Pharmacodynamics of Isavuconazole in an *Aspergillus fumigatus* Mouse Infection Model

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Azole resistance is an emerging problem in *Aspergillus fumigatus* which translates into treatment failure. Alternative treatments with new azoles may improve therapeutic outcome in invasive aspergillosis (IA) even for strains with decreased susceptibility to current azoles. The *in vivo* efficacy of 0.25, 1, 4, 16, 64, 128, 256, and 512 mg/kg of body weight/day prodrug isavuconazonium sulfate (BAL8557) (isavuconazole [ISA]-equivalent doses of 0.12, 0.48, 1.92, 7.68, 30.7, 61.4, 122.9, and 245.8 mg/kg/day, respectively) administered by oral gavage was assessed in an immunocompetent murine model of IA against four clinical *A. fumigatus* isolates: a wild-type isolate (ISA MIC EUCAST, 0.5 mg/liter) and three azole-resistant isolates harboring substitutions in the *cyp51A* gene: G54W (ISA MIC EUCAST, 0.5 mg/liter), M220I (ISA MIC EUCAST, 4 mg/liter), and TR34/L98H (ISA MIC EUCAST, 8 mg/liter). The maximum effect (100% survival) was reached at a prodrug isavuconazonium sulfate dose of 64 mg/kg for the wild-type isolate, 128 mg/kg for the G54W mutant, and 256 mg/kg two times per day (q12) for the M220I mutant. A maximum response was not achieved with the TR34/L98H isolates with the highest dose of prodrug isavuconazonium sulfate (256 mg/kg q12). For a survival rate of 50%, the effective AUC0−24h/MIC EUCAST ratio for ISA total drug was 24.73 (95% confidence interval, 22.50 to 27.18). The efficacy of isavuconazole depended on both the drug exposure and the isavuconazole MIC of the isolates. The quantitative relationship between exposure and effect (AUC0−24h/MIC) can be used to optimize the treatment of human infections by *A. fumigatus*, including strains with decreased susceptibility.

Invasive aspergillosis (IA) caused by *Aspergillus fumigatus* is an important opportunistic fungal infection in immunocompromised patients with an overall mortality ranging between 30 and 88% (1–4). Azole antifungals, such as voriconazole (VRC) and posaconazole (POS), are recommended drugs to manage *Aspergillus* diseases (5, 6). Voriconazole is currently recommended as a first-choice treatment for IA, and posaconazole is indicated for prophylaxis and salvage therapy (5, 6). However, the management of IA has become more complicated due to the emergence of azole resistance in *A. fumigatus* (7–16). Surveillance studies indicate that azole resistance is increasing in multiple European countries and in the Middle East, Asia, and Africa (17–21). Therefore, alternative treatment regimens need to be investigated to improve the outcome of patients with azole-resistant IA.

There are basically two alternative options with respect to the management of azole-resistant IA: treatment with a new antifungal formulation or combination therapy. Recent data suggest that combination therapy using a triazole and an echinocandin may be a beneficial treatment strategy for triazole-resistant isolates (22–24). However, for voriconazole, both *in vitro* interactions and *in vivo* studies indicated that the level of synergistic effect is lost at high voriconazole MICs (MIC of ≥8 mg/liter) (22, 23). As a consequence, this is a major drawback in the treatment of patients with azole-resistant IA. Therefore, it is important to explore the efficacy of new antifungal drugs against azole-resistant IA.

Isavuconazole (ISA) is an investigational broad-spectrum triazole currently being investigated in phase III clinical studies for the treatment of severe invasive fungal infections, including the SECURE (invasive aspergillosis and other filamentous fungi), VITAL (rare fungi), and ACTIVE (candidemia/invasive candidiasis) programs (http://clinicaltrials.gov). Isavuconazole is administered as a water-soluble prodrug, isavuconazonium sulfate, that is available in both intravenous (i.v.) and oral formulations. The prodrug is rapidly converted to the active moiety, isavuconazole, and nonactive metabolite upon administration. It is now granted Food and Drug Administration (FDA) fast-track status in the United States and received "orphan drug" designation in the United States and European Union for the treatment of invasive aspergillosis and mucormycosis (25). FDA also recently designated isavuconazole as a Qualified Infectious Disease Product (QIDP) for the oral and intravenous treatment of invasive aspergillosis, invasive mucormycosis, and invasive candidiasis (news release, 16 July 2014, Basilea, Basel, Switzerland). In Europe, an application also has recently been submitted to the European Medicines Agency (EMA), seeking approval for isavuconazole to be used for the treatment of invasive mold infections (news re-
lease, 17 July 2014, Basle, Basel, Switzerland), on the basis of the SECURE registration study (26).

There are only limited preclinical data on the in vivo efficacy of isavuconazole in azole-resistant IA (27). Therefore, the objective of the present study was to investigate the pharmacodynamics (PD) and dose-response and exposure-response relationships of isavuconazole against wild-type and clinical azole-resistant A. fumigatus isolates harboring different substitutions in the Cyp51A gene in an immunocompetent murine model of disseminated aspergillosis. Survival and reduction in kidney fungal burden determined by real-time quantitative PCR were used as primary and secondary endpoints (respectively) to determine the dose-effect and the exposure-effect relationships of isavuconazole for susceptible as well as azole-resistant isolates in comparison with the other azoles.

(Parts of these results were presented at the 53rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Denver, CO, 10 to 13 September 2013, and at the 24th European Congress of Clinical Microbiology and Infectious Diseases [ECCMID], Barcelona, Spain, 10 to 13 May 2014.)

MATERIALS AND METHODS
Fungal isolates. Four clinical A. fumigatus isolates obtained from patients with proven IA (classified according to European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and National Institute of Allergy and Infectious Diseases Mycoses Study Group [EORTC/MSG] consensus definitions) (28) were used in the experiments: a wild-type isolate without mutations in the cyp51A gene (AZN8196) and three azole-resistant isolates harboring substitutions in the cyp51A gene: G54W (V59-73) and M220I (V28-77) isolates that have become resistant during patient azole therapy and a TR46/L98H (V52-35) isolate that has become resistant through environmental azole exposure. Strain identification and the cyp51A gene substitutions were confirmed by sequence-based analysis as described previously (9). In addition, microsatellite genotyping of the isolates was performed to confirm that they are genetically distinct (29). The isolates were stored in 10% glycerol broth at −80°C and were cultured on Sabouraud dextrose agar (SDA) supplemented with 0.02% chloramphenicol for 5 to 7 days at 35 to 37°C. All isolates were cultured again on SDA for 5 to 7 days at 35 to 37°C before preparation of the inoculum.

Preparation of inoculum for antifungal susceptibility testing. The suspensions of conidia were harvested in normal saline containing 0.025% Tween 20. The appropriate dilutions in normal saline were made to obtain an final inoculum concentration of $2 \times 10^5$ to $5 \times 10^5$ CFU/ml (30).

In vitro antifungal susceptibility testing. In vitro antifungal susceptibility testing (MICs and minimum effective concentrations [MECs]) was performed by using the EUCAST (European Committee for Antimicrobial Susceptibility Testing) (30) and CLSI (Clinical and Laboratory Standards Institute) (31) broth microdilution guidelines. The final concentrations of the antifungal agents ranged from 0.016 to 16 mg/liter for susceptible and from 2 to 1024 mg/liter for azole-resistant isolates in comparison with the other azoles.

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tection of animals used for scientific purposes adopted on 22 September 2010), and all animal procedures were approved by the Animal Welfare Committee of Radboud University (RU-DEC 2012-050).

In all survival studies, the monitoring was performed by experienced individuals blinded to the animal treatment. The infected mice were examined at least three times daily. Clinical inspections focused on dehydration, torticollis, staggering, severe weight loss (a decrease of 15% within 48 h or 20% within 24 h), or body temperature drop to below 33°C. Mice demonstrating these clinical signs were humanely terminated according to strict protocols. On day 15 postinfection, all surviving mice were humanely euthanized under isoflurane anesthesia, and blood and internal organs were collected.

Survival and reduction in fungal burden were the primary end-point in groups of 11 and 3 mice, respectively. The survival in days postinfection was recorded for each mouse in each group and considered primary outcome-effect measure to assess the therapeutic efficacy of isavuconazole (35). On day 3 postchallenge, a quantitative real-time PCR (qPCR) was performed in groups of 3 mice. In these groups, the mice were sacrificed on day 3 postinfection (before they began to die from infection) and the fungal load in the kidney was determined. In the systemic aspergillosis model with i.v. infection, the kidneys are the main target organs, which may indicate intraluminal localization of the fungus in the renal tubules initially protects them from inflammatory cells. The reduction in kidney fungal burden was then correlated with the survival of the remaining 11 mice from each corresponding group at day 15 postinfection.

Determination of fungal burden in kidney. Left and right kidneys from each animal were homogenized using a TissueLyser (Qiagen; TissueLyser Type MM 301) and UV irradiated beads with magnetic metal cores (3 mm) and 30 Hz in 2 min. Tissue samples were transferred to Magna Lyser Green bead tubes (Roche Applied Science). Five hundred microliters of Tris-EDTA (TE) buffer was added, and homogenization was performed for 20 s at 6,500 rpm by using the Magna Lyser instrument. Supernatant was used for DNA isolation by using the automated Magna Pure (MP96) system and the Magna Pure LC total nucleic acid isolation kit according to the manufacturer’s protocol (Roche Applied Science). Phocine herpesvirus (PhHV) was added to all samples as an internal extraction and inhibition control. The concentration of total isolated DNA was measured by using the NanoDrop ND-1000 spectrophotometer (Thermo Scientific). Aspergillus loads were determined by qPCR using the LC480 instrument and the probe master kit (Roche Applied Science).

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visual inspection. Dose/MIC and AUC0–24/MIC ratios were calculated by dividing the dose (milligrams per kilogram) or AUC by the MIC. Dose/MIC and AUC0–24/MIC ratio data were log10 transformed to approximate a normal distribution prior to statistical analysis. The 50% effective PK/PD indexes (EI50, EI80, and EI90) best correlating with efficacy were determined. In addition, the 50% effective doses (ED50) of isavuconazole best correlating with efficacy were determined. For comparison between strains, an F test was performed to define whether ED50 differed among the four groups. Statistical significance was defined as a P value of <0.05 (two-tailed).

RESULTS

In vitro susceptibility. The characteristics and in vitro susceptibilities of the four A. fumigatus isolates are shown in Table 1. All isolates grew well after 48 h of incubation at 35 to 37°C. Variable isavuconazole activity was found in azole-resistant isolates, in which isavuconazole showed cross-resistance to voriconazole, but not with itraconazole and posaconazole. In comparison to a MICEUCAST of 0.5 mg/liter for the wild-type isolate, isavuconazole showed similar activity against the isolate harboring the G54W resistance mechanism (MICEUCAST, 0.5 mg/liter) but reduced in vitro activity against M220I and TR34/L98H isolates, with MICsEUCAST of 4 and 8 mg/liter, respectively. There was no difference in the amphotericin B (AmB) and anidulafungin (AFG) activity between the isolates.

Pharmacokinetics of isavuconazole. A total of 210 samples from 210 mice (3 mice per time point, 10 time points, 7 different dosages) were analyzed. All 210 mice were alive at the time of sample collection. The observed plasma concentrations-versus-time profiles of isavuconazole are shown in Fig. 1. The corresponding pharmacokinetic parameters are tabulated in Table 2 for plasma and in Table 3 for epithelial lining fluid (ELF). The dose-normalized isavuconazole AUC in plasma ranged from 0.54 to 0.84 mg · h/liter/(mg/kg) for single doses (dose expressed as the prodrug, concentration expressed as isavuconazole) ranging from 16 mg/kg to 256 mg/kg, was slightly lower for the 4-mg/kg dose, and could not be reliably determined for lower doses. The concentrations of isavuconazole in ELF correlated well with those obtained in plasma but were lower, including the maximum total drug concentrations (Cmax) of isavuconazole (Table 3). A significant relationship between mean isavuconazole concentrations in plasma and ELF was noted by linear regression analysis ($r^2 = 0.86, P < 0.0001$) (Fig. 2). The penetration of isavuconazole in ELF based on total drug was between 35.8 and 72.5% with a mean of

![FIG 1](https://aac.asm.org/content/59/5/2858.full.html) Plasma concentrations of isavuconazole (BAL4815) following oral administration of the prodrug isavuconazonium sulfate (BAL8557) at 0.25-, 1-, 4-, 16-, 64-, 128-, and 256-mg/kg dosages to immunocompetent infected mice. ISA-equivalent doses are 0.12, 0.48, 1.92, 7.68, 30.7, 61.4, and 122.9 mg/kg, respectively. Each symbol corresponds to the geometric mean and standard deviation of the mean plasma levels for three mice.
54.1% (Table 3). For the ELF data from the 128-mg/kg dose, \( k_{el} \) (terminal elimination rate constant) could not be determined with the consequence that the predicted AUC\(_{0-\infty} \) could not be calculated reliably. Hence, the AUC\(_{0-125} \) in plasma and ELF were determined. The variable penetration of isavuconazole into ELF might be explained due to the difference in lysis of the cells available in interstitial spaces over the course of infection that limits passage through alveolar epithelial cells in various levels; in addition, measurements at the same time in plasma and a third compartment are liable to significant variation.

**Efficacy of isavuconazole. (i) Survival and quantitative PCR as outcome parameter to monitor therapeutic efficacy of ISA.**

Figure 3 shows the survival curves of isavuconazole-treated mice by prodrug dose. The survival curves for all control groups receiving saline by oral gavage showed a mortality of 100%. The results show that for each prodose drug, survival decreased as the MIC increased. Similarly, when the prodrug dose was increased, an improved response was observed. The maximum effect (100% survival) was reached at a prodose of 64 mg/kg for the wild-type isolate (MIC, 0.5 mg/liter), 128 mg/kg for the G54W isolate (MIC, 0.5 mg/liter), and 256 mg/kg for the M220I isolate (MIC, 4 mg/liter). However, the response was lower in the mice infected with the TR34/L98H isolate (MIC, 28.93 mg/kg). For the latter isolate, the maximum dose of isavuconazole (256 mg/kg prodrug) resulted in 27.27% survival in mice. Isavuconazole therapy significantly reduced kidney *Aspergillus* genome copies at day 3 postinfection. The mean number of genome copies detected in untreated animals was \( 3.69 \times 10^5 \) in kidneys (\( n = 3 \), range = \( 3.5 \times 10^2 \) to \( 3.7 \times 10^5 \)) at day 3 postinfection (Fig. 4; see also Fig. S1 in the supplemental material). Further, there was a mean 3- to 4-log reduction of *A. fumigatus* genome copies in infected animals treated with the highest dosage of ISA.

**(ii) Dose-response analysis.**

The dose-response curves for the dosing regimens and control groups of isavuconazole administered to the immunocompetent mice are shown in Fig. 5. Isavuconazole treatment improved the survival of the mice in a dose-dependent manner. A dose-response relationship was observed that depended on the isavuconazole dose and the azole resistance isolates with higher MICs to isavuconazole (\( \approx 4 \) mg/liter) was shifted to the right compared to those infected with isolates with the susceptible profile for isavuconazole (MIC, 0.5 mg/liter), indicating that higher doses of isavuconazole were required to achieve similar efficacy.

The Hill-type model with a variable slope fitted the relationship between the dose and 14-day survival well, with R\(^2\) values of 1 (wild type), 0.99 (G54W isolate), 0.95 (M220I isolate), and 0.91 for the TR34/L98H isolate. The 50% effective dose (ED\(_{50}\)) based on survival was 24.15 mg/kg prodrug isavuconazonium sulfate (BAL8557) (95% confidence interval [CI], 23.96 to 24.33 mg/kg) for the wild type, 28.93 (95% CI, 24.23 to 34.54 mg/kg) for the G54W isolate, 109 (95% CI, 50.69 to 234.6 mg/kg) for the TR34/L98H isolate.

**TABLE 2** Pharmacokinetic parameters of isavuconazole (BAL4815) following oral administration of various dosages administered as prodrug isavuconazonium sulfate (BAL8557) ranging from 0.25 to 256 mg/kg (ISA-equivalent doses ranging from 0.12 to 122.9 mg/kg)\(^a\)

<table>
<thead>
<tr>
<th>Dose group (mg/kg)</th>
<th>( C_{max} ) (mg/liter)</th>
<th>( C_{last} ) (mg/liter)</th>
<th>Half-life (h)</th>
<th>AUC(_{INF, prod} ) (h · mg/liter)</th>
<th>AUC(_{INF, D, prod} ) (h · mg/liter/kg)</th>
<th>Clss_F [liters/h]</th>
<th>Vz_F (liters/kg)</th>
</tr>
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<tbody>
<tr>
<td>0.25</td>
<td>0.12</td>
<td>0.12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.11</td>
<td>0.11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.54</td>
<td>0.08</td>
<td>1.12</td>
<td>0.97</td>
<td>0.24</td>
<td>4.09</td>
<td>6.62</td>
</tr>
<tr>
<td>16</td>
<td>2.31</td>
<td>0.24</td>
<td>2.49</td>
<td>9.68</td>
<td>0.60</td>
<td>1.68</td>
<td>6.04</td>
</tr>
<tr>
<td>64</td>
<td>5.69</td>
<td>0.52</td>
<td>3.00</td>
<td>34.70</td>
<td>0.54</td>
<td>1.87</td>
<td>8.11</td>
</tr>
<tr>
<td>128</td>
<td>9.84</td>
<td>2.42</td>
<td>6.06</td>
<td>107.41</td>
<td>0.84</td>
<td>1.31</td>
<td>11.46</td>
</tr>
<tr>
<td>256</td>
<td>25.62</td>
<td>0.15</td>
<td>3.06</td>
<td>146.68</td>
<td>0.57</td>
<td>1.76</td>
<td>7.75</td>
</tr>
</tbody>
</table>

\( \text{Abbreviations: } C_{max}, \text{ last observed quantifiable concentration}; AUC\(_{INF, prod} \), \text{ predicted area under the plasma concentration-time curve from time zero to infinity}; AUC\(_{INF, D, prod} \), \text{ dose-normalized predicted area under the plasma concentration-time curve from time zero to infinity}; Clss_F, \text{ total systemic clearance}; Vz_F, \text{ volume of distribution.} \)

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**TABLE 3** Penetration ratio of isavuconazole (BAL4815) in ELF compared to plasma based on total drug\(^a\)

<table>
<thead>
<tr>
<th>ISA dose (mg/kg)</th>
<th>Plasma AUC(_{INF, prod} ) (h · mg/liter)</th>
<th>ELF AUC(_{INF, prod} ) (h · mg/liter)</th>
<th>ELF/AUC(_{INF, prod} ) plasma ratio (%)</th>
</tr>
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<tbody>
<tr>
<td>64</td>
<td>34.70</td>
<td>25.14</td>
<td>72.45</td>
</tr>
<tr>
<td>128</td>
<td>107.41</td>
<td>------------------------------------</td>
<td>------------------------------------------</td>
</tr>
<tr>
<td>256</td>
<td>146.68</td>
<td>52.57</td>
<td>35.84</td>
</tr>
</tbody>
</table>

\( \text{Abbreviation: } AUC\(_{INF, prod} \), \text{ predicted area under the plasma concentration-time curve from time zero to infinity.} \)

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**FIG 2** Relationship between isavuconazole (BAL4815) concentrations in plasma and ELF. The line indicates the model fit using linear regression (\( r^2 = 0.86, P < 0.001 \)).
isolate, and 483.8 (95% CI, 103.2 to 2,266 mg/kg) for the TR 34/L98H isolate. Notably, the dose-response curves were significantly different ($P < 0.05$) between the wild-type $A. fumigatus$ isolate with an isavuconazole MIC EUCAST of 0.5 mg/liter and the azole-resistant isolates harboring M220I and TR 34/L98H substitutions in the $cyp51A$ gene with isavuconazole MIC EUCASTs of 4 and 8 mg/liter, respectively (Table 4).

As a comparison, the ED$_{50}$ based on survival was 11.6 mg/kg isavuconazole active moiety (95% confidence interval [CI], 11.5 to 11.7 mg/kg) for the wild type, 13.9 (95% CI, 11.6 to 16.6 mg/kg) for the G54W isolate, 52.3 (95% CI, 24.3 to 112.6 mg/kg) for the M220I isolate, and 232.3 (95% CI, 49.52 to 1,089 mg/kg) for the TR34/L98H isolate.

(iii) Exposure-response analysis. The AUC for each dose (Table 2) was used to calculate the AUC$_{24}$/MIC ratio for each isolate, as shown in Fig. 6. The exposure-response relationship has a sigmoidal shape. Increased isavuconazole exposure was required to obtain maximum efficacy in mice infected with the M220I (MIC, 4 mg/liter) and TR$_{34}$/L98H (MIC, 8 mg/liter) strains compared to those infected with the wild-type and G54W strains (MIC, 0.5 mg/liter).

The Hill equation with a variable slope fitted the relationship between the 24-h AUC/MIC ratio and 14-day survival well ($R^2 = 0.96$), as statistically significant pharmacodynamic indices (PDIs) for isavuconazole single-agent regimens ($P < 0.05$). The 50% effective pharmacodynamic index (total AUC$_{0-24}$/MIC$_{EUCAST}$) for isavuconazole was 24.73 (95% confidence interval, 22.50 to 27.18), to be the PD index most closely predictive of efficacy. Using MICs determined with the CLSI method, the effective AUC$_{0-24}$/MIC$_{CLSI}$ ratio for ISA total drug was 50.48 (95% confidence interval, 44.90 to 56.74).

The relationship between the in vivo efficacy and other PDIs, such as the cumulative percentage of a 24-h period that the drug concentration exceeded the MIC under steady-state PK conditions and the peak-level $C_{max}$/MIC, was also determined (data not shown). However, AUC$_{0-24}$/MIC appeared to be the most important pharmacodynamic index correlating with efficacy.

(iv) Dose fractionation studies. Figure 7 shows the results of the dose fractionation study. There is no difference between exposure-response relationships of the groups treated with various dosing intervals.

DISCUSSION

In the present study, the efficacy of isavuconazole was demonstrated against $A. fumigatus$ wild-type and $cyp51A$ azole-resistant isolates in an immunocompetent murine model of disseminated aspergillosis. Efficacy was dependent both on the drug concentration exceeded the MIC under steady-state PK conditions and the peak-level $C_{max}$/MIC, was also determined (data not shown). However, AUC$_{0-24}$/MIC appeared to be the most important pharmacodynamic index correlating with efficacy.
Isavuconazole was not efficacious. Importantly, a maximal effect of 100% survival was achieved in all strains tested except for the strain containing the TR34/L98H mutation. This is of significant importance, since the prevalence of azole resistance in *A. fumiga-
tus* is increasing, and cross-resistance is a growing concern (12, 21, 32, 45, 46).

A possible limitation of the experimental design used to explore the PK/PD relationships of isavuconazole in our study is that the effects were observed in nonneutropenic animals and the route of infection was dissemination rather than inhalation (the normal route of infection). In addition, a significant difference between tail and intrapulmonary infection is the difference in inoculum size. A lower inoculum size is needed in immunosuppressed models. The effects observed could therefore be an under- or overestimation of the exposure required. However, in our previous studies with azoles, we showed that using survival as a gold standard endpoint in our model provides useful exposure-response relationships. Studies with azoles in neutropenic (27, 47, 48) and nonneutropenic (23, 49, 50) models have shown that the exposure–response relationships are of the same order of magnitude; in fact, a slightly lower drug exposure target may be required in the neutropenic model. This could possibly be because of the lower inoculum used in this model.

Isavuconazole is an investigational broad-spectrum triazole developed for the treatment of severe invasive and life-threatening fungal diseases (25, 51–54). This compound demonstrated *in vitro* activity and *in vivo* efficacy against a broad range of yeasts and molds, including *Aspergillus* spp., *Fusarium* spp., *Candida* spp., the *Mucorales*, *Cryptococcus* spp., and black yeasts and their filamentous relatives (25, 55–63).

In the current study, our model indicated that the primary driver of efficacy appears to be AUC/MIC. For a survival rate of 50%, the effective AUC0–24/MICCLSI ratio for isavuconazole total drug was 50.48 (95% confidence interval, 44.90 to 56.74). Similarly, the exposure-response relationships of isavucona-

![FIG 4 Efficacy of isavuconazole against 4 *A. fumigatus* isolates expressed as *A. fumigatus* DNA load (copies/nanogram of DNA) in kidneys at 72 h postchallenge. For all groups, *n* was 3. Doses were administered as the prodrug isavuconazonium sulfate (BAL8557). The highest dose (256 mg/kg) was used two times per day, corresponding to 512 mg/kg prodrug isavuconazonium sulfate (BAL8557)/day, in groups of animals for which 100% efficacy was not achieved with a once daily dose of 256 mg/kg.](http://aac.asm.org/)
for 7 days. A dose-response relationship was observed for each isolate, with higher doses of isavuconazole achieving a larger microbiologic effect. The static-dose range was 65 to 617 mg/kg/12 h, for which the median total- and free-drug 24-h AUC/MIC ratio PD targets for net stasis were 503 and 5, respectively. The 1-log10 killing-dose range was 147 to 455 mg/kg/12 h, and the corresponding median free-drug AUC/MIC ratio was 11.1 (27). Since the AUC of isavuconazole given 200 mg once daily (q.d.) was reported as approximately 90 mg · h/liter in healthy subjects approaching steady state (64), we therefore conclude that the A. fumigatus strains with MICs of 0.5 mg/liter would be covered, and attainment is most likely reached for strains with MICs of up to 2 or potentially 4 mg/liter.

The efficacy of isavuconazole has also been investigated in an immunosuppressed murine model of disseminated Aspergillus flavus infection (65). Isavuconazole demonstrated impressive antifungal activity against A. flavus infection, leading to prolonged survival, equivalent to similar doses of itraconazole and voriconazole and superior to either drug administered at 10 mg/kg/dose. The excellent efficacy of isavuconazole occurred despite much lower exposure as demonstrated by 4-fold-lower AUCs. Isavuconazole was at least as effective as itraconazole or voriconazole at reducing organ burden and was able to clear all burden in 33 to 83% of mice treated with >15 mg/kg/dose (65).

Three previous studies have examined drug exposure and the efficacy of isavuconazole in a murine model of invasive candidiasis (48, 66–68) and showed a very strong relationship between the PD index AUC/MIC ratio and treatment outcome. One study investigated the efficacy of isavuconazole using a neutropenic mouse model of disseminated C. krusei and Candida tropicalis infections. Isavuconazole was as effective as voriconazole and much more effective than fluconazole at reducing brain burden. All doses of isavuconazole (6, 15, 30, 60, 90, 120, or 150 mg/kg equivalent active compound) reduced brain burden (P < 0.05) in the C. krusei model and kidney burden in the C. tropicalis model (48).

Another study investigated the PK/PD properties of isavuconazole in a neutropenic murine model of invasive candidiasis (IC) against clinical isolates of Candida species, including Candida albicans, Candida glabrata, and C. tropicalis, with both a 24-h and a 96-h treatment duration (48). This study has shown that the pharmacodynamic index most closely correlated with efficacy is the ratio of the 24-h area under the concentration-time curve (AUC) to the MIC, and a target 24-h free-drug AUC/MIC ratio near 25 was associated with 50% of maximal microbiologic efficacy (48).

### Table 4

Comparison of efficacies of isavuconazole among four A. fumigatus isolates based on ED$_{50}$

<table>
<thead>
<tr>
<th>A. fumigatus strain</th>
<th>ISA MIC (mg/liter)</th>
<th>ISA ED$_{50}$ (mg/kg)</th>
<th>Comparison with wild type</th>
<th>P value</th>
<th>F test (df$<em>{n}$, df$</em>{d}$)$^a$</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>0.5</td>
<td>24.15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G54W strain</td>
<td>0.5</td>
<td>28.93</td>
<td>0.16</td>
<td>2.88 (1, 4)</td>
<td>Nonsignificant</td>
<td></td>
</tr>
<tr>
<td>M220I strain</td>
<td>4</td>
<td>109</td>
<td>&lt;0.0001</td>
<td>89.89 (1, 7)</td>
<td>Significant</td>
<td></td>
</tr>
<tr>
<td>TR$_{tsr}$/L98H strain</td>
<td>8</td>
<td>438.8</td>
<td>&lt;0.0002</td>
<td>47.33 (1, 7)</td>
<td>Significant</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ df$_{n}$, degrees of freedom numerator; df$_{d}$, degrees of freedom denominator.
Similarly, using a nonneutropenic murine model of disseminated *Candida albicans* infection, it has been shown that the pharmacodynamic driver most likely to predict the outcome of itraconazole treatment is the AUC/MIC ratio (68).

In addition, in a neutropenic mouse model of intratracheal infection, Luo et al. investigated the efficacy of isavuconazole against a brain isolate of *Rhizopus delemar* (isavuconazole MIC<sub>100</sub> and minimum fungicidal concentration [MFC] values of 0.25 μg/ml) (69). Isavuconazole was effective against isolates with MIC and MFC values ranging between 0.125 and 1.00 μg/ml. A high dose of isavuconazole (215 mg/kg of isavuconazonium sulfate three times daily [t.i.d.]) prolonged the survival time and lowered the tissue fungal burden of cyclophosphamide–cortisone acetate-treated mice. In addition, isavuconazole was as effective as a high-dose liposomal amphotericin B (15 mg/kg, given once daily through tail vein injection) treatment (69).

In the present study, we also found that the dosing frequency did not have an impact on survival. Given the half-life of the drug in mice, which is much shorter than that in humans, a once-daily dose should therefore suffice and be adequate to treat infections in humans. Of note, in clinical studies to date, positive efficacy and safety data have been reported in patients with invasive aspergil-
lrosis (26). Isavuconazole achieved predictable drug levels supporting reliable dosing and a switch from once-daily intravenous to oral administration (26, 70, 71). In addition, no significant food effect has been found with oral administration of isavuconazole (72), which in contrast is a potential advantage of isavuconazole over the oral solution of posaconazole.

In conclusion, our data are an additional promising support for isavuconazole in the treatment of IA. As with the other azole antifungal drugs, the efficacy of isavuconazole treatment in A. fumigatus relies both on drug exposure and on the antifungal susceptibility of the etiological agent. Therefore, the quantitative relationship between exposure and effect (AUC0–24/MIC) can be used to optimize the treatment of human infections by A. fumigatus, including strains with decreased susceptibility.

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