDR1\) in the duodenum causing increased levels of the \(MDR1\) gene product P-gp,\textsuperscript{36} but it is not known if liposomal amphotericin has a similar effect. This results in increased efflux of ciclosporin and reduced plasma levels. Interestingly, ciclosporin is itself an inhibitor of P-gp. The \(MDR1\) gene is polymorphic, raising the possibility of variation in the interaction with amphotericin B. These data suggest a possible interaction between amphotericin B deoxycholate and P-gp, and hence with the genetic polymorphisms seen in this transporter.

Overall, however, there is little evidence to support a clinically significant impact of pharmacogenomic variation upon the therapeutic effects of amphotericin B or its lipid formulations.

Triazole antifungals

The azoles have a common mode of action, inhibiting biosynthesis of ergosterol, critical to the integrity of the fungal cell membrane. Specifically, they inhibit 14\(\alpha\)-sterol demethylase, an enzyme in the conversion pathway of lanosterol to ergosterol;\textsuperscript{37} this demethylase is a member of the CYP450 superfamily found in fungi, designated CYP51A1.

Azoles are both inhibitors and substrates of various human CYP enzymes (Table 4). This may explain, in part, the plethora of drug interactions between azoles and several other groups of drugs, many of which are clinically relevant to the HSCT recipient.\textsuperscript{38}

Fluconazole. Fluconazole is a triazole antifungal, with activity against \(Candida\) and many species of \(Aspergillus\), often with reduced activity against \(Candida\) glabrata and none against \(Candida\) krusei. It is not active against \(Aspergillus\).

Available as oral and i.v. formulations, the oral bioavailability of fluconazole exceeds 90\%,\textsuperscript{39} and is unaffected by gastric pH,\textsuperscript{40} food intake or gastrointestinal disease.\textsuperscript{41} It is hydrophilic with minimal protein binding and therefore achieves good tissue penetration into cerebrospinal fluid, saliva, sputum, vagina, skin\textsuperscript{42} and brain tissue.\textsuperscript{42} Elimination of fluconazole is predominantly renal with no circulating metabolites.\textsuperscript{43}

Fluconazole inhibits, but is not a substrate of, several CYP enzymes (Table 4). It inhibits 2C9, 2C19 and to a lesser extent 3A4,\textsuperscript{44,45} but not CYP1A2,\textsuperscript{46} 2A6 or 2E1. Its ability to inhibit 2C9 and 2C19 causes potential drug interactions particularly relevant to HSCT recipients; for example, the interaction with CY may result in increased formation of toxic metabolites,\textsuperscript{47} although others have noted a protective effect of co-administration.\textsuperscript{48}

Fluconazole is both a substrate\textsuperscript{49} and an inhibitor\textsuperscript{50} of the UGT isoform, UGT2B7, the enzyme involved in its metabolism to the urinary metabolite fluconazole glucuronide.\textsuperscript{43} It also inhibits UGT2B4, hence inhibiting, for example, the glucuronidation of codeine that may potentiate codeine-induced analgesia.\textsuperscript{51}

The limited involvement of CYP and drug transporters in the uptake and metabolism of fluconazole means that their genetic variation has relatively little impact on fluconazole pharmacokinetics.

Itraconazole. Itraconazole is a triazole antifungal, with activity against \(Candida\) species (including some fluconazole-
Table 3  Licensed indications, absorption, distribution, metabolism and elimination of antifungal drugs

<table>
<thead>
<tr>
<th>Antifungal Drug</th>
<th>Licensed indicationsa</th>
<th>Absorption</th>
<th>Distribution</th>
<th>Metabolism</th>
<th>Elimination</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin B</td>
<td>Systemic or deep mycoses; suspected or proven infection in febrile neutropenic patients</td>
<td>Poor oral bioavailability, hence i.v. only—complete absorption</td>
<td>95% protein bound[10]</td>
<td>Very little metabolic breakdown[11]</td>
<td>Largely unchanged in urine (21%) and bile (43%)[31]</td>
<td>Less often used clinically because of nephrotoxicity</td>
</tr>
<tr>
<td>Liposomal amphotericin B</td>
<td>Systemic or deep mycoses; suspected or proven infection in febrile neutropenic patients</td>
<td>Poor oral bioavailability, hence i.v. only—complete absorption</td>
<td>Most remains associated with the liposomes[31]</td>
<td>Very little metabolic breakdown[32]</td>
<td>Slow elimination: after 7 days only 5% excreted in urine and 4% in faeces—remaining drug thought to be in the tissues[33]</td>
<td>Complete elimination from tissues may take several weeks. Aerosolised liposomal amphotericin B has shown promise in preventing aspergillosis[34]. Dose adjustment in renal impairment or use alternative antifungal agent in renal impairment dosage adjustment is required and may be required in hepatic impairment[35].</td>
</tr>
</tbody>
</table>
| Fluconazole      | Invasive candidiasis; treatment and prophylaxis of cryptococcosis; prophylaxis in immunocompromised patients | Good oral bioavailability >90%
[36] | 11–20% protein bound[12] | Limited metabolism[37] | Largely unchanged in urine (80%) with 11% metabolites[38] | In renal impairment dosage adjustment is required and may be required in hepatic impairment[39]. |
| Itraconazole     | Oral and oesophageal candidiasis in immunocompromised patients; prophylaxis in haematology patients and HSCT recipients who are likely to become neutropenic | Gastric pH important; oral bioavailability better with solution than capsules[23,25] | 99% protein bound[27] | Extensive hepatic metabolism via CYP3A4[27] Hydroxy-itraconazole is an active metabolite | As metabolites via urine (35%) and faeces (54%); mainly 3–18% as parent drug detected in faeces[37] | The i.v. prep not recommended in severe renal impairment or haemodialysis; consider dose reduction in hepatic impairment |
| Voriconazole     | Invasive aspergillosis; candidaemia in non-neutropenic patients; fluconazole-resistant invasive candidosis; serious infections caused by Scedosporium and Fusarium. | Good oral bioavailability >90%
[41] | 58% protein bound[35] | Largely hepatic. CYP2C19 and 3A4 are most important[21]. Higher incidence of “poor metabolisers” in Asian, SE Asian and Pacific islander populations[22] | As metabolites in urine (78%) and faeces (23%); only 2% unchanged drug excreted in faeces[33] | The i.v. preparation not recommended in severe renal impairment or haemodialysis; dosage adjustment in hepatic impairment |
| Posaconazole     | Prophylaxis in neutropenic patients undergoing chemotherapy for AML or MDS; patients who have received a HSCT and have GVHD. Invasive aspergillosis in patients intolerant of or refractory to amphotericin B or itraconazole. | Absorption is enhanced by concomitant fatty food intake and acidic gastric environment[49] | 99% protein bound[27] | Mainly via the UGT enzyme pathway, limited role for CYP[49] | 66% excreted unchanged in faeces. Metabolites excreted in faeces (11%) and urine (14%)[49] | Caution in hepatic impairment |
| Caspofungin      | Invasive candidiasis, aspergillosis in patients refractory to or intolerant of amphotericin or itraconazole; empirical therapy in febrile neutropenic patients | Poor oral bioavailability, hence i.v. only—complete absorption | >90% protein bound, mainly to albumin[46] but varies between healthy individuals and patients[99,98] | Peptide hydrolysis and N-acetylation[48] | Metabolites eliminated in urine (41%) and faeces (35%), with little drug excreted unchanged[49] | Dosage adjustment in hepatic impairment |
| Micafungin       | Oesophageal and invasive candidiasis; prophylaxis in adults undergoing allogenic HSCT who are expected to become neutropenic; invasive candidiasis; prophylaxis in children and adolescents undergoing HSCT | Poor oral bioavailability, hence i.v. only—complete absorption | 99% protein bound, mainly to albumin and less to α1-acid glycoprotein[100,101] | Metabolised in liver, but not by CYP[102] | Metabolites excreted in faeces (>90%) and urine (8%); <1% of unchanged drug excreted in urine[103] | Avoid in severe hepatic impairment |
| Anidulafungin    | Invasive candidiasis in non-neutropenic patients | Poor oral bioavailability, hence i.v. only—complete absorption | >99% protein bound[104] | Slow chemical degradation, limited hepatic metabolism[110] | Mainly as metabolites in faeces (>90%) with ~10% excreted as unchanged drug[110] | No dosage adjustment required for renal or hepatic impairment |

Abbreviations: CYP = cytochrome P450; HSCT = haemopoietic SCT; MDS = myelodysplastic syndrome; UGT = uridine 5'-diphospho-glucuronosyltransferase.

aAll licensed indications and dosage adjustments are as in the British National Formulary, March 2011 (http://bnf.org/bnf/index.htm), or Ashley and Currie[49].
Table 4: Interactions of antifungals with cytochrome P450 enzymes and transporters

<table>
<thead>
<tr>
<th>Interaction</th>
<th>Comments</th>
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</thead>
<tbody>
<tr>
<td>Amphotericin B</td>
<td>No inhibition of CYP3A4&lt;sup&gt;460&lt;/sup&gt; or of P-gp in vitro.</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>Potent inhibitor of CYP2C9 and CYP2C19, and less so for CYP3A4. Interaction with other transporters unknown.</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>Substrate for CYP3A4&lt;sup&gt;95&lt;/sup&gt;, potent inhibitor of CYP3A4&lt;sup&gt;460&lt;/sup&gt; and less so for CYP2C9, CYP2C19. Inhibition of P-gp, BCRP, or NTCP.</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>Substrate for CYP2C19, CYP 3A4, CYP2C9&lt;sup&gt;73&lt;/sup&gt; and inhibitor of CYP2C9, CYP3A4 and CYP2B6&lt;sup&gt;110&lt;/sup&gt;. No inhibition of BCRP&lt;sup&gt;113&lt;/sup&gt;.</td>
</tr>
<tr>
<td>Posaconazole</td>
<td>Inhibitor of CYP3A4, but not a substrate of CYP&lt;sup&gt;118&lt;/sup&gt;.</td>
</tr>
<tr>
<td>Caspofungin</td>
<td>Inhibitor of CYP3A4&lt;sup&gt;46&lt;/sup&gt; and NCTP&lt;sup&gt;96&lt;/sup&gt;. Not a substrate for CYP1A2, 2A6, 2C9, 2C19, 2D4 or 3A4. CYP3A4 inhibition by caspofungin causes a 76% decrease in the metabolism of cytarabine. CYP inducers (e.g., rifampin, nevirapine, efavirenz, carbamazepine, dexamethasone and phenytoin) induce the metabolism of caspofungin.</td>
</tr>
<tr>
<td>Micafungin</td>
<td>Slight inhibition of CYP3A4&lt;sup&gt;109&lt;/sup&gt;. No inhibition of P-gp&lt;sup&gt;160&lt;/sup&gt;.</td>
</tr>
<tr>
<td>Anidulafungin</td>
<td>No inhibition of CYP or OATP&lt;sup&gt;109&lt;/sup&gt;.</td>
</tr>
</tbody>
</table>

Abbreviations: BCRP = breast cancer resistance protein; BSEP = bile salt export pump; CYP = cytochrome P450; NCTP = Na–taurocholate cotransporting polypeptide; OAT = organic anionic transporters; OATP = organic anionic transporting polypeptide; P-gp = permeability glycoprotein.

resistant species), *Aspergillus*, *Cryptococcus* and a range of other clinically important fungi.

Itraconazole is available as capsules, an oral solution and an i.v. infusion. The bioavailability of the capsules and oral solution varies significantly with dietary intake. Itraconazole capsules are maximally absorbed with food<sup>42</sup> and acidic drinks (for example, cola<sup>53</sup> or vitamin C drink<sup>54</sup>) at low gastric pH. Oral itraconazole solution has increased bioavailability (∼30-35%) compared with the capsules, but in contrast to the capsules, is maximally absorbed during fasting.<sup>55</sup> Variations in gastric acidity and the widespread use of proton pump inhibitors in HSCT recipients may decrease absorption of itraconazole. Therefore, use of itraconazole oral solution in these patients, which produces higher serum trough concentrations,<sup>56</sup> is preferred.

Itraconazole is also a substrate or inhibitor of several drug transporters (Table 4).

Itraconazole is extensively protein bound, mainly to albumin,<sup>57</sup> and is predominantly metabolised in the liver, mainly by CYP3A4, for which it is both a substrate and an inhibitor.<sup>58</sup> More than 30 metabolites are formed,<sup>57</sup> including hydroxy-itraconazole,<sup>59,60</sup> all of which are inhibitors of CYP3A4 and have a higher affinity for CYP3A4 than itraconazole itself. This may explain the potency of CYP3A4 inhibition seen with itraconazole.<sup>59</sup>

Recently, itraconazole was shown to be a substrate for the phase II enzyme UGT1A4, with an affinity similar to that of the imidazole antifungal agents, and it may also be an inhibitor of UGT1A4.<sup>49</sup>

Itraconazole interacts with several genetically variable drug transporters and CYP enzymes, thus implicating them in the observed variability of itraconazole levels. Moreover, because CYP3A4 and P-glycoprotein also play a crucial role in the metabolism of many other drugs used in HSCT patients, this effect is likely to be compounded. Enhanced understanding of the pharmacogenomics of itraconazole will optimise itraconazole dosing as well as prediction of toxicities with other drugs.

**Voriconazole.** Voriconazole is a triazole antifungal, available as i.v. and oral formulations, with excellent oral bioavailability<sup>61</sup> and highly variable nonlinear pharmacokinetics.<sup>52–64</sup>

Excretion of voriconazole occurs predominantly via metabolites in the urine, with limited faecal excretion.<sup>65</sup> Voriconazole has moderate plasma protein binding, but there are limited data on the interaction of voriconazole with the various drug transporters. Voriconazole may interact with CaMdr1p,<sup>66</sup> the yeast homologue of P-gp, and by implication human P-gp.

Genetic polymorphism in CYP2C19 accounts for 49% of the variance in clearance of voriconazole,<sup>67</sup> with recognition of ’poor metaboliser’ and ‘extensive metaboliser’ phenotypes.<sup>17</sup> Levels of voriconazole in poor metabolisers...
can be 4 to 5 times higher than those in extensive metabolisers. A rapid metaboliser 2C19 phenotype has been described, resulting in levels of voriconazole that are significantly lower than either the extensive or poor metaboliser phenotypes. Interestingly, the coexistence of multiple CYP polymorphisms in the same patient do not necessarily have an additive effect on the metabolism of voriconazole.

Metabolism of voriconazole results in several metabolites, the major circulating metabolite being the N-oxide of voriconazole, formed predominantly by CYP3A4 and 2C19, with a smaller contribution from 2C9. Production of the methyl hydroxylated metabolite is due solely to CYP3A4. CYP450-mediated metabolism of voriconazole is responsible for ~75% of its oxidative metabolism. The remainder is due to flavin-containing mono-oxygenases that also exhibit genetic polymorphism with resultant variations in catalytic activity. Voriconazole is also a low-affinity substrate for UGT1A4.

An understanding of how genetic polymorphism affects voriconazole metabolism has been pivotal in explaining the pharmacokinetic variability observed in patients. The occurrence of genetic variants in the CYP alleles (Table 4) has a significant impact on the levels of drug achieved in those patients and probably upon the therapeutic efficacy of voriconazole.

**Posaconazole.** Posaconazole is a triazole antifungal, currently only available as an oral suspension. Its bioavailability is significantly improved when taken with food (168% compared with the fasting state), especially if the food is high in fat (290% compared with the fasting state). Doses up to 800 mg/day result in dose-proportional pharmacokinetics, but absorption is saturated at 800 mg/day. Absorption of posaconazole is further improved by split-dosing the dose (200 mg four times daily compared with 400 mg twice daily) and administering with an acidic beverage or a nutritional supplement. Concomitant dosing with cimetidine or omeprazole both decrease posaconazole serum trough levels, probably because of reduced gastric acidity, an effect likely to be seen with other H2 receptor agonists or proton pump inhibitors.

Data on interactions with transporters demonstrate that posaconazole is both a substrate and an inhibitor of P-gp. The effect of SNPs in P-gp alleles was studied in 28 black and 28 Caucasian healthy volunteers dosed with 400 mg posaconazole twice daily after a meal. Individuals with a SNP resulting in reduced expression of P-gp (n = 13) demonstrated no difference in the area under the curve compared with those with normal levels of P-gp (n = 43). Therefore, the expression of SNPs in P-gp alleles had no effect on posaconazole pharmacokinetics in healthy individuals, but whether this is also true in HSCT recipients, many of whom may have other issues related to absorption and reduced food intake, is not known. There are as yet no data on the interaction of posaconazole with other transporters in the setting of HSCT.

Posaconazole is extensively bound by plasma proteins mainly by serum albumin.

In contrast to the other triazoles, posaconazole interacts with fewer CYP enzymes (Table 4), possibly explaining its narrower drug interaction range. Posaconazole is excreted largely unchanged; there are no active metabolites and the metabolites seen in urine are glucuronide conjugates, produced by UGT1A4. Genetic variants occur in the alleles for this enzyme, some of which cause reduced catalytic activity. Drug interactions may also occur via the UGT metabolic pathway, including that between posaconazole and phenytoin.

Overall, it is likely that the effect of genetic variation on posaconazole pharmacokinetics is relatively small and more related to absorption than metabolism or elimination.

**Echinocandins**

The echinocandins inhibit β1,3-glucan synthetase, an enzyme involved in fungal cell wall synthesis, leading to increased cell wall permeability and fungal cell lysis. The lack of β1,3-glucan synthetase and cell wall in human cells explains the corresponding lack of toxicity seen with the echinocandins.

**Caspofungin.** Caspofungin is an echinocandin antifungal that is dosed i.v. and is extensively protein bound in plasma, mainly to albumin. Its plasma profile is determined primarily by its rate of distribution from plasma into tissues, rather than by metabolism or excretion. Uptake occurs into hepatocytes via an initial rapid reversible adsorption to the cell surface and a second slow phase of transport across the cell membrane, mediated by organic anion transporter protein 1B1 (OATP1B1). Interactions with other transporters are detailed in Table 4.

Biotransformation of caspofungin occurs primarily through hydrolysis, rather than by oxidative metabolism. An initial open-ring metabolite is found in plasma, with subsequent peptide degradation resulting in low MW products found in urine. Within 4 weeks, 41% of the dose is excreted in urine and 35% in faeces as metabolites.

Caspofungin is the echinocandin with most interaction with the CYP450 system (see Table 4). Rifampin may inhibit the uptake of caspofungin into the tissues via OATP1, with trough levels 14–31% lower after multiple rifampin doses. Genetic polymorphisms of OATP1B1 may affect the efficiency of uptake of caspofungin into the liver. However, there are no other known points at which genetic variability affects caspofungin, and hence pharmacogenomic analysis will be of minor importance in optimising use of this antifungal drug.

**Micafungin.** Micafungin is an echinocandin antifungal that is dosed i.v. It is mainly bound to albumin and binding is independent of micafungin concentration. Hepatocyte uptake is mediated by both active and passive mechanisms. Na–taurocholate cotransporting polypeptide (NTCP) is most important in uptake, whereas secretion across the canalicular hepatocyte membrane is mediated largely by BSEP (bile salt export pump). MRP3 (multidrug resistance protein 3) mediates transport of micafungin across the sinusoidal hepatocyte membrane. Biliary excretion rather than hepatic uptake is thought to be the rate-
limiting step in hepatic elimination of micafungin in humans.\textsuperscript{102} Active transport is utilised for both uptake and excretion of micafungin and may be susceptible to genetic variability, as all the transporters involved in the process are known to be polymorphic.

Metabolism of micafungin occurs mainly in the liver, with inactive metabolites excreted in bile and urine.\textsuperscript{103} Arylsulfatase is involved in micafungin metabolism,\textsuperscript{104} and polymorphisms have been described.\textsuperscript{105} In general, however, pharmacogenomic analysis seems unlikely to add much to the clinical use of micafungin.

\textit{Anidulafungin.} Anidulafungin is an echinocandin antifungal that is dosed i.v. Importantly, anidulafungin has no interactions described with any drug yet studied,\textsuperscript{106} including rifampin.\textsuperscript{107} Anidulafungin is extensively bound by plasma proteins\textsuperscript{108} and undergoes bioransformation, rather than metabolism, undergoing a slow, nonenzymatic degradation to inactive peptides.\textsuperscript{97} Neither phase 1 nor phase 2 hepatic metabolism is involved in elimination of anidulafungin, nor is it a substrate, inducer or inhibitor of CYP450. At physiologically relevant concentrations it does not inhibit OATP.\textsuperscript{109}

Elmination of anidulafungin is via bile in the faeces, with <10% of the drug excreted unchanged in the faeces and <1% in urine.\textsuperscript{107,110}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
\textbf{Antifungal} & \textbf{Target during prophylaxis (mg/L)} & \textbf{Target during treatment (mg/L)} & \textbf{Upper limit of nontoxic range (mg/L)} & \textbf{Studies from which levels are derived} \\
\hline
Itraconazole & 0.5 & 0.5 & 4–5 & Outcome better in aspergillosis,\textsuperscript{102} cryptococcosis\textsuperscript{190} and oral candidosis\textsuperscript{194} with higher serum trough levels. In neutropenic patients, fewer breakthrough infections during prophylaxis with levels >0.5 mg/L.\textsuperscript{106} \\
Voriconazole & 1–2 & 1–2 & 5–6 & Invasive aspergillosis, favourable outcome correlated with levels >2.05 mg/L.\textsuperscript{77} Improved outcome and lower fungal infection-related mortality with trough levels >2.2 mg/L.\textsuperscript{79} \\
Posaconazole & 0.7 & 1.25 & Unknown & Liver toxicity seen in patients with levels >6 mg/L.\textsuperscript{63} and neurotoxicity seen in patients with levels >5.5 mg/L.\textsuperscript{49} \\
\hline
\end{tabular}
\caption{Therapeutic drug monitoring ranges for itraconazole, voriconazole and posaconazole (pre-dose or trough levels)}
\end{table}

Genetic variation of CYP or phase II enzymes or the hepatic transporters is unlikely to affect anidulafungin pharmacokinetics, given their lack of interaction with anidulafungin, and hence pharmacogenomic analysis will not inform clinical use.

\textbf{Therapeutic drug monitoring (TDM)}

TDM is considered necessary for drugs with (1) unpredictable population pharmacokinetics, (2) a narrow therapeutic index and (3) a defined therapeutic range.\textsuperscript{111} Amphotericin B, its lipid formulations and the echinocandins demonstrate predictable pharmacokinetics, and hence TDM is not required. The triazoles are the main agents where TDM is considered helpful in optimising treatment.\textsuperscript{111–113} Although levels of fluconazole can vary significantly between patients, the variability is largely due to renal function, and hence doses are best adjusted according to creatinine clearance.\textsuperscript{114} There is increasing evidence to support TDM in routine use of itraconazole, voriconazole and posaconazole.

The methods available for the measurement of drug levels include bioassays, high-performance liquid chromatography and liquid chromatography–mass spectrometry. Bioassays are inherently unreliable in patients receiving more than one antifungal drug, where the assay will usually only measure total antifungal activity and cannot separate out the activity due to each drug.\textsuperscript{115} The presence of the microbiologically active metabolite of itraconazole, hydroxy-itraconazole, results in artificially elevated results in itraconazole bioassays.\textsuperscript{112} Most laboratories now use either high-performance liquid chromatography or liquid chromatography–mass spectrometry as they are more sensitive, specific and have reduced analytical times. When properly validated, they are capable of measuring the level of an individual drug even in the presence of others. Assessment of a laboratory service for provision of TDM should ensure that the assays are performed regularly and results are communicated in a timely manner to ensure that they are relevant to patient management (see Table 5). The therapeutic ranges used vary between laboratories, but data are accumulating to suggest what the optimal ranges should be.
Table 7  Suggested indications for therapeutic drug monitoring (TDM) of itraconazole, voriconazole and posaconazole

<table>
<thead>
<tr>
<th>Indications for TDM</th>
</tr>
</thead>
<tbody>
<tr>
<td>At 5–7 days after initiation of therapy or dose adjustment</td>
</tr>
<tr>
<td>If an interacting drug is started or stopped*</td>
</tr>
<tr>
<td>During prophylaxis if a breakthrough infection occurs</td>
</tr>
<tr>
<td>Uncertain drug compliance</td>
</tr>
<tr>
<td>Patient exhibits signs or symptoms of toxicity</td>
</tr>
<tr>
<td>Lack of response during treatment</td>
</tr>
<tr>
<td>Reduced oral absorption, for example, diarrhoea</td>
</tr>
<tr>
<td>Patient has inadequate oral nutrition*</td>
</tr>
</tbody>
</table>

*Precise timings of assay will be determined by the individual drug and concomitant medication.

Not usually applicable for voriconazole because absorption is independent of gastric pH.

Table 6 summarises therapeutic ranges for itraconazole, voriconazole and posaconazole, and Table 7 lists the suggested indications for TDM.

Use of TDM is becoming more widespread and data from reference laboratories indicate that therapeutic levels are not achieved in a significant proportion of routine samples. In compliant patients who have levels persistently out of range, there is an argument to support pharmacogenomic analysis of CYPs, for example, 2C19 in the case of voriconazole. In fact, some clinical laboratories are already providing this service.

The inability to predict serum levels from triazole drug doses, the significant potential for drug interactions, the effects of genetic variation on pharmacokinetics and the correlation between improved outcomes with higher serum levels all add to the weight of evidence supporting TDM in HSCT recipients.

Conclusions and future perspectives

Antifungal efficacy in invasive fungal infection is dependent on systemic drug exposure; therefore, maximising the absorption and distribution of antifungals to relevant tissues is essential for favourable treatment outcomes. The potential for genetically induced variation in metabolism of antifungal drugs, and the knock-on effect on systemic drug exposure; therefore, maximising the absorption and distribution of antifungals to relevant tissues is essential for favourable treatment outcomes. The potential for genetically induced variation in metabolism of antifungal drugs, and the knock-on effect on drug interactions, remains largely theoretical, pending systematic laboratory evaluation. Such experience will clarify the relative importance of specific polymorphisms. However, there is already ample evidence of clinically relevant pharmacogenetic influence in the case of the azoles, particularly voriconazole. In contrast, its role in the use of amphotericin B and the echinocandins may be less important, because of their distinct metabolic and elimination pathways.

Genetic variation affects susceptibility to fungal infection, metabolism and drug interactions of antifungals and, it is now emerging, the development of resistance to the azoles in *Aspergillus fumigatus*. Unsurprisingly, mutations of fungal CYP51, a prime target of the triazoles, seem to mediate the predominant resistance pathways.

Large-scale screening for SNPs in relevant human genes, using next-generation sequencing techniques, is now feasible. As scientists unravel the journey of antifungal drugs through the body and the many enzymes and transporters encountered *en route*, we will benefit from a better understanding of how genetic variation affects pharmacokinetics. An individual will have a unique profile of susceptibility to invasive fungal infection, metabolic handling of and response to antifungal therapy. Meaningful analysis of this genomic profile is a tantalising prospect on the horizon, if not quite within our grasp. The transition from horizon to clinic will depend upon a better understanding of the costs and benefits, particularly in the current economic climate.

Conflict of interest

HRA has received speakers fee, travel support or research funding from Pfizer, Gilead UK and Merck Ltd. MHG has received speakers fees or travel support from Pfizer, Gilead UK and Merck Ltd.

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