The combination of two antifungal agents, voriconazole (VRC) and micafungin (MFG), was assessed for synergism against clinically relevant yeasts and molds. So far, only one little in vitro study including 10 isolates of Aspergillus fumigatus has been performed on the specific combination of VRC and MFG (6, 9). In the present study, we have investigated the interaction between VRC and MFG by checkerboard assays against 196 clinical isolates from patients, including fluconazole-resistant strains from human immunodeficiency virus-infected patients (15) (Candida spp. [n]; C. albicans [55], C. dubliniensis [19], C. glabrata [12], and C. parapsilosis [12]; filamentous fungi: A. fumigatus [61], A. flavus [24], A. niger [3], A. nidulans [3], Scedosporium prolificans [3], S. apiospermum [3], and Fusarium solani [1]) C. albicans ATCC 90028, C. dubliniensis CBS 7987, C. parapsilosis ATCC 22013, A. niger ATCC 9642, and S. prolificans ATCC 200549 were used as controls. The assay preparation for Candida was performed in accordance with NCCLS document M27-A2 (17). Since the NCCLS M38-A (16) reference method for testing filamentous fungi does not give recommendations for in vitro testing of echinocandins, minor modifications were necessary. Several preliminary tests demonstrated an incubation temperature of 37°C and an incubation time of 24 h (11, 12) as optimal conditions. Conidia were counted on a hemocytometer. Measuring the metabolic activity by the 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carboxyl]2H-tetrazoliumhydroxide (XTT) colorimetric method was used to determine the MIC endpoints (10). Standard antifungal powder of VRC was provided by Pfizer (New York, N.Y.), while MFG was provided by Fujisawa (now Astellas Pharma Inc., Tokyo, Japan). The final concentration of the antifungal agents ranged from 0.0156 to 4 μg/ml for VRC and from 0.002 to 128 μg/ml for MFG. The interactions were investigated by a checkerboard titteration broth microdilution-based method. 96-well round-bottom plates (Corning BV, Schiphol-Rijk, The Netherlands) were prepared with twofold concentrated RPMI 1640 medium (with 1-glutamine, without bicarbonate) (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) supplemented with 2% glucose (AppliChem GmbH, Darmstadt, Germany). For the filamentous fungi checkerboards, RPMI 1640 medium (Sigma Aldrich Chemie GmbH, Steinheim, Germany) without phenol red for the endpoint determination by the XTT colorimetric method, was used.

For Candida spp., MIC endpoints were determined spectrophotometrically at 540 nm (spectrophotometer MR 700; TECAN-Deutschland GmbH, Crailsheim, Germany) and recorded as the first concentration of the antifungal agent tested alone or in combination at which the absorbance value was 50% less than that in the control well (17). For filamentous fungi, the read-out was performed by recording 50% growth inhibition (MIC) using the XTT (2,3-bis(2-methoxy-4-nitro-5-sulphophenyl)-5-[(phenylamino)carboxyl]-2H-tetrazoliumhydroxide) (AppliChem GmbH, Darmstadt, Germany) colorimetric method (11–13). Absorbance was measured spectrophotometrically at 450 nm (690 nm reference). MIC endpoints were determined as the first concentration of the antifungal agent tested alone or in combination at which the absorbance value was 50% less than that in the control well. For calculation purposes, off-scale MICs were converted to the next higher dilution. In vitro drug interactions were calculated on the basis of fractional inhibitory concentration (FIC) index as previously published 2, 6, 18).

Table 1 summarizes the 50% minimum inhibitory concentration (MIC50) of VRC or MFG alone and the corresponding MIC50 in combination. The median FIC indices (2FIC) and the resulting interaction for the 201 isolates tested are included.

Candida spp. With regard to the MIC50 (Table 1), high susceptibility to VRC (1, 4, 5) and MFG in single use was documented for all Candida isolates. In combination they demonstrated indifferent effects in 97% of the isolates. Due to the combination, a substantial reduction in the VRC MIC50 was detectable, while no reduction was found for MFG. However, only three of the tested strains demonstrated synergistic effects. Johnson et al. (6) proposed that the combination of azoles (VRC) with echinocandins (MFG) has not been particularly impressive in vitro. Since echinocandins administered alone are highly active against most Candida spp. (8, 14), the fungicidal activity may be difficult to improve in combination.
The MICs (data not shown) of VRC and MFG are so low that a possible synergism was below the detection limit of our system. The fact that there is only a significant reduction for the VRC MIC$_{50}$ emphasizes the high activity of MFG against Candida spp. The reduction of the VRC MIC$_{50}$ results from the high activity of MFG alone and is not an interaction effect. Pfaffer et al. (20) demonstrated this effect for caspofungin. To further analyze the potential use of MFG as single therapy as Arikan et al. (3, 7) demonstrated this effect for caspofungin. To further analyze the potential use of MFG as single therapy, Pfaller et al. (20) demonstrated this effect for caspofungin. To further analyze the potential use of MFG as single therapy, as add-on therapy. Clinical studies to investigate the lack of toxicity of both antifungals makes them an attractive option as add-on therapy. Clinical studies to investigate the effectiveness of MFG combined with VRC are warranted.

### Filamentous fungi
For all filamentous isolates, respectable reductions of the MFG MIC$_{50}$ were obtained (Table 1). The combination of VRC and MFG led to a significant reduction of the MIC$_{50}$S. The interaction between VRC and MFG (Table 1) was synergistic in 64% of all molds and 79% for the A. fumigatus isolates. For S. prolificans, a pathogen with enhancing frequency of occurrence, causing various types of human infections, for three out of four tested isolates synergistic effects were observed. The majority of all indifferent FIC indices were close to the cutoff from synergism to indifference. Antagonism was not observed. In contrast to the results for the Candida spp., the combination was generally synergistic, in accordance to the investigations of Johnson et al., Manavathu et al., and Petraitis et al. (6, 9, 19). It must be noted that clinical data from in vitro studies of the activities of the VRC-MFG combination against filamentous fungi have been poorly evaluated. According to the investigations of Manavathu et al., interactions between the two drugs were additive (9). Data from in vitro studies are sparse. An in vivo animal study demonstrated similar efficacy for monotherapy and combination therapy; however, the activity was neither enhanced nor reduced with the two-drug combination (3). Thus, current data suggest an absence of harm or a negative interaction when an azole is combined with an echinocandin for the treatment of invasive aspergillosis (3, 7). The discrepancies of the activity between in vitro and in vivo studies have to be further investigated. For the most important clinical pathogen, A. fumigatus, we observed almost 80% synergistic effects. In conclusion, the combination of VRC and MFG was synergistic in vitro against filamentous fungi including Scedosporium spp. and F. solani. The relative lack of toxicity of both antifungals makes them an attractive option as add-on therapy. Clinical studies to investigate the effectiveness of MFG combined with VRC are warranted.

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