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Activity of Posaconazole and Comparator Antifungal Agents Tested Against Filamentous Fungi

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Introduction

- The epidemiology of invasive filamentous fungal disease has changed in recent years. Although *Aspergillus fumigatus* is the most frequent pathogen worldwide, infections are increasingly due to Mucorales, *Fusarium, Scedosporium* species, and non-*fumigatus* species of *Aspergillus*.
- These emergent fungal pathogens have different susceptibility patterns that may affect clinical practices.
- Posaconazole is a broad-spectrum triazole antifungal that exhibits potent antifungal activity against a variety of yeasts and moulds.
- Posaconazole is approved by US-FDA for prophylaxis of invasive *Aspergillus* and *Candida* infections and for treatment of oropharyngeal candidiasis, including those infections refractory to itraconazole and/or fluconazole.
- We evaluated the *in vitro* activities of posaconazole and comparator antifungal agents against 2,554 isolates of filamentous fungi, including 2,100 *Aspergillus* species and 454 non-*Aspergillus* moulds (98 *Fusarium*, 81 Mucorales, and 76 *Scedosporium* species isolates) collected worldwide from clinically significant infections from 2010 to 2018.

Materials and Methods

- A total of 2,554 non-duplicate fungal isolates were collected prospectively (1/patient) from 75 medical centers.
- Only isolates determined to be significant by local criteria as the reported probable cause of infection were included in the program.
- Isolates were submitted to matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) using the MALDI Biotyper (Bruker Daltonics, Billerica, Massachusetts, USA).
- Isolates that were not identified by proteomic methods were identified using previously described sequencing-based methods.
- Susceptibility testing was performed for posaconazole, itraconazole, voriconazole, caspofungin, anidulafungin, micafungin, and amphotericin B according to the CLSI M38 document (2018).
- CLSI epidemiological cutoff value (ECV) interpretive criteria were applied (M59, 2020).
- Since ECVs for posaconazole are not available for *A. fumigatus* and the CLSI method, we calculated the ECVs based on this isolate collection for posaconazole, itraconazole, and voriconazole. Calculations were performed according to the CLSI M57 document (2016).
- Quality control (QC) was performed as recommended by CLSI using the following strains: *Candida parapsilosis* ATCC 22019, *Candida krusei* ATCC 6258, *Aspergillus flavus* ATCC 204304, and *Aspergillus fumigatus* ATCC MYA-3626.
- Isolates displaying echinocandin MIC>ECV were sequenced for *cyp51* mutations by the MiSeq Sequencer (Illumina).
- Each sample was assembled using a reference-guided assembly in DNASTAR SeqMan NGen v.14.0 (Madison, Wisconsin, USA) and compared to sequences available in the literature.

Results

- Organism group and distribution per geographic origin and specimen type are shown in Figures 1 and 2, respectively.
- Posaconazole ($MIC_{50/90}$, 0.25/0.5 mg/L) showed comparable activity to itraconazole ($MIC_{50/90}$, 0.5/1 mg/L) and voriconazole ($MIC_{50/90}$, 0.5/0.5 mg/L) against *A. fumigatus* (Table 1).
- The posaconazole ECV generated using *A. fumigatus* isolates from this collection at 97.5% was 0.5 mg/L (Figure 3).
  - ECV values calculated at 95%, 97.5%, and 99% for posaconazole, itraconazole, and voriconazole are displayed in Figure 3.
- Categorical agreement between posaconazole and the other azoles tested against *A. fumigatus* ranged from 98.2–98.7%.
- Posaconazole ($MIC_{50/90}$, 0.5/0.5 mg/L) exhibited similar activity to voriconazole ($MIC_{50/90}$, 0.5/1 mg/L) and itraconazole ($MIC_{50/90}$, 0.5/1 mg/L) against *Aspergillus* section *Flavi* and other *Aspergillus* groups (Table 1).
- Most (>95%) of the *Aspergillus* species isolates tested were wild-type (WT) to all azoles and echinocandins (Table 1).
- Among the isolates of *A. fumigatus*, the rate of non-wildtype (NWT) strains varied across the different geographic regions (Table 3).
  - The frequency of azole NWT strains of *A. fumigatus* from Europe increased steadily from 2010 to 2018.
  - There was no consistent trend for an increased frequency of NWT strains from other geographic regions.
- Among the 27 azole-NWT *A. fumigatus* strains, 18 (66.7%) displayed mutations in the *cyp51A* that encoded the sterol 14α-demethylase, which is the target of the azoles (Table 4).
  - Most of these isolates were from European countries; additionally, 8 isolates came from 1 Italian hospital.
- The azoles and echinocandins showed poor activity against *Fusarium* and *Scedosporium* species (Table 2).
- Posaconazole ($MIC_{50/90}$, 1/2 mg/L) and amphotericin B ($MIC_{50/90}$, 1/2 mg/L) were the most active agents against the Mucorales isolates (Table 2).

Conclusions

- Posaconazole exhibited excellent activity against most species of *Aspergillus* and was comparable to itraconazole and voriconazole.
- Most *Aspergillus* species remain susceptible to triazoles. Although there was no evidence for an increasing frequency of NWT strains among *A. fumigatus* isolates from North America, Latin America, or the Asia-Pacific region, we confirm an increase in the rate of NWT strains to all 3 triazoles among isolates from Europe.
- Most of the azole-NWT strains of *A. fumigatus* displayed CYP51A alterations.
- Azoles and echinocandins have limited activity against *Fusarium* and *Scedosporium* species.

Figure 1 Distribution of main species and organism groups of mould isolates collected from 2010–2018

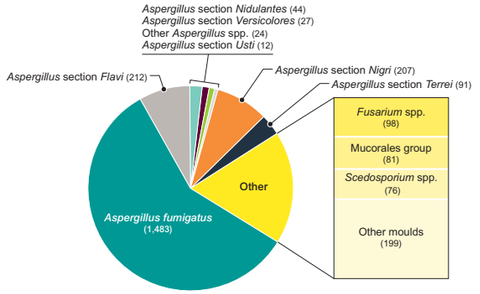


Table 1 Antimicrobial activity of posaconazole and comparator agents tested against *Aspergillus* spp. isolates using the CLSI method

Antimicrobial agent (No. of isolates)	MIC_{50}	MIC_{90}	ECV[a] %WT	ECV[a] %NWT
A. fumigatus (n=1,483)				
Posaconazole	0.25	0.5	97.9[b]	2.1[b]
Itraconazole	0.5	1	98.2	1.8
Voriconazole	0.5	0.5	99.0	1.0
Caspofungin	0.015	0.03	100.0	0.0
Anidulafungin	0.015	0.03		
Micafungin	≤0.008	0.015		
Amphotericin B	1	2	99.1	0.9
A. section *Flavi* (n=212)				
Posaconazole	0.5	0.5	97.6	2.4
Itraconazole	0.5	1	99.5	0.5
Voriconazole	0.5	1	100.0	0.0
Caspofungin	0.015	0.03	100.0	0.0
Anidulafungin	≤0.008	0.015		
Micafungin	0.015	0.03		
Amphotericin B	2	2	100.0	0.0
A. section *Nigri* (n=207)				
Posaconazole	0.5	1	100.0	0.0
Itraconazole	1	2	98.5	1.5
Voriconazole	1	2	99.5	0.5
Caspofungin	0.015	0.03	100.0	0.0
Anidulafungin	≤0.008	0.015		
Micafungin	≤0.008	0.03		
Amphotericin B	1	1	100.0	0.0
A. section *Terrei* (n=91)				
Posaconazole	0.25	0.5	100.0	0.0
Itraconazole	0.5	1	100.0	0.0
Voriconazole	0.5	0.5	100.0	0.0
Caspofungin	0.015	0.03	98.9	1.1
Anidulafungin	0.015	0.03		
Micafungin	≤0.008	0.015		
Amphotericin B	2	>2		

[a] ECV criteria published in CLSI M59 (2018).
[b] Based on the ECV calculated for this study.

Table 2 Antimicrobial activity of posaconazole and comparator agents tested against mould isolates other than *Aspergillus* spp. using the CLSI method

Antimicrobial agent [a] (No. of isolates)	MIC_{50}	MIC_{90}
Fusarium solani species complex (n=49)		
Posaconazole	>8	>8
Itraconazole	>8	>8
Voriconazole	8	>8
Caspofungin	>4	>4
Anidulafungin	>4	>4
Micafungin	>4	>4
Amphotericin B	2	2
Mucorales (n=81)		
Posaconazole	1	2
Itraconazole	2	8
Voriconazole	>8	>8
Caspofungin	>4	>4
Anidulafungin	>4	>4
Micafungin	>4	>4
Amphotericin B	1	2
Scedosporium apiospermum/S. boydii (n=65)		
Posaconazole	1	2
Itraconazole	2	8
Voriconazole	0.5	1
Caspofungin	2	>4
Anidulafungin	4	4
Micafungin	0.5	>4
Amphotericin B	2	>2

[a] Clinical breakpoint criteria or ECV interpretation unavailable.

Table 3 Activity of azole agents against *Aspergillus fumigatus* by geographic region

	North America (n=757)		Europe (n=493)		Asia-Pacific (n=171)		Latin America (n=62)	
Antifungal agent	MIC_{50}/MIC_{90}	(%NWT)[a]	MIC_{50}/MIC_{90}	(%NWT)[a]	MIC_{50}/MIC_{90}	(%NWT)[a]	MIC_{50}/MIC_{90}	(%NWT)[a]
Posaconazole	0.25/0.5	2.0[b]	0.25/0.5	2.8[b]	0.25/0.5	0.6[b]	0.25/0.5	1.6[b]
Itraconazole	1/1	1.2	0.5/1	3.4	0.5/1	0.6	0.5/1	0.0
Voriconazole	0.5/0.5	0.3	0.5/0.5	2.4	0.5/0.5	0.6	0.5/0.5	0.0

NWT, non-wildtype
[a] ECV criteria published in CLSI M59 (2020).
[b] Based on the ECV (at 97.5%) calculated for this study.

Table 4 Summary of CYP alterations detected among non-wildtype *Aspergillus* spp. isolates

Organism	No. of isolates	Region (no. of isolates)	MIC range according to CLSI method (mg/L): Posaconazole	Itraconazole	Voriconazole	Aminoacid substitutions: CYP51A	CYP51B
Aspergillus flavus species complex	3	NA (2); APAC (1)	0.5-1	2-8	1-2	WT	WT
Aspergillus fumigatus	1	NA	0.5	2	0.5	A9T	WT
Aspergillus fumigatus	1	EU	1	>8	0.5	F219I	WT
Aspergillus fumigatus	1	NA	0.5	2	1	F46Y, M172V, E427K	WT
Aspergillus fumigatus	1	EU	0.5	2	1	F46Y,M172V,N248T,D255E,E427K	WT
Aspergillus fumigatus	3	NA	0.5-1	2	0.5-1	I242V	WT
Aspergillus fumigatus	11	EU	0.5-4	4->8	1->8	L98H, TR34	WT
Aspergillus fumigatus	1	NA	0.5	2	2	WT	Q42L
Aspergillus fumigatus	8	NA (3); EU (4); APAC (1)	0.25-4	2-8	0.5-8	WT	WT
Aspergillus niger species complex	1	EU	1	8	4	K77Q	WT

Figure 2 Organism distribution by region and specimen type

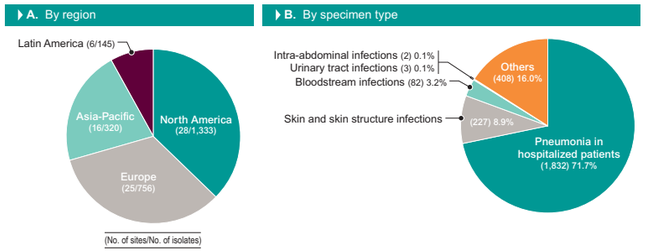
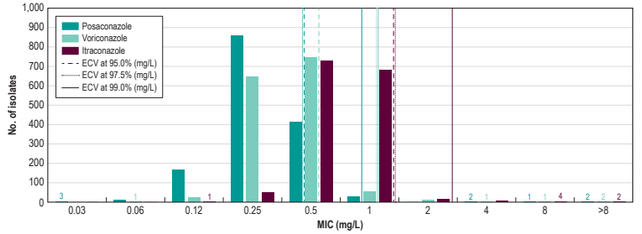


Figure 3 MIC distribution of posaconazole, itraconazole, and voriconazole against *Aspergillus fumigatus* isolates collected from 2010–2018 and proposed ECV



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