Combination of Voriconazole and Anidulafungin for Treatment of Triazole-Resistant *Aspergillus fumigatus* in an *In Vitro* Model of Invasive Pulmonary Aspergillosis

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Voriconazole is a first-line agent for the treatment of invasive pulmonary aspergillosis. Isolates with elevated voriconazole MICs are increasingly being seen, and the optimal treatment regimen is not defined. We investigated whether the combination of voriconazole with anidulafungin may be beneficial for the treatment of *A. fumigatus* strains with elevated voriconazole MICs. We used an *in vitro* model of the human alveolus to define the exposure-response relationships for a wild-type strain (voriconazole MIC, 0.5 mg/liter) and strains with defined molecular mechanisms of triazole resistance (MICs, 4 to 16 mg/liter). All strains had anidulafungin minimum effective concentrations (MECs) of 0.0078 mg/liter. Exposure-response relationships were estimated using galactomannan as a biomarker. Concentrations of voriconazole and anidulafungin were measured using high-performance liquid chromatography (HPLC). The interaction of voriconazole and anidulafungin was described using the Greco model. Fungal growth was progressively inhibited with higher drug exposures of voriconazole. Strains with elevated voriconazole MICs required proportionally greater voriconazole exposures to achieve a comparable antifungal effect. Galactomannan concentrations were only marginally reduced by anidulafungin monotherapy. An additive effect between voriconazole and anidulafungin was apparent. In conclusion, the addition of anidulafungin does not markedly alter the exposure-response relationship of voriconazole. A rise in serum galactomannan during combination therapy with voriconazole and anidulafungin should be interpreted as treatment failure and not attributed to a paradoxical reaction related to echinocandin treatment.

Invasive pulmonary aspergillosis (IPA) is a frequent cause of death in immunocompromised patients. Voriconazole is a first-line treatment, but there are increasing reports of triazole resistance in *Aspergillus fumigatus*, which is associated with increased mortality (17, 42). Other therapeutic options for IPA include lipid formulations of amphotericin B and the echinocandins (46). However, the echinocandins are not fungicidal in laboratory animal models and are associated with relatively poor outcomes for profoundly immunocompromised patients (12, 37, 39, 45). Furthermore, the use of all formulations of amphotericin B may be limited by infusional toxicity and nephrotoxicity (8), which may be detrimental to patient outcomes. Further insights into potentially effective regimens for treatment of triazole-resistant isolates are urgently required.

*In vitro* studies, laboratory animal studies, and a limited amount of clinical data support the use of combination antifungal therapy with a triazole and an echinocandin to treat IPA (6, 27, 29, 38, 39, 41). The rationale for this strategy is predominantly related to the attainment of maximal antifungal effect, which may not be possible with the use of either agent alone. The potential for minimization of drug toxicity and prevention of the emergence of antifungal resistance are generally secondary considerations. Currently, there is no experimental evidence that supports the combination of a triazole with an echinocandin for treating isolates of *A. fumigatus* with elevated voriconazole MICs.

Here, we use a well-validated *in vitro* model of the human alveolus to describe the pharmacodynamics of voriconazole in combination with anidulafungin against both wild-type and resistant strains of *A. fumigatus*. This model has enabled us to explore the potential benefits offered by combining voriconazole with an echinocandin to treat isolates of *A. fumigatus* with elevated voriconazole MICs.

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**MATERIALS AND METHODS**

*Aspergillus* isolates and *in vitro* susceptibility testing. The strains used in this study are summarized in Table 1. A previously described *A. fumigatus* transformant expressing green fluorescent protein (GFP) was used as the wild type (14, 47). Isolates (L98H and G138C) with reduced susceptibility to voriconazole and carrying previously defined substitutions (17) within the triazole target Cyp51A were also studied. They were obtained from the Regional Mycology Reference Laboratory, University Hospital South Manchester.

Voriconazole MICs were determined for each isolate using both CLSI and European Committee for Antimicrobial Susceptibility Testing (EUCAST) methodologies (5, 9). Maximum voriconazole concentrations of 32 mg/liter for CLSI and 64 mg/liter for EUCAST were tested (microscopy was performed to confirm that the voriconazole did not crystallize or precipitate during incubation). Anidulafungin minimum effective concentrations (MECs) were determined for each isolate using the CLSI M38-A2 methodology (5) in three separate independently conducted experiments.
In vitro model of the human alveolus. A previously described cell culture model of the human alveolus containing an air-liquid interface was used (14, 24). Briefly, this model consists of a cellular bilayer constructed of human pulmonary artery endothelial cells (HPAECs) (Lonza Biologies, Slough, United Kingdom) and human alveolar epithelial cells (A549; LGC Standards, Teddington, United Kingdom) on opposing sides of the polyester membrane of a Transwell insert (Corning Life Sciences, Lowell, MA). The cellular bilayer delineates an air-filled alveolar compartment (above the cellular bilayer) and the fluid-filled endothelial compartment (below the cellular bilayer), which mimics the pulmonary vasculature.

Aspergillus conidia were added to the alveolar compartment to mimic airborne infection. The inoculum for each strain was prepared as described previously (24). A desired final density of 1 × 10⁶ conidia/ml was verified using quantitative cultures, and 100 μl of the conidial suspension was placed in the alveolar compartment of each well insert (i.e., the absolute number of inoculated conidia was 10⁶). After 6 hours of incubation at 37°C, the residual fluid from the conidial suspension was removed from each alveolar compartment, and the well inserts were transferred into 24-well plates containing antifungal agents to mimic systemic drug administration (i.e., antifungal agents were added only to the endothelial compartment). The inserts were then incubated for a further 24 h before sampling at 30 h postinoculation. The 6-hour delay in drug administration was employed to replicate early treatment of IPA and was based upon previous studies (14, 18, 24).

Antifungal agents. The clinical formulations of voriconazole (Pfizer, Walton Oaks, United Kingdom) and anidulafungin (Pfizer) were separately reconstituted in EBM-2 medium (Lonza Biologies) with 2% fetal bovine serum (FBS) (Lonza Biologies). Serial dilutions in EBM-2 with 2% FBS were performed to create the range of concentrations required for each experiment. Three hundred microliters of voriconazole solution and 300 μl of anidulafungin solution were then added to each well of a fresh tissue culture plate to produce combinations of the two drugs at various concentrations.

Pharmacokinetics of voriconazole and anidulafungin. Voriconazole and anidulafungin were added to the endothelial compartment to mimic systemic drug administration. The pharmacokinetics in this compartment were defined by taking samples at 0, 1, 4, 8, and 24 h after the addition of each agent. Experiments were carried out in an infected model system, with three replicates performed for each treatment regimen at each time point. The following drug concentrations were studied: voriconazole at 1, 4, and 32 mg/liter; anidulafungin at 0.031, 0.125, and 1 mg/liter; and combinations of voriconazole and anidulafungin at 4 and 0.125 mg/liter and 32 and 1 mg/liter, respectively. Pharmacokinetics experiments were performed separately from the pharmacodynamics experiments.

Pharmacodynamics of voriconazole and anidulafungin combinations. The antifungal effect of combinations of voriconazole and anidulafungin were assessed by measuring concentrations of galactomannan in the endothelial compartment after 24 h of drug exposure. The concentrations used in this study were based on preliminary dose-finding experiments. For the GFP-producing strain, the initial concentration ranges of both voriconazole and anidulafungin used within the endothelial compartment were 0 to 1 mg/liter. For the strains containing the L98H and G138C mutations, voriconazole concentration ranges of 0 to 16 mg/liter and 0 to 32 mg/liter, respectively, were used. Three replicates were performed for each drug combination in each experiment, and all experiments were performed twice.

Fluorescence microscopy. Fluorescence microscopy was performed to provide a visual assessment of the growth of the GFP strain after 24 h of drug exposure. After sampling at 24 h, the well insert membranes were gently washed in PBS and fixed by placing them in 4% paraformaldehyde (Sigma-Aldrich, Gillingham, United Kingdom). The membranes were then removed from the well insert using a scalpel and mounted with a glass slide and coverslip before viewing. The alveolar compartment side of each membrane was assessed. Images were collected on an Olympus BXS1 upright microscope (Olympus, Southend-on-Sea, United Kingdom) using a 10× UPlanFL N objective and a fluorescein isothiocyanate (FITC) filter set and captured using a Cool snap ES camera (Photometrics, Tuscon, AZ) through MetaVew Software ( Molecular Devices [United Kingdom] Limited, Wokingham, United Kingdom). The images were then processed and analyzed using ImageJ (http://rsb.info.nih.gov/ij).

HPLC. Voriconazole and anidulafungin levels in media were measured using high-performance liquid chromatography (HPLC) with a Shimadzu Prominence (Shimadzu, Milton Keynes, United Kingdom). An extracted sample (20 μl) was injected onto a Kinetex 2.6 μm C₅ 100A 75- by 4.6-mm column (Phenomenex, Macclesfield, United Kingdom). A standard curve encompassing 0.0625 to 8 mg/liter and 0.006 to 10 mg/liter, respectively, was constructed in the respective matrix from stock solutions of voriconazole and anidulafungin at 1,000 mg/liter in methanol (Fisher Scientific, Loughborough, United Kingdom). The internal standard was diazepam at 1 μg/liter (Sigma-Aldrich). A gradient method was used, with starting concentrations of 80% 0.1% trifluoroacetic acid (TFA) in water and 20% 0.1% TFA in acetonitrile (Fisher Scientific) changing to 35% and 65%, respectively, over 10 min, with an overall run time of 13 min and a flow rate of 1 ml/min.

Voriconazole and the internal standard were detected using UV at 254 nm; they eluted after 3.9 and 4.7 min, respectively. The coefficient of variation (CV%) was <5% over the concentration range of 0.0625 to 8 mg/liter. The limit of detection was 0.0625 mg/liter. The intra- and interday variation was <5%. Anidulafungin was detected using fluorescence with excitation at 273 nm and emission at 464 nm. They eluted after 3.4 and 4.5 min, respectively. For plasma, the CV% was <2.4% over the concentration range of 0.05 to 10 mg/liter. The limit of detection was 0.05 mg/liter. The intra- and interday variation was <2.4%. For the remaining matrix, the CV% was <4.7% over the concentration range of 0.006 to 10 mg/liter. The limit of detection was 0.006 mg/liter. The intra- and interday variations are <5%.

Galactomannan. Galactomannan was measured by commercially available double-sandwich enzyme-linked immunosorbent assay (ELISA) (Platelia Aspergillus kit; Bio-Rad, Hemel Hempstead, United Kingdom) according to the manufacturer’s instructions with a single modification to the volume of the sample, as previously described (18).

Data analysis and mathematical modeling. The combination of the administration of voriconazole and anidulafungin was modeled using the Greco model, which is in turn based upon the concept of Loewe additivity (10, 16). This model takes the following form: 1

\[
\frac{E_{\text{VCZ}}}{(1 + \text{IC}_{\text{VCZ}}/\text{E}_{\text{VCZ}})} + \frac{E_{\text{AFG}}}{(1 + \text{IC}_{\text{AFG}}/\text{E}_{\text{AFG}})} = \frac{E_{\text{VCZ}}}{(1 + \text{IC}_{\text{VCZ}}/\text{E}_{\text{VCZ}})} + \frac{E_{\text{AFG}}}{(1 + \text{IC}_{\text{AFG}}/\text{E}_{\text{AFG}})}
\]

where IC_{VCZ} and IC_{AFG} are the concentrations of voriconazole and anidulafungin that produces 50% of the maximum effect; and E_{VCZ} and E_{AFG} are the respective slope parameters for the two drugs; IC_{VCZ} is the initial concentration for voriconazole that produces 50% of the maximum effect; and IC_{AFG} is the initial concentration of anidulafungin that produces 50% of the maximum effect; and α is the interaction parameter.

The first two terms on the right side of the equation describe the effect of voriconazole and anidulafungin alone (Loewe additivity); the third is
the interaction term and contains the interaction parameter, \( \alpha \). If \( \alpha \) is positive but the 95% confidence interval (CI) crosses zero, the combined effect is additive. If \( \alpha \) is positive and the lower bound of its 95% CI excludes zero, synergy is present. If \( \alpha \) is negative and the upper bound of its 95% CI excludes zero, antagonism is present.

**RESULTS**

**Susceptibility testing.** The MICs for voriconazole against *A. fumigatus* determined using both CLSI and EUCAST methodology are summarized in Table 1. In general, EUCAST methodology resulted in MICs that were one dilution higher than with CLSI methodology. The MECs for anidulafungin are also summarized in Table 1.

**Pharmacokinetics of voriconazole and anidulafungin.** The concentrations of voriconazole in the endothelial compartment did not significantly decrease over the 24-h experimental period (Fig. 1). The anidulafungin concentrations progressively declined over the course of the experiment. No pharmacokinetic interaction was apparent when the two drugs were combined.

**Pharmacodynamics of voriconazole and anidulafungin combinations.** Voriconazole induced a concentration-dependent decline of galactomannan in the endothelial compartment for all the *Aspergillus* strains tested (Fig. 2). The strains with elevated voriconazole MICs required proportionally greater voriconazole exposures to achieve a comparable antifungal effect. A voriconazole area under the concentration-time curve over 24 h in the steady state (AUC)/MIC ratio of 15 was required to achieve near-maximal effect as monotherapy and in combination with anidulafungin (Fig. 3). The addition of anidulafungin did not markedly alter the exposure-response relationship of voriconazole. Galactomannan levels were mildly reduced by anidulafungin monotherapy but not fully suppressed.

An additive effect between voriconazole and anidulafungin was demonstrated using the Greco model for all strains (Fig. 3 and Table 2). No antagonism was evident at any dose combination tested.

**Microscopy.** Fluorescence microscopy of the wild-type GFP strain also suggested a combination effect between voriconazole and anidulafungin (Fig. 4). A concentration-dependent decline in fungal biomass was evident with increasing voriconazole exposure. Greater anidulafungin exposure resulted in the formation of increasingly smaller “clumps” of *Aspergillus* and prominent changes in the morphology of the hyphae, which became shortened and broad. The combination of voriconazole and anidulafungin appeared to be beneficial at lower voriconazole exposures.

**DISCUSSION**

The use of combination therapy to treat IPA with voriconazole and an echinocandin is conceptually attractive because of their different mechanisms of action. IPA is a relatively common syndrome in immunocompromised patients, and mortality remains high despite treatment with currently recommended first-line agents (11). Elevated MICs for voriconazole in clinical isolates of

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**FIG 1** Pharmacokinetics of voriconazole (A) and anidulafungin (B) in the endothelial compartment of the *in vitro* model of the human alveolus. Voriconazole and anidulafungin were administered to the endothelial compartment at time zero. The respective agents were administered alone (solid lines) or in combination (dashed lines). The data points represent the means and standard deviations of three replicates.

**FIG 2** Fitted surfaces describing the effect of the combination of voriconazole (VCZ) and anidulafungin for three strains of *A. fumigatus*: the wild-type GFP strain (left), L98H (middle), and G138C (right). The drug concentrations are the initial endothelial concentrations. The combination is additive for each strain.
scribing the interaction of voriconazole and anidulafungin were as follows: $E_{\text{con}}$, 7.32 (7.23 to 7.41); $E_{\text{M0VCZ}}$, 4.88 (4.81 to 4.96); $m_{\text{VCZ}}$, 2.92 (2.88 to 2.96); $E_{\text{M0AFG}}$, $7.40 \times 10^5$ ($-4.67 \times 10^6$ to $6.15 \times 10^6$); $m_{\text{AFG}}$, 0.518 ($-0.0932$ to 1.13); $\alpha$, $0.200 \times 10^{-7}$ ($-22.7$ to 22.7). $E_{\text{con}}$ is the galactomannan index in the absence of any antifungal therapy; $E_{\text{M0VCZ}}$ is the initial AUC/MIC ratio of voriconazole in the endothelial compartment that produces half-maximal effect; $m_{\text{VCZ}}$ is the slope function for voriconazole; $E_{\text{M0AFG}}$ is the initial AUC/MIC ratio of anidulafungin in the endothelial compartment that produces half-maximal effect; $m_{\text{AFG}}$ is the slope function for anidulafungin; $\alpha$ is the interaction term.

A. fumigatus are increasingly seen (17, 42). Theoretically, combination therapy could improve efficacy through the attainment of maximal antifungal effect. Such an approach may enable undetected triazole resistance to be overcome. The risk of a submaximal antifungal effect resulting from subtherapeutic voriconazole concentrations may also be minimized. Voriconazole fails to achieve target plasma concentrations following standard regimens in a proportion of patients, who could potentially be protected by a second agent until dosage alterations are made (13, 43).

Previous studies on the effects of voriconazole and echinocandin combinations against Aspergillus have focused on wild-type isolates of A. fumigatus. The outcomes of in vitro interaction studies using broth microdilution checkerboard designs are variable, reporting either a synergistic or an additive effect, depending on the study endpoint and the mathematical definitions for the drug interaction effect (6, 7, 25, 28, 34–36, 38, 40). Most studies assess the reductions of fungal growth achieved by various concentrations of the test agents, either through visual assessment or by using a spectrophotometer. However, the measurement of these in vitro interactions is complicated by the mode of action of the echinocandins against Aspergillus. Unlike voriconazole, the echinocandins do not produce complete inhibition of fungal growth but instead cause partial inhibition in which the fungus produces short, stubby, and excessively branched hyphae (1, 23). As a consequence, defining a suitable growth endpoint when testing triazole and echinocandin combinations is problematic.

The combination of voriconazole and an echinocandin has been investigated in several laboratory animal models of IPA (4, 20, 25, 38, 44), although none have examined strains with elevated MICs. In the majority of cases, a combination results in reductions in the Aspergillus tissue burden compared with monotherapy without significant prolongation of survival. Petraitis et al. demonstrated a synergistic interaction between voriconazole and standard dosages of anidulafungin in a rabbit model of IPA using Bliss independence drug interaction analysis (38). Significant reductions in the residual fungal burden, galactomannan antigenemia,

### TABLE 2 Parameter estimates and 95% confidence intervals describing the interaction of voriconazole and anidulafungin in three isolates of A. fumigatus

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>$E_{\text{con}}$</td>
<td>Wild type (7.22–7.44)</td>
</tr>
<tr>
<td>$E_{\text{M0VCZ}}$</td>
<td>L98H (8.29–8.44)</td>
</tr>
<tr>
<td>$m_{\text{VCZ}}$</td>
<td>G138C (8.03–8.29)</td>
</tr>
<tr>
<td>$E_{\text{M0AFG}}$</td>
<td>0.164 (0.158–0.169)</td>
</tr>
<tr>
<td>$m_{\text{AFG}}$</td>
<td>1.93 (1.91–1.95)</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>2.51 (2.46–2.56)</td>
</tr>
<tr>
<td>$m_{\text{VCZ}}$</td>
<td>12.82 (8.00–17.7)</td>
</tr>
<tr>
<td>$m_{\text{AFG}}$</td>
<td>4.49 (2.86–6.11)</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>8.94 (2.96 to 2.96)</td>
</tr>
</tbody>
</table>

$^a E_{\text{con}}$ is the galactomannan index in the absence of any antifungal therapy; $E_{\text{M0VCZ}}$ is the initial concentration of voriconazole in the endothelial compartment that produces half-maximal effect; $m_{\text{VCZ}}$ is the slope function for voriconazole; $E_{\text{M0AFG}}$ is the initial concentration of anidulafungin in the endothelial compartment that produces half-maximal effect; $m_{\text{AFG}}$ is the slope function for anidulafungin; $\alpha$ is the interaction term. Because the estimate for the interaction term ($\alpha$) is close to zero and its lower 95% confidence bound crosses zero, the interaction between administration of voriconazole and anidulafungin is additive.

$^b$ Value was fixed to obtain the final parameter estimates; hence, no 95% confidence limits are available.

![FIG 3](image-url) **FIG 3** Effect induced by the combination of voriconazole and anidulafungin, expressed as the AUC/MIC ratio of each drug, for the 3 strains of A. fumigatus combined. The parameter estimates and their 95% confidence intervals describing the interaction of voriconazole and anidulafungin were as follows: $E_{\text{con}}$, 7.32 (7.23 to 7.41); $E_{\text{M0VCZ}}$, 4.88 (4.81 to 4.96); $m_{\text{VCZ}}$, 2.92 (2.88 to 2.96); $E_{\text{M0AFG}}$, $7.40 \times 10^5$ ($-4.67 \times 10^6$ to $6.15 \times 10^6$); $m_{\text{AFG}}$, 0.518 ($-0.0932$ to 1.13); $\alpha$, $0.200 \times 10^{-7}$ ($-22.7$ to 22.7). $E_{\text{con}}$ is the galactomannan index in the absence of any antifungal therapy; $E_{\text{M0VCZ}}$ is the initial AUC/MIC ratio of voriconazole in the endothelial compartment that produces half-maximal effect; $m_{\text{VCZ}}$ is the slope function for voriconazole; $E_{\text{M0AFG}}$ is the initial AUC/MIC ratio of anidulafungin in the endothelial compartment that produces half-maximal effect; $m_{\text{AFG}}$ is the slope function for anidulafungin; $\alpha$ is the interaction term.

![FIG 4](image-url) **FIG 4** Fluorescence microscopy of the wild-type GFP strain within the alveolar compartment after 24 h of exposure to combinations of voriconazole and anidulafungin (initial endothelial compartment drug concentrations are shown). Perforations in the well insert membrane are visible in the background of each image. Scale bar, 100 μm.
anidulafungin made a small additional contribution to the reduction when voriconazole was used in place of caspofungin. In our model, liquid culture of caspofungin resulted in increased levels of galactomannan in a patient reported by Klont et al. in which increased concentrations of galactomannan is released (3). This paradoxical effect has not been demonstrated on the tips and branch points of hyphae, from which galactomannan may cause a paradoxical increase in circulating galactomannan concentrations with disease progression and the response to treatment (2, 22, 26, 32, 33). Importantly, the relationship between galactomannan and the antifungal effect of echinocandins may be different than for other antifungal agents. The treatment of aspergillosis due to isolates of A. fumigatus with elevated voriconazole MICs. We have recently used a dynamic model of the human alveolus to demonstrate that A. fumigatus Cyp51A mutants with decreased susceptibility to voriconazole can be treated only with drug exposures that are likely to be toxic to humans (19). Our current data suggest that the addition of anidulafungin to voriconazole does not significantly alter the exposure-response relationships for isolates of A. fumigatus with these resistance mutations. Voriconazole concentrations were the major determinant of response in our model, as assessed both by levels of galactomannan and by using microscopy endpoints with the GXP-expressing wild type.

We used galactomannan as a biomarker to assess fungal growth and the antifungal effect of voriconazole and anidulafungin. Previous work using this model has correlated galactomannan concentrations with disease progression and the response to treatment with posaconazole (18). There is also evidence from clinical studies that galactomannan can be used as a marker for clinical response to treatment (2, 22, 26, 32, 33). Importantly, however, the relationship between galactomannan and the antifungal effect of echinocandins may be different than for other antifungal agents. The treatment of aspergillosis with an echinocandin may cause a paradoxical increase in circulating galactomannan concentrations (15, 21). This could be explained by the mode of action of echinocandins, which show a preferential effect on the tips and branch points of hyphae, from which galactomannan is released (3). This paradoxical effect has not been demonstrated in all studies (31) but was apparent in an in vitro experiment reported by Klont et al. in which increased concentrations of caspofungin resulted in increased levels of galactomannan in a liquid culture of A. fumigatus (21). The effect was not seen when voriconazole was used in place of caspofungin. In our model, anidulafungin made a small additional contribution to the reduction of galactomannan achieved by voriconazole and produced a similarly small reduction when administered as monotherapy. The contribution of anidulafungin to the antifungal effect of the combination may be underestimated using galactomannan as a measure of the antifungal effect. Determining whether this is also true for resistant strains would require the construction of additional transformed strains expressing GFP. While the effects of anidulafungin appeared more pronounced using a microscopy endpoint (Fig. 4), the agent still failed to cause hyphal death. This highlights the difficulty of measuring the antifungal activity of echinocandins against Aspergillus using experimental models and conventional biomarkers. Notably, voriconazole was able to suppress galactomannan in all Aspergillus strains when administered at sufficiently high concentrations, regardless of the magnitude of anidulafungin drug exposure. Consequently, failure to suppress serum galactomannan in a patient treated for aspergillosis with a combination of a triazole and an echinocandin suggests inadequate therapy rather than a paradoxical effect that can be safely ignored.

We acknowledge several limitations of this study. First, we used a static in vitro model, which mimics very early invasive disease rather than established pulmonary infection, where the drug exposure-response relationships may be different. Furthermore, the pharmacokinetics of both antifungal agents in this model are not necessarily humanlike. Moreover, we did not study the additional antifungal effect of pulmonary alveolar macrophages that serve as innate immune effector cells even in immunocompromised hosts. Nevertheless, we have demonstrated an additive interaction for the combination of voriconazole and anidulafungin against A. fumigatus, with voriconazole primarily responsible for the antifungal effect. Our results reinforce the need to consider alternative agents, such as amphotericin B, for the treatment of IPA when voriconazole resistance mutations are present, rather than necessarily relying on a voriconazole and echinocandin combination. A rise in serum galactomannan during combination therapy with these agents should be interpreted as treatment failure, and alternative antifungal regimens should be sought.

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