APX001 Pharmacokinetic/Pharmacodynamic Target Determination against *Aspergillus fumigatus* in an *In Vivo* Model of Invasive Pulmonary Aspergillosis

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ABSTRACT APX001, the prodrug of APX001A, is a first-in-class antifungal agent that has a potent activity against *Aspergillus fumigatus*. The goal of current study was to determine the pharmacodynamic (PD) index and target of APX001 in an immunocompromised murine model of invasive pulmonary aspergillosis against 6 *A. fumigatus* isolates. Minimum effective concentration (MEC) values ranged from 0.03 to 0.06 mg/liter. Dose fractionation was performed against isolate AF293 using total doses of APX001 ranging from 81 to 768 mg/kg of body weight/day fractionated into every 3-, 6-, and 8-h regimens over a 96-h treatment duration. Efficacy was assessed by *A. fumigatus* quantitative PCR (qPCR) of conidial equivalents from lung homogenates. Nonlinear regression analysis using the Hill equation demonstrated that the 24-h area under the concentration-time curve (AUC)/MEC ratio was the pharmacokinetic (PK)/PD index that best correlated with efficacy (coefficient of determination [R^2] = 0.79). Treatment studies with the remaining strains utilized regimens of 40 to 1,536 mg/kg of APX001 administered every 3 h for a 96-h duration. Exposure-response relationships for all strains were similar, and the median free drug AUC/MEC PK/PD targets for stasis and 1-log-kill endpoints were 47.6 and 89.4, respectively. The present studies demonstrated *in vitro* and *in vivo* APX001A/APX001 potency against *A. fumigatus*. These results have potential relevance for clinical dose selection and evaluation of susceptibility breakpoints.

KEYWORDS APX001, *Aspergillus*, pharmacodynamics

Invasive pulmonary aspergillosis (IPA), most frequently caused by *Aspergillus fumigatus*, is a leading cause of mortality in immunocompromised patients (1, 2). The triazoles voriconazole and isavuconazole are considered first-line agents and have led to significant improvements in the treatment of IPA (3, 4). However, outcomes remain suboptimal and therapeutic challenges persist. For example, first, resistance to triazoles has been well documented in numerous geographical locations (5–10). Alternative therapy, such as liposomal and deoxycholate formulations of amphotericin B, is associated with a poorer outcome and significant toxicity. Second, patients with IPA are often on numerous other medications, and therefore, there can often be significant drug-drug interactions with triazoles (11). Therefore, the development of compounds for the treatment of IPA that target novel cellular functions is of major importance and is earnestly needed.

APX001 (formerly E1211) is the methyl-phosphate prodrug of the active moiety, APX001A (formerly E1210), and is a first-in-class small-molecule antifungal drug candi-

Citation Zhao M, Lepak AJ, Marchillo K, Vanhecker J, Sanchez H, Ambrose PG, Andes DR. 2019. APX001 pharmacokinetic/pharmacodynamic target determination against *Aspergillus fumigatus* in an *in vivo* model of invasive pulmonary aspergillosis. *Antimicrob Agents Chemother* 63:e02372-18. <https://doi.org/10.1128/AAC.02372-18>.

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Received 6 November 2018

Returned for modification 3 December 2018

Accepted 15 January 2019

Accepted manuscript posted online 22 January 2019

Published 27 March 2019

TABLE 1 *In vitro* activities of APX001A and select comparator antifungal agents, *in vivo* fitnesses in the animal model, and genetic mutations associated with drug resistance

<i>A. fumigatus</i> strain	MEC/MIC ^a (mg/liter)			<i>In vivo</i> fitness (CE ^b /96 h) in untreated mice	Comment
	APX001A	Isavuconazole	Posaconazole		
AF293	0.03	1	0.5	1.04	Wild type
AF41	0.06	0.25	0.25	1.76	Wild type
EMFR S678P	0.03	0.25	0.25	2.03	<i>Fks1</i> S678P (echinocandin; MIC, >16 mg/liter)
F11628	0.06	8	8	1.90	<i>Cyp51</i> G138C
AF72	0.06	2	2	1.97	<i>Cyp51</i> G54E
F14532	0.03	1	1	2.42	<i>Cyp51</i> M220T

^aThe MEC value was determined for APX001A, whereas the MIC value was determined for isavuconazole and posaconazole.

^bCE, conidial equivalents.

date (12–14). APX001A inhibits the fungal enzyme *Gwt1* of the glycosylphosphatidylinositol (GPI) biosynthesis pathway by preventing inositol acylation during synthesis of GPI-anchored proteins (15). This leads to pleiotropic effects, including compromised cell wall integrity, and a reduction in fungal virulence attributes, including biofilm and germ tube formation, all of which ultimately contribute to severe fungal growth defects. APX001A/APX001 has potent broad *in vitro* and *in vivo* activity against major fungal pathogens, including *Candida*, *Cryptococcus*, *Aspergillus*, *Scedosporium*, and *Fusarium* strains, regardless of their azole resistance and echinocandin resistance, which is consistent with the distinct mechanism of action (16–25).

Laboratory animal models that mimic human disease play an essential role in the characterization of pharmacological exposures associated with efficacy and/or safety prior to clinical use. Importantly, the results obtained with well-designed animal models used in antimicrobial development are generally highly predictive of clinical efficacy (26). This is particularly important for invasive fungal infections, which are often more difficult to study in clinical trials, are relatively less common than other infections, and usually involve very complex, often immunocompromised patient populations. Therefore, an understanding of antifungal pharmacokinetic (PK)/pharmacodynamic (PD) properties and exposure-response relationships underpins the design of antifungal regimens to be advanced to clinical studies that are safe and effective. Here, we describe the use of a well-established neutropenic mouse model of IPA to characterize the PK/PD relationships of APX001 against *A. fumigatus* and provide a basis for the design of clinical dosing strategies.

RESULTS

***In vitro* susceptibility testing and *in vivo* fitness.** The minimum effective concentration (MEC) values of APX001A, genetic mutations, and relative fitness in the *in vivo* murine model of each isolate are shown in Table 1. APX001A exhibited similar potency against wild-type and *Cyp51* and *Fks1* mutant strains with similar MEC values (0.03 to 0.06 mg/liter). The organisms also demonstrated similar *in vivo* fitness in the animal models, as defined by relatively equal increases in the lung organism burden in untreated mice over the study period.

Pharmacokinetics. Data from our prior PK study of APX001 were used for the current study analysis (27). The area under the concentration-time curve (AUC) and peak concentrations ($C_{\max}$) over the dose range were linear (coefficient of determination [R^2] = 0.96), including when accounting for drug accumulation due to the frequent administration. Thus, for dose levels that were not directly measured, the AUC and $C_{\max}$ were estimated using linear extrapolation or interpolation. A plasma protein binding value for mice of 98.3%, based upon prior unpublished data from the sponsor, was used in the current study to calculate free drug concentrations for analysis.

PK/PD index determination. At the start of therapy, mice had $4.85 \pm 0.12 \log_{10}$ conidial equivalents (CE)/ml of lung homogenate, and the organism burden increased to $5.61 \pm 0.26 \log_{10}$ CE/ml of lung homogenate after 96 h in untreated control mice.

FIG 1 Relationship between the APX001 dosing interval and efficacy against strain AF293 in a murine neutropenic invasive pulmonary aspergillosis model. Each symbol represents the mean $\pm$ SD for four animals. The error bars represent the standard deviation. The dashed horizontal line represents net stasis over the treatment period. Points above the line represent a net increase in burden, and points below the line represent a net decrease in burden.

Escalating doses of APX001 resulted in dose-dependent killing (Fig. 1). The highest doses studied reduced the organism burden by approximately 0.5 log₁₀ CE/ml of lung homogenate compared to that at the start of therapy. Visually, the similarity of the dose-response curves for each of the three dosing fractionations in Fig. 1 suggests that AUC/MEC is likely the most predictive PK/PD parameter of efficacy. To determine this statistically, the dose-response data were modeled using each of the three PK/PD indices in relation to the microbiological effect and applying nonlinear regression analysis using the Hill equation. This is shown in Fig. 2. The strongest relationship was noted when efficacy results were correlated with the AUC/MEC ratio, with an R^2 value of 0.79. Regression with C_{max} /MEC and the percentage of the time that the plasma free drug concentrations exceeded the MEC (%T>MEC) resulted in less strong relationships, with R^2 values of 0.52 and 0.23, respectively.

Treatment efficacy and pharmacodynamic magnitude determination. Dose-response results for all strains are shown in Fig. 3. There was congruence among the dose-response curves, which would be expected, given the narrow MEC range observed *in vitro*. In general, at the highest APX001 dose studied, a 1- to 2-log kill was observed. The dose-response data were modeled to characterize the relationship between the 24-h free-drug AUC/MEC ($f\text{AUC}/\text{MEC}$) and treatment effect (Fig. 4). A sigmoid exposure-response relationship was observed for each isolate, with a strong fit for $f\text{AUC}/\text{MEC}$ and treatment effect ($R^2 = 0.80$), similar to the dose fractionation results. The dose and $f\text{AUC}/\text{MEC}$ needed to produce growth suppression compared to the growth at the start of therapy (static dose) and in the regimens associated with a 1-log reduction in organism burden (1-log₁₀ kill) for each isolate are reported in Table 2. The median static dose $f\text{AUC}/\text{MEC}$ ratio was 47.6. The 1-log₁₀-kill $f\text{AUC}/\text{MEC}$ ratio was roughly 2-fold higher than the stasis PD target, with a median value of 89.4.

DISCUSSION

A. fumigatus is one of the most prevalent fungal pathogens causing life-threatening disease in immunocompromised patients. Despite advances in medical care and the development of highly effective triazole therapy (e.g., voriconazole and isavuconazole), outcomes in patients with invasive pulmonary aspergillosis remain suboptimal. The reasons for this are multifactorial; however, of major concern are the rising reports of triazole resistance and the clinical implication that this has on patient outcome. For example, recent studies have demonstrated that triazole-resistant *A. fumigatus* infection is associated with 50% to 100% mortality (28–30). Although numerous mechanisms

FIG 2 Pharmacodynamic regression of the *in vivo* dose fractionation study with APX001 against strain AF293. Each symbol represents the mean for four mice. The dose data are expressed as the AUC from 0 to 24 h/MEC (A), $\text{C}_{\text{max}}/\text{MEC}$ (B), and the percentage of the time that the plasma free drug concentration exceeds the MEC (%T>MEC) (C). The horizontal dashed line represents the net stasis of the infectious burden from the start of therapy. Points above the line represent an increase in burden (i.e., net growth), whereas those below the line represent a decrease in burden. The coefficient of determination (R^2), based on the Hill equation, is shown in the upper corner with the associated PD parameters, including maximal effect (E_{max}), point of 50% maximal effect (ED_{50}), and slope of the line (N).

FIG 3 Dose-response curves for each isolate. Each symbol represents the mean and standard deviation for four mice. Six total drug dose levels of APX001 were given orally. The horizontal dashed line represents the net stasis of the burden from the start of therapy. Points above the line represent an increase in burden, whereas those below the line represent a net decrease in burden.

have been implicated in triazole resistance (31), by far the most common and clinically relevant are mutations in the *Cyp51* gene, which encodes the enzyme 14 α -sterol-demethylase, a key enzyme in the ergosterol biosynthesis pathway and the cellular target of triazole therapy (32). Other significant factors that limit triazole effectiveness include adverse effects or intolerance, irregular and unpredictable pharmacokinetic exposures (due to nonlinear pharmacokinetics) necessitating therapeutic drug monitoring, and drug-drug interactions. For all of these reasons, the continued development of novel therapies for invasive aspergillosis is necessary.

APX001, the prodrug of APX001A, is a first-in-class broad-spectrum antifungal agent in clinical development for the treatment of life-threatening invasive fungal infections. In this study and in previous studies (19, 20, 22), APX001A has been shown to exhibit *in vitro* activity against *A. fumigatus*, including those strains with triazole resistance mutations in the *Cyp51* gene and with echinocandin resistance mutations in the *Fks1*

FIG 4 Pharmacodynamic regression analysis of the PK/PD index free drug AUC/MEC and the *in vivo* effect of APX001 against six *A. fumigatus* strains. Each symbol represents the mean for four mice. The drug exposure data are expressed as the APX001A 24-h free drug AUC/MEC. The line drawn through the data points is the best-fit line based upon the sigmoid E_{max} formula (Hill equation). The horizontal dashed line represents the net stasis of the burden from the start of therapy. Points above the line represent an increase in burden, whereas those below the line represent a decrease in burden.

TABLE 2 Dose and total drug and free drug AUC/MEC needed to achieve net stasis and 1-log-kill endpoints for each *A. fumigatus* strain^a

Organism	MIC (mg/liter)	Stasis			1-log kill		
		24-h total dose (mg/kg)	24-h tAUC/MIC	24-h fAUC/MIC	24-h total dose (mg/kg)	24-h tAUC/MIC	24-h fAUC/MIC
F14532	0.03	420	5,220.5	88.75	1,052.9	9,811.2	166.79
EMFR	0.03	362.5	4,409.8	74.97	1,114.2	10,184.5	173.14
AF41	0.06	305.5	1,803.4	30.66	922.3	4,507.8	76.63
F11628	0.06	492.1	3,118.1	53.01	1,024.6	4,819.3	81.93
AF72	0.06	402.3	2,485.0	42.25	1,168.7	5,258.2	89.39
AF293	0.03	128.6	1,113.8	18.93	NA		
Mean		351.8	3,025.1	51.43	1,056.5	6,916.2	117.58
Median		382.4	2,801.6	47.63	1,052.9	5,258.2	89.39
SD		125.7	1,561.0	26.54	93.5	2,828.8	48.09

^atAUC/MEC, total AUC/MEC; fAUC/MEC, free AUC/MEC; NA, not achieved.

gene. For example, in a study by Pfaller et al., the MEC range for APX001A against *Cyp51* mutants was 0.03 to 0.12 mg/liter, within the range observed for wild-type strains (22). We included a selection of *Cyp51* mutants in our study and observed similar results (APX001A MEC, 0.03 to 0.06 mg/liter). It is noteworthy that we also included a laboratory-engineered *FKS* mutant with echinocandin resistance and demonstrated the similar *in vitro* potency of APX001A against this strain and wild-type strains, as has been seen in prior analyses of resistant isolates (17, 27). Thus, in keeping with the known novel mechanism of action compared to that of existing agents, there was no evidence of cross-resistance in our study.

Previous *in vivo* studies demonstrating efficacy in various immunocompromised animal models of fungal infections, including candidiasis, aspergillosis, and fusariosis, have been performed (16–19, 27, 33). The first invasive aspergillosis study (19) differed significantly from the current studies presented, in that it was performed with a single *A. flavus* isolate, the infection utilized a relatively low inoculum (3.0×10^4 conidia), and survival as opposed to organism burden, which was used in the present study, was the endpoint. A more recent invasive aspergillosis study was conducted using a single *A. fumigatus* isolate and demonstrated a significant reduction in the lung fungal burden (4.2 to 7.6 log₁₀ conidial equivalents/gram tissue) (33). While this study is important as a proof of principle, it did not have enough strains or adequate dose ranging of drug exposures to allow for the reliable determination of exposure-response relationships, which is necessary to determine optimal clinical dosing regimens and susceptibility breakpoints.

The current study is the first with an IPA model to utilize a dose ranging design with PK/PD analyses correlating drug exposure, MEC, and a measure of the fungal burden. A number of compelling study findings were observed. First, the results are consistent with previous findings in an invasive candidiasis murine model demonstrating that AUC/MEC is the most predictive PK/PD index of the microbiological effect (27). Second, the APX001 dose-response relationships were very similar across a diverse set of *A. fumigatus* strains, as supported by the high *R*² value (0.80) and relatively close fit to the individual stasis and 1-log-kill targets (Table 2). Additionally, we found marked *in vivo* killing activity, with a 1- to 2-log₁₀ kill for all but one strain. This potency was perhaps surprising, given that echinocandins, which also impact the cell wall, lead to stunted growth *in vitro* (i.e., MEC measurement) and *in vivo* usually achieve only a net static effect against *A. fumigatus* with little to no observable decrease in the fungal burden from that at the start of therapy (34). In fact, the concentration-dependent decrease in the *A. fumigatus* fungal burden in the murine IPA model for APX001 is comparable to that for posaconazole and isavuconazole in this model (35, 36). We speculate that this degree of activity may be linked to APX001A pleiotropic effects on cell wall integrity, fungal growth, and the potential for the induction of an endoplasmic reticulum stress response (37). Finally, by including a dynamic measure of fungal burden and using a dose ranging study, we were able to elucidate the pharmacodynamic target exposures

(AUC/MEC) for two treatment outcomes. The median total drug AUC/MEC ratio required to achieve a stasis effect for *A. fumigatus* was 2,802, and that required to achieve a 1-log kill effect was 5,258. The stasis targets are within the range of AUC/MIC stasis target values that we observed in a murine disseminated candidiasis model with *Candida glabrata* and *Candida auris* but notably lower than the values observed with *Candida albicans* (27). This finding is also reminiscent of that from a PK/PD study with mold-active triazoles, where lower AUC/MEC targets and more fungicidal activity were observed for posaconazole and isavuconazole against *A. fumigatus* than against *C. albicans* (35, 36, 38, 39).

As a first-in-class antifungal, the preclinical PK/PD target for APX001 that correlates with the clinical response is unknown. Nonetheless, we observed potent *in vivo* activity of APX001 that was comparable to that of triazoles previously tested in this well-established murine model of IPA. The study demonstrates that APX001 has concentration-dependent *in vivo* efficacy against wild-type, azole-resistant, and echinocandin-resistant *A. fumigatus* isolates. The AUC/MEC ratios were very highly associated with *in vivo* activity. The PK/PD target exposures derived in this study should be useful for designing optimized drug dosing regimens for continued clinical development of this promising antifungal.

MATERIALS AND METHODS

Organisms, media, and antibiotic. Six *A. fumigatus* isolates were chosen, including three isolates with *Cyp51* mutations and one laboratory isolate with an *Fks1* mutation. The organisms were grown and subcultured on potato dextrose agar (PDA; Difco Laboratories, Detroit, MI). The organisms were chosen based on similar fitness, as determined by growth in the lungs of untreated animals over 96 h (Table 1). APX001A for *in vitro* studies and APX001 for *in vivo* studies were supplied by the study sponsor (Amplix Pharmaceuticals, Inc., San Diego, CA). APX001A was prepared on the day of use by dissolving with 700 mM HCl and subsequent dilution in RPMI to the required concentrations. APX001 was prepared in 0.21 M NaOH at a concentration of 100 mg/ml and then diluted with sterile 5% glucose solution to the required concentrations.

In vitro susceptibility testing. The MECs of APX001A for the various isolates were determined using a broth microdilution method, in accordance with the guidelines presented in Clinical and Laboratory Standards Institute (CLSI) document M38-A2 (40). All MEC assays were performed in duplicate on three separate occasions. The MEC values of APX001A were defined as the lowest concentration that led to the growth of small, rounded, compact hyphal forms compared to the hyphal growth seen in the growth control well. All MEC results were determined at 48 h, as recommended by CLSI document M38-A2 (40). The median MEC for replicate assays was reported and utilized in the PK/PD analysis.

Infection model. Six-week-old, specific-pathogen-free, female ICR/Swiss mice weighing 23 to 27 g were used for all studies (Harlan Sprague-Dawley, Indianapolis, IN). Animals were maintained in accordance with the criteria of the Association for Assessment and Accreditation of Laboratory Animal Care. All animal studies were approved by the Animal Research Committee of the William S. Middleton Memorial VA Hospital.

A neutropenic, corticosteroid-immunosuppressed murine IPA model was utilized as previously described (34–36). Briefly, mice were rendered neutropenic (polymorphonuclear cell counts, $<100/\text{mm}^3$) by the subcutaneous (s.c.) injection of 150 mg/kg of body weight cyclophosphamide on days -4 , -1 , and $+3$ to ensure the maintenance of neutropenia until the end of the study (96 h). Additionally, cortisone acetate was administered at 250 mg/kg s.c. on day -1 . Throughout the 4-day experiment, mice were also given ceftazidime at 50 mg/kg/day s.c. to prevent opportunistic bacterial infection.

Organisms were prepared by subculturing on PDA 5 days prior to infection and incubated at 37°C. On the day of infection, the inoculum was prepared by flooding the culture plate with 5 ml of normal saline containing 0.05% Tween 20. Gentle agitation was used to release the conidia. The conidial suspension was collected and quantified by use of a hemocytometer (Bright-Line; Hausser Scientific, Horsham, PA). The suspension was diluted in saline to a final concentration of 1×10^7 conidia/ml. Viability was confirmed by plating the suspension and performing CFU counts.

Infection was produced in animals using an aspiration pneumonia model that has been successfully utilized in previous studies (34–36). Briefly, mice were anesthetized with a combination of ketamine and xylazine. Fifty microliters of the conidial suspension was pipetted into the anterior nares with the mice, held upright to allow aspiration into the lungs. Drug treatment commenced 2 h after the initiation of infection.

Tissue fungal burden. Pulmonary fungal burden was determined by real-time quantitative PCR (qPCR) using previously reported methods (34–36, 41, 42). Briefly, animals were euthanized if showing signs of distress or at the end of the 96-h experiment. Both lungs were immediately removed by aseptic technique and placed into sterile Whirl-Pak bags (Nasco, Fort Atkinson, WI) containing 2 ml of sterile 0.9% normal saline. Samples were then homogenized or frozen until homogenization could occur. Homogenization of lung tissue occurred in two steps. First, the lungs were manually homogenized using direct pressure to yield a primary homogenate (43). One milliliter of the primary homogenate was then transferred to a sterile 2-ml screw-cap microcentrifuge tube (Sarstedt, Newton, NC) with 700 μl of 425- to 600- μm acid-washed glass beads (Sigma-Aldrich, St. Louis, MO). The primary homogenate was

mechanically disrupted by vigorous agitation in a BioSpec Mini-BeadBeater (Bartlesville, OK) for 90 s at 4,200 rpm to yield a secondary homogenate. This secondary homogenate was stored at -20°C until DNA extraction.

DNA was extracted from secondary homogenates with a DNeasy Blood and Tissue kit (Qiagen, Valencia, CA) following the manufacturer's protocol and stored at -20°C until the day of qPCR analysis. Extracted DNA was subjected to quantitative, real-time PCR. qPCR plates were prepared on the day of the assay. Standard amounts of conidia were prepared by the use of hemocytometer counts and were spiked into blank uninfected lungs, used for generating standard curves. Samples were assayed in duplicate using a Bio-Rad CFX96 real-time system (Bio-Rad, Hercules, CA). A single-copy gene, *Fks1*, was chosen for quantitation (41). Primer sequences included 5'-GCCTGGTAGTGAAGCTGAGCGT-3' for the forward primer and 5'-CGGTGAATGTAGGCATGTTGTCC-3' for the reverse primer, and the probe sequence was 5'-6-carboxyfluorescein-AGCCAGCGGCCGCAATG-MGB-3' (Integrated DNA Technologies, Coralville, IA). The *Fks1* mutation in strain EMFR S678P was not located in the primer-probe area of the genome and has previously been shown to not affect the quantitation reaction for that isolate (35). The primer-probe set was validated for all isolates by determining the kinetics and quantitative results for known quantities of conidia over the dynamic range (10^2 to 10^8) (34–36). The cycling conditions were as follows: activation, 50°C for 2 min; heat inactivation, 95°C for 10 min for 1 cycle; denaturation, 95°C for 15 s; annealing and extension, 65°C for 1 min for 40 cycles. Quantitation standards were run in conjunction with each set of samples. The threshold cycle (C_T) of each sample was interpolated from a five-point standard curve of C_T values prepared by spiking uninfected mouse lungs with known amounts of conidia (10^3 to 10^7) from each isolate being tested. Results were reported as the number of conidial equivalents (CE) per milliliter of lung homogenate.

Pharmacokinetics. Murine APX001 pharmacokinetic data, including the AUC and C_{max} , were derived from our previous study (27).

PK/PD index determination. A dose fractionation study design was undertaken to determine the PK/PD index (AUC/MEC, C_{max} /MIC, or time above the MEC) that was predictive of efficacy for APX001 against *A. fumigatus*. Four doses (80, 192, 512, and 768 mg/kg) of APX001 were fractionated into dosing regimens in which the drug was administered every 3 h (q3h), every 6 h (q6h), and every 8 h (q8h). Mice were infected with isolate AF293 as described above and administered APX001 by oral gavage. After 96 h the mice were euthanized, and the lungs were collected for DNA quantification. To determine which PK/PD index was most closely linked with efficacy, the number of CE per milliliter of lung homogenate at the end of 96 h of therapy was correlated with (i) the 24-h free drug AUC/MEC, (ii) the free drug C_{max} /MEC ratio, and (iii) the percentage of time over 24 h during which the plasma free drug levels exceeded the MEC ($\%T > \text{MEC}$) for each of the dosage regimens studied. The correlation between efficacy and each of the three PK/PD indices was determined by nonlinear regression based on the Hill equation. The coefficient of determination (R^2) was used to estimate the variance that might be due to regression with each of the PK/PD indices.

PK/PD index magnitude studies. Dose-response experiments were performed for the five remaining *A. fumigatus* isolates using the invasive pulmonary aspergillosis model, as described above. Six dose levels (consisting of 5, 10, 24, 64, 96, and 192 mg/kg/3 h) were administered by the oral route. The treatment duration was 96 h. The dose-response relationships were quantified, and the relationship between the PK/PD index and treatment efficacy was determined using the sigmoid maximal effect (E_{max}) model. These PK/PD relationships were examined utilizing the plasma free drug concentrations from pharmacokinetic studies. The coefficient of determination (R^2) from this model was used to assess the strength of this relationship. The doses required to produce a net static effect (static dose) and a 1-log-kill effect compared to the number of CE per milliliter of lung homogenate at the start of therapy for multiple pathogens in the invasive pulmonary aspergillosis model were determined utilizing the plasma free drug concentrations and the following equation: $\log_{10} D = \log_{10} [E / (E_{\text{max}} - E)] / N + \log_{10} \text{ED}_{50}$, where D is the drug dose, E is the growth (measured by qPCR and represented as the number of CE per milliliter of lung homogenate) in untreated control mice, E_{max} is the maximal effect, N is the slope of the dose-response relationship, and ED_{50} is the dose needed to achieve 50% of the maximal effect.

ACKNOWLEDGMENTS

This study was supported by a research grant from Amplyx Pharmaceuticals.

We kindly thank David Perlin for providing the engineered echinocandin-resistant mutant strain of *A. fumigatus*.

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