**Therapeutic drug monitoring for invasive mould infections and disease: Pharmacokinetic and pharmacodynamic considerations**

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**Abstract**

Therapeutic drug monitoring (TDM) may be required to achieve optimal clinical outcomes in the setting of significant pharmacokinetic variability, a situation that applies to a number of anti-mould therapies. The majority of patients receiving itraconazole should routinely be managed with TDM. Voriconazole exhibits highly variable inter-individual pharmacokinetics, and a trough concentration of 1.0–5.5mg/L is widely accepted although it is derived from relatively low-quality evidence. The case for TDM of posaconazole is currently in a state of flux following introduction of a newer tablet formulation with improved oral bioavailability, but it may be indicated when used either for prophylaxis or treatment of established disease. The novel broad-spectrum azole drug isavuconazole does not currently appear to require TDM but 'real-world' data are awaited and TDM could be considered in selected clinical cases. For both polyene and echinocandin agents, there are insufficient data regarding the relationship between serum concentrations and therapeutic outcomes to support the routine use of TDM. A number of practical challenges to the implementation of TDM in the treatment of invasive mould infections remain unsolved. The delivery of TDM as a future standard of care will require real-time measurement of drug concentrations at the bedside and algorithms for dosage adjustment. Finally, measures of pharmacodynamic effect are required in order to delivery truly individualised therapy for patients.

**Introduction**

Dose is a notoriously poor measure of drug exposure, both in individual patients and in larger patient populations. The use of a standard fixed regimen often results in considerable pharmacokinetic variability. While it is possible to estimate pharmacokinetic variability using population pharmacokinetic modelling approaches, the sources of observed variance cannot generally be fully explained by fixed effects (covariates) such as weight, height, ethnicity, pharmacogenetics and organ dysfunction. This limitation is further compounded by the physiological changes which are associated with various stages of illness. Thus, a fixed dosing strategy or a 'one-size-fits-all' approach will always result in some patients experiencing low drug exposure, with an increased probability of concentration-dependent therapeutic failure, while others have higher exposures than intended, placing them at increased risk of toxicity. In the management of infections, the clinical consequences of this variability vary according to the drug-pathogen combination.

The rationale for therapeutic drug monitoring (TDM) of antifungal therapies is the same as for any other therapeutic agent. First, for TDM to be of potential benefit, the drug should have largely unpredictable pharmacokinetic behaviour so that dose alone, or in conjunction with covariates such as renal function or weight, is inadequate to predict safe and effective drug exposure. Second, the drug should have a narrow therapeutic index so that under- or over-exposure could render the treatment ineffective or toxic, respectively. Third, the drug should have a defined concentration range which is associated with a satisfactorily high probability of both safety and efficacy.1 In general, the anti-*Aspergillus* triazoles (i.e. itraconazole, voriconazole, posaconazole) and flucytosine fulfil these criteria. In contrast, the echinocandins and amphotericin B generally do not.2 An understanding of antifungal TDM has been relatively mature for some time, with the possible exception of isavuconazole for which there is relatively limited information.

Here, we review current knowledge regarding TDM of antifungal agents.

**Application of TDM to commonly used antifungal agents**

*Itraconazole*

Itraconazole was the first orally bioavailable antifungal agent with activity against moulds such as *Aspergillus* species. It is indicated for the treatment of oral and oesophageal candidiasis, prophylaxis against fungal infections in the setting of prolonged neutropenia, and for treatment of invasive aspergillosis and cryptococcosis infections that are refractory to first-line therapy.3 The safety profile of itraconazole is less favourable than fluconazole and it has been associated with cardiotoxicity, gastrointestinal intolerance, neurological deficits and hepatitis.3,10 Itraconazole is available as oral capsules, oral solution and an iv preparation, although the latter is not available in the USA.

Itraconazole exhibits highly variable and non-linear pharmacokinetics, the basis for which is multifactorial. Itraconazole solution has an oral bioavailability that is approximately 30% higher than capsules. The absorption of itraconazole from capsules is largely dependent on gastric acidity and as such, capsules should always be administered with food or even an acidic beverage (e.g. cola).10-12 Itraconazole is extensively protein bound in plasma, highly lipophilic and only moderately water soluble.13 It undergoes oxidative metabolism, primarily by the CYP3A4 isoenzyme, which the drug also inhibits, and as a result drug-drug interactions are important.14-16 Alterations in hepatic metabolism may also contribute to pharmacokinetic variability.2

Itraconazole was developed before the advent of modern pharmacodynamic approaches. The pharmacodynamic drug exposure target is quantified in terms of Cmin (i.e. trough concentration) rather than AUC (or AUC:MIC ratio). The Cmin is a relatively pragmatic measure of drug exposure. The PK profile of itraconazole is relatively flat meaning that deviations from true Cmin values are unlikely to be clinically significant. A Cmin range of 0.5–1mg/L, when measured using HPLC/ mass spectrometry,17 is generally used although this target is somewhat arbitrary. Pharmacodynamic targets are based on studies performed in a variety of clinical settings. In neutropenic patients receiving itraconazole for the prevention of invasive fungal infections, Cmin <0.5mg/L is associated with an increased likelihood of breakthrough infections18-20 as well as significantly higher mortality.20 When used for the treatment of oropharyngeal candidiasis, serum itraconazole concentrations <1mg/L are predictive of therapeutic failure.21 Clinical outcomes for patients with cryptococcal meningitis and respiratory coccidioidomycosis are improved with higher steady-state itraconazole concentrations.22 Finally, when itraconazole is administered in patients with invasive aspergillosis23 or histoplasmosis,24 higher Cmin values tend to lead to better clinical outcomes. Importantly, therapeutic targets for TDM of itraconazole were derived in an era before triazole resistance emerged in *Aspergillus*. Such practice is illustrative of the limitations that result from setting pharmacodynamic targets without accounting for the susceptibility of the target organism. The appropriate Cmin target for the treatment of *Aspergillus* with non wild-type MICs is unknown,17 although the majority of isolates with common resistance mechanisms tend to have very high MIC values and are unlikely to be treatable with dosage escalation and a well-demonstrated upper toxicity boundary.10

The majority of patients receiving itraconazole should routinely be managed with TDM. The rationale for this is five-fold: (1) high inherent pharmacokinetic variability of all itraconazole formulations; (2) evidence of clinically relevant drug exposure-response relationships (as summarised above); 3) evidence of drug exposure-toxicity relationships;25 4) the potential for drug-drug interactions14-16 and 5) potential issues with compliance given the unpalatability of itraconazole suspension.10,17 The accumulation of itraconazole occurs slowly and target trough concentrations are only reached after 7–15 days of dosing.26 These estimates are further complicated by overt nonlinear pharmacokinetics in at least some patients, who experience early toxicity due to high Cmin values. Therapeutic Cmin values can only be confidently achieved with the use of an oral loading dose or use of an iv formulation, although this is a relatively uncommon scenario with the advent of new antifungal agents.27 A suggested strategy for TDM of itraconazole is to measure the trough concentration after the first week of therapy and then at regular intervals (e.g. every 1-2 weeks) according to the clinical context (e.g. when interacting drugs are started or discontinued, or if there are concerns about gastrointestinal absorption or compliance). Cmin should be re-estimated 5–7 days following dose adjustment to ensure that target concentrations have been achieved.17

*Voriconazole*

Voriconazole is a structural congener of fluconazole which has a broader spectrum of antifungal activity but maintains the high oral bioavailability of fluconazole.28 It is indicated for the treatment of invasive aspergillosis, invasive candidiasis exhibiting fluconazole resistance, and infections caused by *Scedosporium* spp. and *Fusarium* spp.3 Voriconazole can be used for the prevention of invasive fungal diseases in haematopoietic stem cell transplant recipients.29,30 Voriconazole is available as a tablet, oral suspension, and powder for infusion.3

Voriconazole exhibits highly variable inter-individual pharmacokinetics.31 This variability can be attributed to many factors, including pharmacogenetic polymorphisms, drug-drug interactions, altered gastrointestinal absorption, inflammation and body weight.32 Voriconazole undergoes extensive hepatic metabolism via CYP3A4, CYP2C9 and CYP2C19. Important pharmacogenetic determinants of clearance in CYP2C19 are responsible for ethnic differences in pharmacokinetics.33 Voriconazole exhibits classical nonlinear (Michaelis-Menten) pharmacokinetics in adults as a result of saturable clearance.32 In contrast, young children display linear pharmacokinetics and have higher weight-corrected clearance34 and higher weight-based dosages (9 mg/kg twice daily) are required to achieve pharmacodynamic targets that are associated with therapeutic success.35 There is scant data from neonates and considerable uncertainty about the appropriate regimen in this setting.36 Voriconazole is not widely used in this population because *Aspergillus* is such an uncommon pathogen.

The British Society of Medical Mycology (BSMM) recommends a voriconazole Cmin target for TDM of between 1.0 and 5.5mg/L when the drug is used to treat established invasive infection.17 This target is widely accepted although it is derived from relatively low-quality evidence, based on studies which were limited by the difficulty of estimating voriconazole exposure in individual patients and in controlling for confounding factors that may have an impact upon clinical outcome.17 The majority of studies assessing the utility of voriconazole TDM have been relatively small, were generally conducted at a single centre and are retrospective, and the methodological approaches were somewhat variable.32,37 Two meta-analyses suggest that a target voriconazole concentration of ≥1.0mg/L is predictive of clinical success,32,38 although the more recent of these reported some uncertainty about this estimate.32 For prophylactic use, the target concentration for TDM is less clear. The achievement of serum voriconazole concentrations within the therapeutic range does not appear to predict successful prophylaxis when using absence of breakthrough invasive fungal infection and/or fungal colonisation as the outcome measure.32  If the MIC of the invading pathogen is known, the recommended target for TDM is a Cmin:MIC ratio of 2.0–5.0 (when the MIC is measured according to CLSI methodology). This has been demonstrated using an experimental pharmacodynamic model39 and in a retrospective study.40

Changes in voriconazole concentration occur more quickly than those of itraconazole and posaconazole. Initial serum sampling for TDM is recommended within the first 2–5 days of therapy. If clearance mechanisms are saturated in a particular patient, the initial level may not predict future concentrations, so subsequent sampling should be performed.17,28 This strategy also applies to dose adjustments, initiation or discontinuation of interacting drugs, and changes in clinical circumstances. The timing of repeat sampling to determine voriconazole concentrations is difficult to rigorously define. If a patient is unstable or critically unwell and there is some uncertainty about voriconazole concentrations then repeat sampling may be warranted every 3-5 days. In other circumstances less intensive monitoring may be appropriate.

*Posaconazole*

Posaconazole has broad antifungal activity against medically important fungal pathogens including *Candida* spp., *Aspergillus* spp., *Cryptococcus neoformans* and the Mucorales.41 It is licensed for use in fusariosis as salvage therapy; in invasive aspergillosis that is refractory or intolerant to first-line agents; for chromoblastomycosis and mycetoma when there is resistance and/or intolerance of itraconazole; and for coccidioidomycosis where there is resistance to, or intolerance of amphotericin, itraconazole or fluconazole.3 Posaconazole is available as a solid tablet, an oral suspension and an iv formulation.

Posaconazole is structurally similar to itraconazole. It is highly protein bound and widely distributed in tissues.42 Posaconazole suspension exhibits linear pharmacokinetics with daily doses up to 800mg, beyond which further dose increases do not result in proportional increases in drug exposure.43 It has a Tmax of 5 hours. The oral bioavailability of posaconazole suspension is increased in an acidic environment and in the presence of food.43,44 The long terminal half-life of posaconazole (approximately 34 hours) means that steady-state serum concentrations are only achieved after 1 week.17 Metabolism is primarily by glucuronidation rather than oxidation. Posaconazole inhibits CYP3A4 activity, and as such has a number of clinically relevant drug-drug interactions.45

The case for TDM of posaconazole is currently in a state of flux following introduction of a newer tablet formulation which has improved oral bioavailability. TDM of posaconazole may be indicated when used either for prophylaxis or treatment of established disease. Cmin should be measured 7 days after initiation of therapy or a dosage adjustment. When posaconazole suspension is used, TDM is especially indicated if there are concerns about gastrointestinal absorption and if there is uncertainty about compliance. The oral bioavailability of posaconazole tablets and capsules is better than the suspension although considerable variability is still seen,46 suggesting that TDM should be considered. The tablet and oral suspension formulations of posaconazole are not considered interchangeable due to different dosing and pharmacokinetics.47 For patients with established disease, the probability of a clinical response increases with increasing drug exposure.48-50 For salvage therapy, target trough concentrations should be >1.0mg/L, though this does not incorporate an estimate of MIC.17

*Isavuconazole*

Isavuconazole is a novel broad-spectrum azole drug with activity against yeasts, moulds and dimorphic fungi including *Aspergillus* spp., Mucorales, *Candida* spp., and *Cryptococcus* spp.51-53 It has been approved by the EMA and the FDA for treatment of invasive aspergillosis, and by the EMA for invasive mucormycosis in cases where amphotericin B is inappropriate.51 Isavuconazole is administered as a prodrug (isavuconazonium sulphate) and is available in both oral and iv formulations.54

Isuvaconazole has been shown to be non-inferior to voriconazole for the treatment of invasive disease caused by *Aspergillus* spp. and other filamentous fungi in a Phase III, randomised controlled trial.55 A multicentre, double-blind Phase II trial has demonstrated non-inferiority of isavuconazole versus fluconazole for the treatment of uncomplicated oesophageal candidiasis.56 In the treatment of mucormycosis, the efficacy of isavuconazole is similar to that of amphotericin B and the former is well tolerated.57 Isavuconazole displays high oral bioavailability (nearly 100%), is relatively rapidly absorbed with a Cmax of 2–3 hours after oral administration, and is highly protein bound (around 98%).58 It undergoes hepatic metabolism and has a long half-life (approximately 130 hours). Accordingly, a loading dose of 200 mg isavuconazole is given three times a day for two days, followed by a maintenance dose of 200 mg once daily, with the aim of reaching steady state concentrations at day 3.59 Dose adjustment is not required in renal impairment.58 Relative to other azole antifungals, the pharmacokinetics of isavuconazole is predictable52,60 and are linear up to dosages of 600mg/day. Isavuconazole is better tolerated than voriconazole with statistically fewer visual, skin or subcutaneous tissue and hepatobiliary disorders.55

The case for TDM of isavuconazole is uncertain at the present time. Analysis of clinical trial data does not reveal any relationship between various measures of drug exposure (e.g. AUC, Cmin) and efficacy endpoints (all-cause mortality, clinical response) or safety endpoints), although this information is only available in abstract form at present.61 It is important to recognise that these analyses were performed on the Phase III clinical trial using an optimised regimen. Consequently, there is little opportunity to observe concentration-dependent clinical failures and therefore identify a threshold for TDM. Whether the same conclusion holds with more "real world" data remains to be seen. TDM could be considered in selected clinical cases where drug exposure needs to be confirmed (e.g. severe gut disease from graft-versus-host disease [GvHD] where oral absorption may be problematic, treatment of central nervous system disease, or treatment of a non wild-type fungal pathogen). TDM may also be indicated in circumstances where there is currently little information (e.g. dosing in children or adolescents).

Concentrations of isavuconazole can be determined in a small number of reference laboratories in Europe. There are no algorithms that can be used to adjust dosage. One consideration that is especially pertinent to dosage adjustment of isavuconazole is that although concentrations will begin to change immediately after the dose or dosing interval is amended, the impact of changes will not be fully apparent for 4 weeks (the time to a new steady state), after which many clinically relevant issues will have either progressed or resolved.

*Flucytosine*

Flucytosine therapy is a standard-of-care for cryptococcal meningitis. Flucytosine (5-FC) is a pyrimidine analogue that has no intrinsic antifungal activity, but which is taken up by susceptible fungal cells and deaminated to the active metabolite, 5-fluorouracil (5-FU), by the fungal enzyme cytosine deaminase.62 5-FU is further converted to metabolites which inhibit fungal RNA and DNA synthesis.63 5-FU is active against most *Candida* spp. and *C. neoformans* and has some activity against *Aspergillus* spp. Administration of 5-FC should always be combined with other antifungal agents because of the significant risk of resistance acquisition when used as monotherapy.62 It is indicated as an adjunct to amphotericin for the treatment of some cases of severe systemic candidiasis and is a routine component of induction therapy for cryptococcal meningitis.3

5-FC displays excellent bioavailability (approximately 90%) following oral administration. Protein binding is negligible (2–4%).64 5-FC readily penetrates the blood brain barrier and achieves effective concentrations in the vitreous, peritoneal and synovial fluid, making it valuable for treatment of deep *Candida* infections that are otherwise refractory to first-line agents.17,63,65 Since hepatic metabolism is minimal and more than 90% of the drug is excreted unchanged in urine, dose reduction is required in renal impairment.17,66 In patients with normal renal function, Cmax is reached within 1–2 hours at steady state. The half-life is 3–4 hours in patients with normal renal function but this can increase to several days in those with renal impairment or anuria.67

5-FC exhibits significant inter-patient pharmacokinetic variability.68 A precise target range for serum concentrations of 5-FC for TDM is not universally agreed. Recommendations from the BSMM are based on *in vitro* evidence that yeasts exposed to trough concentrations less than 20–40mg/L develop resistance, and that peak concentrations >100mg/L are associated with myelotoxicity and hepatotoxicity.69,70 The impact of trough level on clinical outcomes is unknown. Moreover, target concentrations for 5-FC in combination with other antifungal agents are not defined. Published data on TDM of 5-FC has revealed alarmingly infrequent achievement of concentrations in this putative therapeutic range, with two studies from the UK revealing <20% of concentrations within range,68,71 and a further study achieving only 64.3% of concentrations within range.72

TDM is standard of care for the use of 5-FC. Serum concentrations should be determined at 72 hours post-therapy initiation; after dose adjustment; if there is uncertain compliance with oral therapy; and if there are clinical or laboratory signs of toxicity. The BSMM recommends an upwards dose adjustment of 50% if levels are sub-therapeutic.17

*Polyenes*

The polyenes comprise amphotericin B deoxycholate (DAmB), licensed for systemic fungal infections; liposomal amphotericin B (LAmB), licensed for severe or deep mycoses where toxicity precludes use of conventional amphotericin B, for infection in febrile neutropenic patients unresponsive to broad-spectrum antifungals and for aspergillosis; and amphotericin B lipid complex (ABLC), for severe invasive candidiasis and systemic fungal infections refractory to conventional amphotericin or where toxicity precludes its use.3

Despite over 50 years of clinical use of DAmB, and 20 years' use of LAmB and ABLC, relatively little is understood about the pharmacology of amphotericin B and the requirement for TDM.73-75 The Cmax and AUC: MIC ratio are recognised as the indices which predict clinical response.76 However, there is currently insufficient evidence to support the routine use of TDM for polyenes.77

*Echinocandins*

The echinocandin class comprises anidulafungin, caspofungin and micafungin. These drugs inhibit synthesis of 1,3-β-D-glucan, an essential component of the fungal cell wall.78 Anidulafungin is indicated for the treatment of invasive candidiasis. Caspofungin can additionally be used to treat invasive aspergillosis and systemic fungal infections in neutropenic patients. Micafungin is licensed for the treatment of invasive and oesophageal candidiasis, and for prophylaxis in the neutropenic phase of bone marrow transplantation.3 The echinocandins offer potential for use against azole-resistant fungal pathogens.78

All echinocandins have low oral bioavailability and are only available for parenteral use.3,78 They exhibit linear pharmacokinetics following iv administration, and distribute into tissues. Central nervous system penetration is possible but only with use of higher dosages than are licensed for the treatment of bloodstream infection.78,79 Drug interactions are less clinically important for the echinocandins than for other agents since they are weak substrates for, and do not inhibit, cytochrome P450 enzymes. Neither are they substrates for the P-glycoprotein transport systems.78,80 As is the case with the polyenes, there is insufficient data regarding the relationship between echinocandin serum concentrations and therapeutic outcomes to support the routine use of TDM for these agents.17,78

**Advancing the field: What are the immediate challenges for individualised antifungal therapy?**

Much has been written about antifungal TDM, and the current state of knowledge is relatively stable. Several guidelines have been developed. What then are the remaining challenges to ensure TDM becomes a practical and more widely used adjunct to the routine use of antifungal agents?

*Challenge 1. Measuring drug concentrations at the bedside.*

In many institutions, the turnaround time for antifungal drug measurement is too slow to be clinically helpful. Measurement requires relatively expensive equipment and highly trained laboratory personnel. TDM services are often centralised, leading to delays in transportation and reporting. A radical change in approach is required to enable measurement of drug concentrations at the bedside or in the clinic. This, in turn, would allow rapid modifications to the antifungal regimen which would be expected to provide a far more significant clinical benefit than waiting a week or longer, as is currently often the case.

*Challenge 2. Development of algorithms for dosage adjustment*

There is a distinct lack of published guidance on how to adjust the dosage of antifungal agents in response to a level that is outside a specified therapeutic range. Some authors suggest changing the dose by 50%. Even for agents with relatively simple pharmacology (e.g. fluconazole, 5-FC), such an approach is crude at best. We have for some time been developing software that enables precise calculation of the regimen which is required to shift a patient from their current state to a new, safer and more effective state. Our approach uses a multiple model approach (BestDose), which is a natural extension of the nonparametric adaptive grid algorithms developed by the Laboratory of Applied Pharmacokinetics in Los Angeles. Other approaches are certainly possible, although the complex pharmacology of almost all antifungal agents (e.g. nonlinear pharmacokinetics) is an obstacle to the use of simple dosage calculators based on simple covariates. Furthermore, the problem of extreme variability in pharmacokinetics becomes significantly heightened in special populations such as neonates and children.

The development of software (or other dosage selection tools) is not trivial. Safe use will require expansion of the currently limited pool of clinicians who are well trained in clinical pharmacology. In addition, there are significant regulatory hurdles to ensure that software developed in academic settings fulfils the necessary standards for use in patients. Nevertheless, we are clear that this is the only path forward. Taking time and effort to measure drug concentrations in the laboratory with a coefficient of variation <10% and then guessing the dosage seems haphazard at the least, and in our view is completely unacceptable for clinical practice.

*Challenge 3. Real-time pharmacodynamic monitoring in patients with invasive mould disease*

Current paradigms of drug concentration measurement and dosage adjustment are somewhat crude and counter to notions of truly individualised therapy. Considerable energy has been expended on developing population pharmacokinetic models which estimate drug exposure in individual patients with a high degree of precision. Interpretation of the result is then made with reference to a concentration target range that is constructed from a *population* of patients (usually determined using logistic regression models or classification and regression tree analysis). Such an approach is necessarily a violation of any principle of individualised dosing, whereby a patient should receive the right dose for their given disease. Some patients will require more drug, others will require less. Consider the differences in a patient that has been neutropenic for 6 weeks who has disseminated aspergillosis with bulky disease near the mediastinum and a small CNS lesion on CT, compared to a patient who received an allogeneic transplant 120 days ago and is well and currently an outpatient, but has a small nodule on a CT chest. Clearly these patients are different and require different intensities of therapy. Yet, a TDM laboratory will report the same range for antifungal therapy and dosage adjustment.

Recently, we have been investigating the use of galactomannan (GM) to follow the pharmacodynamic effect of antifungal therapy. This is not necessarily a new concept. While much early work on GM focused on its role as a diagnostic modality, there are many reports which suggest that high unremitting GM levels are associated with poor clinical outcomes, including death.81-84 Thus, circulating GM is a means by which patients can "tell" their physician how much drug they need, without recourse to therapeutic ranges that are constructed from populations of patients. Accordingly, a patient with unremittingly high GM levels needs more drug, regardless of the measured plasma drug concentration and whether it is deemed "therapeutic". If this is not possible because of toxicity, or if dosage escalation and an increase in serum concentration does not result in normalisation of the biomarker, then a second drug needs to be added or the therapy needs to be changed altogether. Such an approach constitutes true individualised therapy. Both a fixed dosing strategy and a therapeutic concentration range is a 'one size fits all' approach, and neither is consistent with any notion of individualised antifungal therapy.

This is also an argument for the development of biomarkers with prognostic value that can be used to follow the disease course. Medical mycology is full of such possibilities, some of which are already established (e.g. use of quantitative CSF counts of 1,3-beta-D-glucan to monitor therapy for cryptococcal meningitis).85 Most fungi produce a myriad of secondary metabolites that can potentially be used to track the effect of antifungal therapy, and the same could apply to transcriptomic and metabolomic approaches as they are further developed. Volumetric radiological analyses are a further extension of this idea.

**Conclusion**

Much remains to be done to ensure that patients with rapidly advancing life-threatening fungal infections are treated in an optimal manner. Such challenges include the development of methods to measure plasma concentrations of antifungal agents at the bedside in a timely manner, algorithms to enable rapid and precise dosage adjustment, and mechanisms by which the treatment of infection can be followed and managed in real-time. The last point remains an important challenge to enable true individualised therapy for patients with invasive fungal diseases.

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