In-vitro pharmacokinetic/pharmacodynamic model data suggest a potential role of new formulations of posaconazole against *Candida krusei* but not *Candida glabrata* infections

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**A R T I C L E   I N F O**

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**A B S T R A C T**

Posaconazole exhibits in-vitro activity against *Candida* *glabrata* and *Candida* *krusei*. Epidemiological cut-off values set by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI) are 1/1 and 0.5/0.5 mg/L, respectively, but clinical breakpoints have not been established to date. This study explored the pharmacodynamics (PD) of posaconazole in a validated one-compartment in-vitro pharmacokinetic (PK)/PD model, and determined the probability of PK/PD target attainment (PTA) for the available formulations. Five *C. glabrata* and three *C. krusei* isolates with posaconazole minimum inhibitory concentrations (MICs) of 0.06–2 and 0.03–0.25 mg/L, respectively, were tested in the PK/PD model simulating different time–concentration profiles of posaconazole. The exposure–effect relationship $\frac{AUC_{0-24}}{MIC}$ was described for EUCAST/CLSI methods, and PTA was calculated in order to determine PK/PD susceptibility breakpoints for oral solution (400 mg q12h), and intravenous (i.v.)/tablet formulations (300 mg q24h). Fungicidal activity (–2log kill) was found against the most susceptible *C. glabrata* isolate alone, and against all three *C. krusei* isolates. The responding EUCAST/CLSI PK/PD targets ($\frac{AUC_{0-24}}{MIC}$) were 102/79 for *C. glabrata* and 12/8 for *C. krusei*. Mean PTA was high (≥95%) for *C. glabrata* isolates with EUCAST/CLSI MICs ≤0.03/0.03 mg/L for oral solution and ≤0.125/≤0.125 mg/L for i.v. and tablet formulations for the wild-type population. For *C. krusei* isolates, mean PTA was high (≥95%) for EUCAST/CLSI MICs ≤0.25/≤0.5 mg/L for oral solution and ≤1/≤2 mg/L for i.v. and tablet formulations for the wild-type population. The use of posaconazole to treat *C. glabrata* infections is questionable. Intravenous and tablet formulations may be therapeutic options for the treatment of *C. krusei* infections, and oral exposure can be optimized with therapeutic drug monitoring (trough levels >0.6–0.9 mg/L).

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1. Introduction

Azole-resistant candida infections remain a significant clinical challenge, particularly when involving a multi-drug-resistant phenotype. *Candida glabrata* and *Candida krusei* account for 5–30% of all bloodstream fungal infections, and are intrinsically less susceptible or resistant to fluconazole [1]. *C. glabrata* has become the most common non-*Candida albicans* pathogen, ranking second to *C. albicans* as the cause of invasive candidiasis in the USA [2] and northern Europe [3,4]. *C. krusei* infections are associated with higher mortality in both intensive care unit (ICU) and non-ICU patients [5].

*C. krusei* is intrinsically resistant to fluconazole, whereas *C. glabrata* acquires fluconazole resistance rapidly afterazole exposure due to overexpression of efflux pumps, or the azole target gene erg11 demonstrating cross-resistance to other azoles such as voriconazole [6]. In recent years, the emergence of resistance to echinocandins (8–15%) [7,8] has limited alternative treatment options for these infections to amphotericin B, which is only available...
as an intravenous (i.v.) formulation, thus prohibiting outpatient or stepdown treatment. Moreover, multi-drug-resistant isolates of C. glabrata and C. krusei demonstrating resistance to several classes of antifungal drugs, including amphotericin B, have been reported occasionally, leaving no therapeutic options against these infections [9,10].

Posaconazole is an extended-spectrum triazole licensed for first-line therapy of oropharyngeal candidiasis and prophylaxis of invasive fungal infections, including candida infections. It exhibits in-vitro activity against C. glabrata and C. krusei isolates, with identical epidemiological cut-off values (ECOFF/ECV) set by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [20] and the Clinical and Laboratory Standards Institute (CLSI) [24]: 1 mg/L for C. glabrata and 0.5 mg/L for C. krusei. Of note, posaconazole appears to be active against some strains resistant to fluconazole and voriconazole, and displays greater in-vitro fungicidal activity against C. krusei strains [11,12]. Nevertheless, the clinical significance of this enhanced antifungal activity is unknown due to the absence of established clinical data. In addition, as posaconazole in oral solution demonstrates significant pharmacokinetic (PK) variability, with many patients having low serum drug exposure, its role in the treatment of C. glabrata and C. krusei infections was limited. However, the recently developed i.v. [13] and delayed-release tablet [14] formulations have less variable PK, and higher exposures potentially represent a new option for the treatment and prevention of azole-resistant candida infections.

This study investigated the pharmacodynamics (PD) of posaconazole against C. glabrata and C. krusei isolates using a previously validated in-vitro PK/PD dilution model simulating the PK of posaconazole [15]. PK/PD susceptibility breakpoints were determined for EUCAST and CLSI methodologies, and the area under the concentration–time curve (AUC) and trough plasma levels of posaconazole for optimal treatment were determined in relation to minimum inhibitory concentrations (MICs).

### 2. Materials and methods

#### 2.1. Candida isolates

Five clinical C. glabrata (three fluconazole-resistant with EUCAST MIC >16 mg/L; two fluconazole-susceptible, increased exposure with MICs of 2 and 16 mg/L, respectively) and three clinical C. krusei isolates with posaconazole EUCAST [16] and CLSI [17] MICs ranging from 0.06 mg/L to 2 mg/L for C. glabrata and from 0.03 to 0.25 mg/L for C. krusei were studied. All isolates were susceptible (wild-type) to micafungin. The median (range) EUCAST and CLSI MICs are shown in Table 1. The isolates were stored in normal sterile saline with 10% glycerol at −70 °C, and revived by subculturing on Sabouraud dextrose agar (SDA) plates supplemented with gentamicin and chloramphenicol (SGC2; bioMérieux, Marcy l’Etoile, France) to ensure purity and viability. Inoculum suspensions were prepared in normal sterile saline from 24-h cultures and adjusted to a final inoculum of 10⁴ colony-forming units (CFU)/mL in the in-vitro model using a counting chamber. The number of CFU was confirmed by quantitative cultures on SDA plates. The in-vitro PK/PD model used 10⁴ CFU/mL as this is the starting inoculum in animal models of experimental candidiasis.

#### 2.2. Antifungal drugs and medium

Pure posaconazole powder (Merck Sharp & Dohme, Kenilworth, NJ, USA) was dissolved in sterile dimethyl sulfoxide (Carlo Erba Reactifs–SDS, Val de Reuil, France), and stock solutions of 3.2 mg/mL were stored at −70 °C until use. The medium used in the in-vitro PK/PD model was RPMI 1640 (with L-glutamine, without bicarbonate) buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS, AppliChem GmbH, Darmstadt, Germany), supplemented with 100 mg/L chloramphenicol (AppliChem GmbH, Darmstadt, Germany).

#### 2.3. In-vitro PK/PD model

A previously validated one-compartment PK/PD dilution model simulating in-vivo PK was used [15]. Briefly, the model consists of a 100-mL culture vessel (conical glass flask) containing fresh RPMI-1640 medium to an initial volume of 30 mL for each C. glabrata or C. krusei isolate and posaconazole dosing regimen. The culture vessel is connected to a peristaltic pump (Minipuls Evolution; Gilson Inc., Middleton, WI, USA), adding fresh medium in order to dilute its content at a rate equal to the clearance of posaconazole in human plasma. Preliminary experiments using dialysis tubes in order to avoid dilution of fungi were unsuccessful in simulating the PK of posaconazole due to posaconazole binding to the cellulose tubes. Therefore, a one-compartment dilution model was used in this study, with the volume of the internal compartment increased over time to approximately 120 mL at 48 h [15].

#### 2.4. In-vitro pharmacokinetics

Previously reported steady-state posaconazole plasma concentration–time profiles in patients treated with posaconazole were simulated in the in-vitro PK/PD model targeting free (unbound) maximum plasma concentrations (Cmax) of 0.15, 0.85 and 2.25 mg/L q12h and a half-life (t1/2) of 36 h [18]. Higher posaconazole exposure of Cmax 5 mg/L was also evaluated in order to better describe the exposure–efficacy relationship. The simulated time–concentration profiles were chosen to simulate different 24-h drug exposures observed clinically that will help to

### Table 1

<table>
<thead>
<tr>
<th>Isolate no.</th>
<th>Reference code$^a$</th>
<th>EUCAST MIC (mg/L)</th>
<th>CLSI MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida glabrata 3</td>
<td>SSI-64.37</td>
<td>0.06 (0.06–0.125)</td>
<td>0.125 (0.03–0.25)</td>
</tr>
<tr>
<td>Candida glabrata 11</td>
<td>SSI-60.02</td>
<td>0.25 (0.25–0.5)</td>
<td>0.25 (0.25–1)</td>
</tr>
<tr>
<td>Candida glabrata $^b$</td>
<td>SSI-61.31</td>
<td>0.5 (0.25–2)</td>
<td>0.5 (0.25–1)</td>
</tr>
<tr>
<td>Candida glabrata 6$^e$</td>
<td>SSI-63.71</td>
<td>1 (1–2)</td>
<td>1 (0.25–2)</td>
</tr>
<tr>
<td>Candida glabrata 8$^e$</td>
<td>SSI-6039</td>
<td>2 (0.5–4)</td>
<td>2 (0.5–2)</td>
</tr>
<tr>
<td>Candida krusei 1</td>
<td>SSI-7327</td>
<td>0.03 (0.01–0.125)</td>
<td>0.06 (0.06–0.125)</td>
</tr>
<tr>
<td>Candida krusei 7</td>
<td>SSI-6300</td>
<td>0.06 (0.03–0.125)</td>
<td>0.125 (0.125)</td>
</tr>
<tr>
<td>Candida krusei 4</td>
<td>SSI-7326</td>
<td>0.25 (0.06–0.25)</td>
<td>0.25 (0.06–0.25)</td>
</tr>
</tbody>
</table>

$^a$ Fluconazole-resistant C. glabrata isolates.
$^b$ SSI, Staten Serum Institute, new coding system.
describe the exposure–effect relationship of posaconazole, rather than to simulate certain time–concentration profiles of specific dosing regimens. As AUC/MIC is the PK/PD driver for posaconazole, AUC\textsubscript{0-24} is more important than the exact shape of simulated time–concentration profiles. Repeated sampling of 100 μL was made from the culture vessel and posaconazole levels were measured with a microbiological agar diffusion bioassay using a wild-type azole-susceptible *Aspergillus fumigatus* AZN8196 (CLSI MIC 0.03 mg/L), as described previously [15]. In order to prevent any loss of drug, samples were spiked with 25% human serum and stored at -70 °C until testing [15]. IntereXperimental variability was assessed in replicate experiments.

2.5. In-vitro pharmacodynamics

To estimate the fungal load inside the culture vessel for each posaconazole dosing regimen, 200-μL samples were collected at regular intervals up to 48 h, serially diluted 10-fold in normal saline and subcultured on SAB plates. Plates were incubated at 30 °C for 24 h and colonies were counted at each dilution. The quantitative cultures of the dilution that yielded 10–50 colonies were used to determine the log\textsubscript{10} CFU/mL at each time point. Time-kill curves were constructed by plotting log\textsubscript{10} CFU/mL over time.

2.6. In-vitro PK/PD analysis

The PK/PD index fAUC\textsubscript{0-24}/MIC ratio was calculated for each simulated dose, isolate and experiment. The drug exposure–response relationship, expressed as 48-h log\textsubscript{10}CFU/mL reduction for each dosing regimen and isolate compared with values at the start of therapy vs. fAUC\textsubscript{0-24}/MIC, was analysed with non-linear regression analysis using a sigmoidal model with variable slope (Emax model), as described previously [15]. As the volume of the in-vitro PK/PD model increased over time, a similar analysis was performed using the actual log\textsubscript{10}CFU after multiplying CFU/mL with the volume at each time point. The PK/PD index corresponding to 50% of Emax-Emin (E\textsubscript{50}) was determined for each species. All data were analysed using GraphPad Prism Version 5.0 for Windows (GraphPad Software, San Diego, CA, USA).

2.7. Monte Carlo simulation

In order to bridge in-vitro PK/PD data with human PK, Monte Carlo simulation analysis was performed using the ‘random number generator’ function of Excel (Microsoft Corp., Redmond, WA, USA) for 5000 patients receiving either oral posaconazole 400 mg q12h or the new i.v. or tablet formulations of posaconazole 300 mg q24h. These regimens correspond to steady-state mean±standard deviation (SD) tAUC\textsubscript{0-24} (trough levels) of 17.26±14.82 mg h/L (0.72±0.63 mg/L) for oral q12h dosing [18], 34.3±12.3 mg h/L (Cmin 1.07±0.58 mg/L, Cavg 1.42±0.60 mg/L) for i.v. dosing, and 35.14±31.4 (Cavg 1.46±0.55 mg/L) for tablet dosing [13,14]. The probability of target attainment (PTA) of E\textsubscript{50} AUC\textsubscript{0-24}/MIC for the simulated oral q12h, and i.v. and tablet q24h dosing regimens was determined for different MICs ranging from 0.015 to 8 mg/L for each of the two methodologies. Published MIC distribution data for *C. glabrata* and *C. krusei* isolates with CLSI [19] and EUCAST [20] were used. The present authors have shown previously that the static exposure of posaconazole was the same with and without serum, and hence fAUC/MIC can be equated to tAUC/MIC from a PK/PD perspective [15].

2.8. Trough levels and MIC correlation

The trough levels in human plasma to attain E\textsubscript{50} for oral q12h and i.v. and tablet q24h regimens were calculated for different MICs. Corresponding trough levels associated with these target values were determined for isolates with different EUCAST and CLSI MICs based on steady-state AUC/trough plasma ratios published previously for oral (AUC/trough=13) [18] and i.v. and tablet (AUC/trough=32) [13,14] dosing regimens.

3. Results

3.1. In-vitro pharmacokinetics

Fig. 1 shows the different time–concentration profiles of posaconazole simulated in the in-vitro model. The mean±SD fCmax (mean of all 12-h experiments±SD) values were 0.56±0.23, 1.67±0.77 and 3.43±1.80 mg/L, with mean±SD AUC\textsubscript{0-24} (mean of all 24-h experiments±SD) values of 4.82±0.89, 15.09±6.50 and 20.86±2.33 mg h/L, respectively, and mean±SD t\textsubscript{1/2} of 15.6±7.7 h for all species and isolates. The lower average t\textsubscript{1/2} was due to a rapid decline in drug concentration. However, as AUC/MIC is the PK/PD driver, this deviation did not have any impact on the PK/PD relationship as the AUCs were determined for each simulated dose, and any deviation from target values was adjusted.

3.2. In-vitro pharmacodynamics

*C. glabrata* (Fig. 2) and *C. krusei* (Fig. 3) isolates grew equally well from mean±SD 4.01±0.01 log\textsubscript{10}CFU/mL at t=0 h to 8.60±0.02 log\textsubscript{10} CFU/mL at t=48 h in drug-free controls. For *C. glabrata* isolates, no antifungal effect (4 log\textsubscript{10}CFU/mL increase) was found against isolates with EUCAST/CLSI MICs of 1 and 2 mg/L at any concentration tested, whereas a fungicidal effect (2.5 log\textsubscript{10}CFU/mL reduction) was only found against the isolate with the lowest EUCAST/CLSI MIC of 0.06/0.125 mg/L at fCmax 2.25 mg/L (Fig. 2). On the other hand, for *C. krusei* isolates, posaconazole reduced the fungal burden by 1.3–1.8 log\textsubscript{10}CFU/mL for isolates with EUCAST/CLSI MICs of 0.03/0.06 and 0.06/0.125 mg/L, while the same effect was only observed for the isolate with EUCAST/CLSI MIC of 0.25 mg/L at fCmax 5 mg/L (Fig. 3). Regrowth occurred for some isolates and concentrations (e.g. *C. krusei* 7 at fCmax=2.25 mg/L) which merits further investigation. When dilution of CFUs was taken into account and log\textsubscript{10}CFU was analysed, time-kill curves were shifted upwards by <0.5 log\textsubscript{10}CFU due to a maximal four-fold increase in the volume of the central compartment at 48 h (Figs S1 and S2, see online supplementary material). The 48-h change in log\textsubscript{10}CFU/mL vs. fAUC\textsubscript{0-24}/MIC relationship for the *C. glabrata* and *C. krusei* isolates is displayed in Fig. 4. The in-vitro PK/PD relationship followed a sigmoid curve for *C. glabrata* (R\textsuperscript{2}=0.64–0.74) and *C. krusei* (R\textsuperscript{2}=0.91–0.95), with mean [95% confidence interval (CI)] EUCAST/CLSI E\textsubscript{50} of 102 (58–179)/79 (45–138) and 12 (7–19)/8 (4–15).

![Fig. 1. Time–concentration profile of simulated q12h oral dosing regimen of posaconazole against Candida glabrata and Candida krusei isolates in the in-vitro pharmacokinetic/pharmacodynamic model with target fCmax of 0.85, 2.25 and 5 mg/L and half-life of 15.6±7.7 h. Errors bars represent standard errors.](image-url)
fAUC/MIC, respectively (Fig. 4). As MICs following CLSI recommendations are slightly different from those following EUCAST recommendations for both C. glabrata and C. krusei isolates, both relationships are shown. Similar PK/PD targets were found when log_{10} CFUs were analysed taking into account the dilution of CFUs (Fig. S3, see online supplementary material).

3.3. Probability of target attainment

Figs 5 and 6 show PTA using the PK/PD targets of 102/79 for EUCAST/CLSI and C. glabrata and 12/8 for EUCAST/CLSI for C. krusei. Fig. 5 shows PTA for C. glabrata isolates, respectively. PTA is shown for the standard oral
400 mg q12h dosing regimen, as well as for the newer i.v. and tablet 300 mg q24h dosing regimens. For *C. glabrata* isolates, fAUC/MIC of 102/79 for EUCAST/CLSI was attained for isolates with EUCAST/CLSI MIC ≤0.03 mg/L for most simulated patients (≥95%) treated with the standard oral 400 mg q12h dosing regimen, and for isolates with EUCAST/CLSI MIC ≥0.125 mg/L for most (≥95%) simulated patients treated with the i.v. or tablet 300 mg q24h dosing regimen. PTA was 0–1% for the current EUCAST/CLSI epidemiological cut-off value of 1 mg/L for both the standard oral, i.v. and tablet formulations of posaconazole.

On the other hand, for *C. krusei* isolates, fAUC/MIC of 12/8 for EUCAST/CLSI were attained for isolates with EUCAST/CLSI MICs ≤0.25/≤0.5 mg/L and ≤1/≤2 mg/L for most (≥95%) simulated patients treated with the standard oral 400 mg q12h dosing regimen or the i.v. or tablet 300 mg q24h dosing regimen, respectively. However, considering the lower 95% CI of mean PTA, this was >95% for isolates with EUCAST/CLSI MICs of ≤0.5 and ≤1 mg/L for the i.v. and tablet 300 mg dosing regimens alone.

3.4. Trough levels and MIC correlation

Posaconazole trough levels required to attain the PK/PD targets determined in the in-vitro model for the two species are shown inFig. 7. For *C. glabrata* isolates, the PK/PD target could be attained safely for isolates with EUCAST/CLSI MICs of 0.25 mg/L with stable trough levels of 0.61–1.1 mg/L (upper 95% CI limit 1.7 mg/L) for all dosing regimens. For isolates with EUCAST/CLSI MICs of 0.5 mg/L, stable trough levels of 1.2–1.6 mg/L (upper 95% CI limit
Fig. 6. Target attainment rates for 5000 patients receiving either (A) standard oral posaconazole 400 mg q12h or (B) intravenous or tablet formulations 300 mg q24h for which the area under the curve was simulated with Monte Carlo analysis for different Clinical and Laboratory Standards Institute (CLSI) minimum inhibitory concentrations (MICs). Dotted lines represent the 95% confidence interval (CI) of probability of target attainment (PTA) calculated using the 95% CI limit of EL₅₀ obtained from non-linear regression analysis of exposure–effect relationships for each Candida species. Horizontal lines represent 95% PTA. Grey lines indicate CLSI MIC distributions. ECV, CLSI epidemiological cut-off value.

Fig. 7. Target values for therapeutic drug monitoring of posaconazole. (A) Oral 400 mg q12h dosing regimen for Candida glabrata and Candida krusei isolates. Standard oral dosing is sufficient to cover the entire C. krusei wild-type distribution for both European Committee on Antimicrobial Susceptibility Testing (EUCAST)/Clinical and Laboratory Standards Institute (CLSI) [epidemiological cut-off value (ECOFF)/epidemiological cut-off value (ECV)] = 0.5 mg/L, but not the C. glabrata wild-type distribution for EUCAST/CLSI (ECOFF/ECV = 1 mg/L). (B) Intravenous (i.v.) or tablet 300 mg q24h dosing regimens. C. krusei isolates with EUCAST/CLSI MICs up to 4 mg/L can be treated adequately, whereas for C. glabrata isolates with EUCAST/CLSI MICs of 0.5 mg/L, pharmacokinetic/pharmacodynamic targets can be attained with stable trough levels of 1.2–1.6 mg/L for i.v. and tablet formulations.
2.8 mg/L) for the i.v. formulation will be required, whereas the required concentrations will not be clinically achievable for oral dosing; the difference in target trough levels is due to the different AUC/ trough ratios for the two dosing regimens. Isolates with EUCAST/CLSI MICs >0.5 mg/L would require clinically unachievable concentrations with either formulation, thus verifying the 0% PTA mentioned above.

On the other hand, C. krusei isolates with EUCAST/CLSI MICs up to 2/2 mg/L for oral solution and EUCAST/CLSI MICs up to 4/4 mg/L for i.v. and tablet formulations can be treated effectively by targeting trough levels of 0.6–0.9 (upper 95% CI limit 1.5 mg/L) and 1.0–2.1 (upper 95% CI limit 3.3 mg/L), respectively (Fig. 7).

4. Discussion

This study showed that the probability of attaining the PK/PD target for posaconazole was very low for wild-type C. glabrata clinical isolates (MIC ≤1 mg/L) for all three formulations. For C. krusei, PTA against wild-type isolates (MIC ≤0.5 mg/L) was significantly higher than 95% for the i.v. and tablet formulations of posaconazole, whereas for non-wild-type isolates, the PK/PD targets were attained with oral solution against isolates with MICs up to 2 mg/L with trough levels >0.6–0.9 mg/L, and with i.v. and tablet formulations against isolates with MICs up to 4 mg/L with trough levels >1.0–2.1 mg/L. To date, there are no clinical EUCAST or CLSI breakpoints for C. glabrata and C. krusei. Based on the findings of this study, C. glabrata does not appear to be a good target for posaconazole, whereas for C. krusei, a PK/PD EUCAST/CLSI susceptibility breakpoint of 1/2 mg/L was proposed for i.v. and tablet formulations which are 1–2 two-fold dilutions above EC025/ECV.

Posaconazole produced a fungicidal effect (≥1 log10 CFU/mL reduction) against the C. glabrata isolate with the lowest EUCAST/CLSI MIC (0.06/0.125 mg/L) and high drug exposure >0.85 mg/L, which corresponds to >14–17 x MIC. This is in line with previous in vitro studies with C. glabrata isolates, where a small or no killing effect was found with 8–16 x MIC of posaconazole [21,22]. For C. krusei isolates, a 0.5–1 log10 CFU/mL reduction and a >1 log10 CFU/mL reduction were found at concentrations ≥0.15 and ≥0.85 mg/L, which corresponds with >5 x and >28 x MIC for EUCAST and >2.5 and >14 x MIC for CLSI, respectively. In previous in vitro time-kill static studies against C. krusei isolates with CLSI MICs of 0.12–0.5 mg/L, posaconazole had a fungicidal effect at ≥1–4 x MIC, indicating PD differences between stable and decreasing concentrations of posaconazole, possibly due to differences in minimal fungicidal concentrations or post-antifungal effects [12,21].

For C. glabrata isolates, the PK/PD susceptibility breakpoints for EUCAST and CLSI were the same (0.03 mg/L for oral solution and 0.125 mg/L for i.v. and tablet formulations). This is in line with the same modal MIC and epidemiological cut-off values of the EUCAST and CLSI methodologies [23]. On the other hand, for C. krusei isolates, the PK/PD susceptibility breakpoints for the EUCAST methodology were 1 two-fold dilution lower than the CLSI PK/PD breakpoints (0.25 and 0.5 mg/L for oral solution, and 1 and 2 mg/L for i.v. and tablet formulations, respectively) in line with the 1 two-fold lower EUCAST modal MIC compared with the CLSI modal MIC in the same large multi-centre comparative study [23]. Although the official ECV is the same as the ECOFF for C. krusei at 0.5 mg/L [24], which is two dilutions higher than the modal MIC for EUCAST and one dilution higher than the modal MIC for CLSI, weighted analysis of multi-centre MIC data indicated a CLSI ECV of 1 mg/L [25]. The clinical relevance of E50 used in the present study is unknown. As found previously in similar in-vitro PK/PD studies of posaconazole against C. albicans where posaconazole had a fungicidal effect (2 log kill), analysis based on El50 showed that an increase of approximately 0.5 log10 CFU/mL (1 log10 CFU taking into account the dilution of fungi) from initial inoculum (near stasis effect) resulted in a PK/PD susceptibility breakpoint that was equal to the EUCAST susceptibility breakpoint for posaconazole and C. albicans [15]. On the contrary, voriconazole had no fungicidal effect against C. albicans (1 log increase from initial inoculum), yet the El50 which corresponded to an increase of approximately 2 log10 CFU/mL from initial inoculum resulted in a PK/PD susceptibility breakpoint that was equal to the susceptibility breakpoint of EUCAST for voriconazole and C. albicans, validating the clinical relevance of El50 [26]. Although voriconazole had a fungicidal effect against C. glabrata and C. krusei isolates (0.5–1 log kill maximal effect), the corresponding El50 values indicated that these species are not a good target for voriconazole [27]. The present authors have shown previously that the in-vitro El50 in serum-free media corresponded to near stasis in animal neutropenic models [15,26]. Furthermore, due to the peculiar PK characteristics of posaconazole, total drug levels are pharmacodynamically equally active as free drug levels [15].

The in-vitro findings of this study are compatible with animal models of experimental C. glabrata and C. krusei infections. The same in-vitro PD range of effects for C. krusei isolates was found in animal studies with immunocompromised mice, showing almost 100% survival after approximately 7 days of treatment with wild-type isolates with posaconazole (MICs of 0.125 and 0.25 mg/L) [28]. On the other hand, for C. glabrata isolates, posaconazole was not fungicidal against wild-type isolates with MICs of 0.5 and 1 mg/L [29,30], as found in the present study.

Clinical cases of C. glabrata and C. krusei infection treated with posaconazole are limited. A C. krusei infection of the lumbar spine was treated successfully with caspofungin and posaconazole after failing to respond to liposomal amphotericin B prophylaxis and treatment with voriconazole and caspofungin monotherapy against an isolate with MIC of 0.5 mg/L [31]. On the contrary, posaconazole failed to clear blood cultures of a C. glabrata isolate with MIC of 0.5 mg/L in a neutropenic patient with endovascular infection despite improvement [30]. Breakthrough C. glabrata but not C. krusei infections following posaconazole prophylaxis with oral solution have been described [32]. Of note, the two breakthrough C. glabrata isolates had MICs of 1 and 2 mg/L for which PTA is 0% based on the present study. Breakthrough infections with wild-type C. glabrata isolates have been reported in neutropenic patients, as predicted in the present study based on the low PTA for wild-type isolates [32]. High clinical success rates (71–100%) were found when posaconazole was used against fluconazole/itraconazole-refractory oropharyngeal and oesophageal candidiasis including C. glabrata and C. krusei infections, probably due to the strong local effect of the oral solution [32].

An important caveat when treating invasive infections is that posaconazole reaches steady state after 5–7 days of therapy; this might be detrimental given that the mortality rate for candida bloodstream infections increases with every day of delayed therapy [33]. AUC0–24 on day 1 with a loading dose of 300 mg q12h for the i.v. and tablet formulations were one-half and one-third of AUC0–24 on days 8–14, respectively [13,14]. This delay would probably be acceptable for C. krusei infections as the PK/PD susceptibility breakpoint is 1–2 two-fold dilutions higher than the ECOFF/ECV values. Perhaps starting with the i.v. formulation and switching to the tablet formulation could accelerate achievement of steady state. Alternatively, higher (2–3 x) loading doses (600 mg q12h for i.v. formulation and 900 mg q12h for tablet formulation on day 1 providing sufficient exposure is attained), early therapeutic drug monitoring on day 2 for dose adjustment [34], or combination therapy with an echinocandin could increase efficacy until steady state is reached. However, further clinical studies are required to elucidate the role of posaconazole in the treatment of invasive C. glabrata and C. krusei isolates.
In conclusion, this in-vitro PK/PD model suggests a potential role for i.v. and tablet formulations of posaconazole against C. kru-
selii but not C. glabrata infections. Further studies are warranted to investigate its clinical potential, including strategies for rapid attainment of therapeutic levels via initial i.v. treatment before switching to the tablet formulation, and increasing loading tablet dose(s).

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Supplementary materials


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