**In vitro** activity of miltefosine in combination with voriconazole or amphotericin B against clinical isolates of *Scedosporium* spp.

*Scedosporium* spp. are significant fungal pathogens in both immunocompromised and immunocompetent individuals. The effectiveness of antifungal therapy against *Scedosporium* spp. is limited due to their multidrug resistance to most widely used antifungal chemotherapies such as azoles, polyenes and echinocandins (Tortorano et al., 2014). Voriconazole, sometimes combined with surgery, appears to be the only drug significantly active against scedosporiosis. However, the mortality due to scedosporiosis remains high and finding more effective antifungal strategies is necessary. Antifungal combination treatment of drugs belonging to different classes may be a useful approach. So far, azoles in combination with echinocandins or polyenes or terbinafine, as well as polyenes in combination with other drugs, have been tested in **in vitro** with various results (Afeltra et al., 2002; Cuenca-Estrella et al., 2008; Lackner et al., 2014; Meletiadis et al., 2003; Rodriguez et al., 2009; Troke et al., 2008; Yustes & Guarro, 2005).

Miltefosine, an alkyl phosphocholine compound first developed as an anticancer agent and known to be active against *Leishmania* spp. and *Trypanosoma cruzi*, has also shown broad-spectrum antifungal activity in **in vitro** (Tong et al., 2007; Widmer et al., 2006) including against *Scedosporium* spp. In **in vitro** data on miltefosine combination therapy remain scarce and results are contradictory (Biswas et al., 2013; Imbert et al., 2014). Moreover, no clinical studies guiding the use of this new compound have been published, and only three cases illustrating the use of miltefosine alone (Ferguson et al., 2013) or in combination with voriconazole and terbinafine (Kesson et al., 2009; Trubiano et al., 2014) have been reported. The aim of this study was to evaluate the **in vitro** activity of miltefosine in combination with voriconazole or amphotericin B against clinical isolates of *Scedosporium* spp.

Eleven clinical isolates of *Scedosporium* spp. from two university hospitals (Hôpital Henri Mondor and Hôpital Européen Georges Pompidou, Paris, France) were tested in this study. Isolates were identified to species level using molecular and/or matrix-assisted laser desorption/ionization time-of-flight mass spectrometry as described previously (Sittler et al., 2013). The MICs of miltefosine, amphotericin B and voriconazole alone and in combination were evaluated using a two-dimensional broth chequerboard method according to Clinical and Laboratory Standards Institute standard M38-A2 as described by Dannaoui et al. (2004). The end point was defined as 100 % inhibition of growth for all three drugs alone and in combination compared with the control after 72 h of incubation at 35 °C. Based on preliminary results, the concentration range of both amphotericin B and voriconazole (0.03–16 mg l⁻¹) was chosen to allow detection of synergy. The **in vitro** susceptibility testing was performed in duplicate for each strain and results were read by two investigators. Strains ATCC 6258 (*Candida krusei*) and ATCC 22019 (*Candida parapsilosis*) were used as quality controls. Combinations were evaluated by calculating the fractional inhibitory concentration index (FICI) as follows: FICI = (MICdrug A comb./MICdrug A alone) + (MICdrug B comb./MICdrug B alone), where drugs A and B were miltefosine and amphotericin B or voriconazole, respectively. Interpretation of the FICI was as follows: ≤0.5, synergism; between 0.5 and 4, indifference; >4, antagonism. The results of antifungal combination testing are shown in Table 1. For all 11 isolates (comprising three *Scedosporium prolificans*, seven *Scedosporium apiospermum / Pseudallescheria boydii* and one *Scedosporium aurantiacum*), single drug susceptibility testing showed geometric mean MICs of 26.2, 7.9 and 3.3 mg l⁻¹ for amphotericin B, voriconazole and miltefosine, respectively, which is consistent with other studies (Cuenca-Estrella et al., 2008; Biswas et al., 2013). As expected, *S. prolificans* isolates showed the highest MICs when compared with other species. Both antifungal combinations tested showed indifferent interaction for all strains, exhibiting FICI values of 0.53–2.5 and 0.75–2.25 for miltefosine/amphotericin B and miltefosine/voriconazole combinations, respectively. To the best of our knowledge, this is the first study evaluating the combination of miltefosine with amphotericin B against *Scedosporium* spp.

There are few data concerning the **in vitro** activity of miltefosine in combination with azoles against *Scedosporium* spp. and they are contradictory: Imbert et al. (2014) reported no interaction when testing four *S. apiospermum* isolates using a 100 % inhibition end point, while Biswas et al. (2013) reported synergy between miltefosine and voriconazole (FICI=0.5) in one out of two *S. prolificans* isolates tested. Two studies reported the successful use of miltefosine in a combination treatment in humans, in association with terbinafine and voriconazole in an osteomyelitis case, and in association with voriconazole alone in a case of disseminated infection (Kesson et al., 2009; Trubiano et al., 2014). However, these studies cannot provide the results of **in vitro** miltefosine/voriconazole interactions for comparison.

The current study provides more **in vitro** susceptibility data for miltefosine alone and in combination against *Scedosporium* spp. It confirms the good **in vitro** activity of this molecule against various species of *Scedosporium*, with MICs ranging from 2 to 4 mg l⁻¹ in our series (Biswas et al., 2013; Widmer et al., 2006). Although no synergy was observed, there was a major reduction in amphotericin B and voriconazole MICs when they were used in combination with miltefosine (the geometric mean decreased from 26.2 to
Table 1. Combination of miltefosine with amphotericin B and voriconazole against 11 Scedosporium clinical isolates

<table>
<thead>
<tr>
<th>Species (n)</th>
<th>VRC MIC (mg l⁻¹)</th>
<th>FICI MIL + VRC</th>
<th>AMB MIC (mg l⁻¹)</th>
<th>FICI MIL + AMB</th>
<th>MIL MIC (mg l⁻¹)</th>
<th>FICI MIL + MIL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scedosporium prolificans</td>
<td>3</td>
<td>0.03–32</td>
<td>0.03–8</td>
<td>1.00–1.25</td>
<td>1–4</td>
<td>1–4</td>
</tr>
<tr>
<td>Scedosporium apiospermum/Pseudallescheria boydii</td>
<td>7</td>
<td>0.03–16</td>
<td>0.03–0.125</td>
<td>1.00–1.25</td>
<td>1–4</td>
<td>1–4</td>
</tr>
<tr>
<td>Scedosporium aurantiacum</td>
<td>11</td>
<td>0.03–32</td>
<td>0.03–8</td>
<td>1.00–32</td>
<td>0.03–8</td>
<td>0.03–8</td>
</tr>
</tbody>
</table>

0.5 mg l⁻¹ for amphotericin B, and from 7.9 to 0.8 mg l⁻¹ for voriconazole), even with S. prolificans, which is consistent with the data from Biswas et al. (2013), who reported a 100-fold decrease in voriconazole MICs using a voriconazole/miltefosine combination against S. prolificans (Biswas et al., 2013). In vitro synergy has only been reported once using the association miltefosine/voriconazole against Scedosporium spp. (Biswas et al., 2013), and our series tends to indicate that an indifferent interaction is most commonly observed. However, as Scedosporium spp. are susceptible to both drugs independently and no antagonism has been reported between these two compounds (Biswas et al., 2013; Imbert et al., 2014), their combination in salvage therapy seems legitimate (Kesson et al., 2009; Trubiano et al., 2014). Further animal and clinical studies are needed to assess the place of miltefosine in antifungal therapy against Scedosporium spp.

Acknowledgements

During the past 5 years, E.D. has received money for board membership from Astellas and Innothera, grants from Gilead, Ferrer and Bio-Rad, payments for lectures from Gilead, MSD and Schering, and travel expenses from MSD, Astellas and Schering.

Fabrice Compain, ¹
Françoise Botterel, ²,³
Emilie Sitterlé, ²,³
André Paugam, ⁴
Marie-Elisabeth Bougnoux ⁵ and Eric Dannaoui ⁷,⁹

¹Université Paris-Descartes, Faculté de Médecine, APHP, Hôpital Européen Georges Pompidou, Unité de Parasitologie–Mycologie, Service de Microbiologie, Paris, France
²APHP, Hôpital Henri Mondor, Unité de Parasitologie–Mycologie, Service de Microbiologie, Créteil, France
³Dynamyc Research Group, Université Paris-Est Créteil, Faculté de Médecine, Créteil, France
⁴Université Paris-Descartes, Faculté de Médecine, APHP, Hôpital Cochin, Service de Parasitologie–Mycologie, Paris, France
⁵Université Paris-Descartes, Faculté de Médecine, APHP, Hôpital Necker Enfants-Malades, Unité de Parasitologie–Mycologie, Service de Microbiologie, Paris, France

Correspondence: Eric Dannaoui (eric.dannaoui@egp.aphp.fr)

Abbreviation: FICI, fractional inhibitory concentration index.


