In *Vitro* Combination of Voriconazole and Miltefosine against Clinically Relevant Molds

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Invasive fungal infections caused by filamentous fungi are a major threat for immunocompromised patients. Innate/acquired resistance to antifungal drugs might necessitate combination therapies. We assessed the potential combination of voriconazole with miltefosine, an original drug with antifungal activity against 33 clinically relevant mold isolates, including both azole-susceptible and -resistant *Aspergillus*. Using complete inhibition as an endpoint, interactions were indifferent for 32/33 isolates. An alternative 50% inhibition endpoint showed synergistic interactions for 14/33 isolates. Antagonism was absent.

Invasive fungal infections (IFI) due to filamentous fungi are a major threat for immunocompromised patients. *Aspergillus fumigatus* is the most common IFI, but other species, such as *Aspergillus flavus*, *Aspergillus niger*, or the naturally azole-resistant *Aspergillus ustus*, are also frequently retrieved (1). Currently, the antifungal armamentarium for systemic filamentous infection is restricted to 3 classes: azoles, echinocandins, and polyenes. Echinocandins are only fungistatic against filamentous fungi, and breakthrough of mold IFI during echinocandin treatment has been reported (2). Broad-spectrum antifungal polyene is associated with frequent adverse effects. Moreover, the recent emergence of azole-resistant *Aspergillus* strains is disquieting and potentially a threat for human health (3, 4). Finally, genera such as *Scedosporium* or *Fusarium* exhibit low susceptibility to all antifungals. To overcome acquired or innate antifungal drug resistance and improve IFI management, combinations of drugs belonging to different classes have been tested and may be useful (5–7).

Miltefosine is an alkylphosphocholine with antineoplastic and especially antiparasitic properties. Despite its very frequent gastrointestinal side effects and a strict contraindication in pregnant women, the drug is now widely used for leishmaniasis treatment (9). However, its use for fungal infections in humans is extremely rare. In the present *in vitro* study, we investigated the potential synergy of a combination of voriconazole and miltefosine against different clinically relevant molds.

We used 33 clinical isolates collected in two French hospitals (Pitié-Salpêtrière and Hôpital Européen Georges Pompidou, Paris) (Table 1): 12 *A. fumigatus* isolates with wild-type cyp51A, 5 cyp51A-mutated *A. fumigatus* isolates (4 with the TR34/L98H alteration and one with the newly described sole Y121F alteration [10]), 3 *A. ustus* isolates, 3 *A. flavus* isolates, 3 *Aspergillus* section *Nigri* isolates (2 *A. niger* and 1 *A. fumigatus*), 4 *Scedosporium apiospermum* isolates, and 3 *Fusarium solani* isolates. Identification was confirmed by molecular sequencing (internal transcribed spacer [ITS] region, beta-tubulin, and calmodulin genes). For miltefosine, MICs were determined using the EUCAST method. A complete inhibition endpoint was determined visually and by spectrophotometric analysis. Alternatively, as previously reported by Widmer et al. (9), the MIC for miltefosine was defined as the concentration producing at least 50% inhibition after 48 h of incubation at 35°C for *Aspergillus* (72 h for *Fusarium* and *Scedosporium*). This alternative endpoint was determined uniquely by spectrophotometric analysis. We used the checkerboard method to test combinations of voriconazole and miltefosine. The MICs of each drug alone and the combinations of the two were determined concomitantly on the same plate. The volume of each drug dispensed was 50 μl to reach a volume of 100 μl per well. Each well was then inoculated with 100 μl of a suspension containing 2 × 10⁵ to 5 × 10⁵ CFU/ml, yielding a final inoculum of 1 × 10⁵ to 2.5 × 10⁵ CFU/ml per well and a final concentration between 0.5 and 32 mg/liter for miltefosine and between 0.008 and 4 mg/liter for voriconazole. Interaction was determined by calculating the fractional inhibitory concentration index (FICI) as follows: FICI = (MIC of voriconazole combination/MIC of voriconazole alone) + (MIC of miltefosine combination/MIC of miltefosine alone). A FICI value of ≤0.5 indicated synergy between the two drugs, whereas a value of >4 indicated antagonism. Values between 0.5 and 4 indicated indifference (11). Each isolate was tested at least two times. The *Candida parapsilosis* strain ATCC 22019 was used as a quality control.

The duplicates gave similar results (i.e., with ≤1 2-fold dilution difference, except for 2 isolates with two 2-fold dilution differences and one isolate with five 2-fold differences) and identical FICI interpretations (except for one isolate) (see the supplemental material for detailed per-isolate data). The results of antifungal synergy testing are summarized in Table 1. When the 100% inhibition endpoint was used, for the non-cyp51A-mutated *A. fumigatus* isolates, the geometric mean MICs for voriconazole and miltefosine were 0.73 mg/liter (range, 0.25 to 1 mg/liter) and 10.7 mg/liter, respectively. For the cyp51A-mutated isolates, the geometric mean MICs were 4 mg/liter for voriconazole. Interaction was determined by calculating the fractional inhibitory concentration index (FICI) as follows: FICI = (MIC of voriconazole combination/MIC of voriconazole alone) + (MIC of miltefosine combination/MIC of miltefosine alone). A FICI value of ≤0.5 indicated synergy between the two drugs, whereas a value of >4 indicated antagonism. Values between 0.5 and 4 indicated indifference (11). Each isolate was tested at least two times. The *Candida parapsilosis* strain ATCC 22019 was used as a quality control.

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TABLE 1. Combination of miltefosine and voriconazole against 33 clinically relevant mold isolates

<table>
<thead>
<tr>
<th>Organism</th>
<th>Combined MIC range (mg/liter)</th>
<th>MIC determination</th>
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<tbody>
<tr>
<td><strong>Combination of miltefosine and voriconazole against 33 clinically relevant mold isolates</strong></td>
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<td></td>
</tr>
<tr>
<td><strong>Alone</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>4–8 (4.29)</td>
<td></td>
</tr>
<tr>
<td>A. niger</td>
<td>2–4 (3.25)</td>
<td></td>
</tr>
<tr>
<td>A. flavus</td>
<td>1–2 (1.62)</td>
<td></td>
</tr>
<tr>
<td><strong>Combined</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>4–8 (4.6)</td>
<td></td>
</tr>
<tr>
<td>A. niger</td>
<td>0.5–1 (0.62)</td>
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</tbody>
</table>

**Note:** FICI values indicate synergy (0.375–0.5), indifference (0.5–4), and antagonism (>4).
ACKNOWLEDGMENTS
S.I. performed the experiments and participated in the writing of the manuscript. M.P. performed several experiments. I.M. performed molecular analyses. E.D. furnished several fungal isolates, participated in scientific discussions, and participated in the writing of the manuscript. D.M. and A.D. participated in the writing of the manuscript. A.F. designed the study and wrote the article.

REFERENCES