# Activity of Posaconazole and Comparator Antifungal Agents Tested Against Filamentous Fungi

### 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

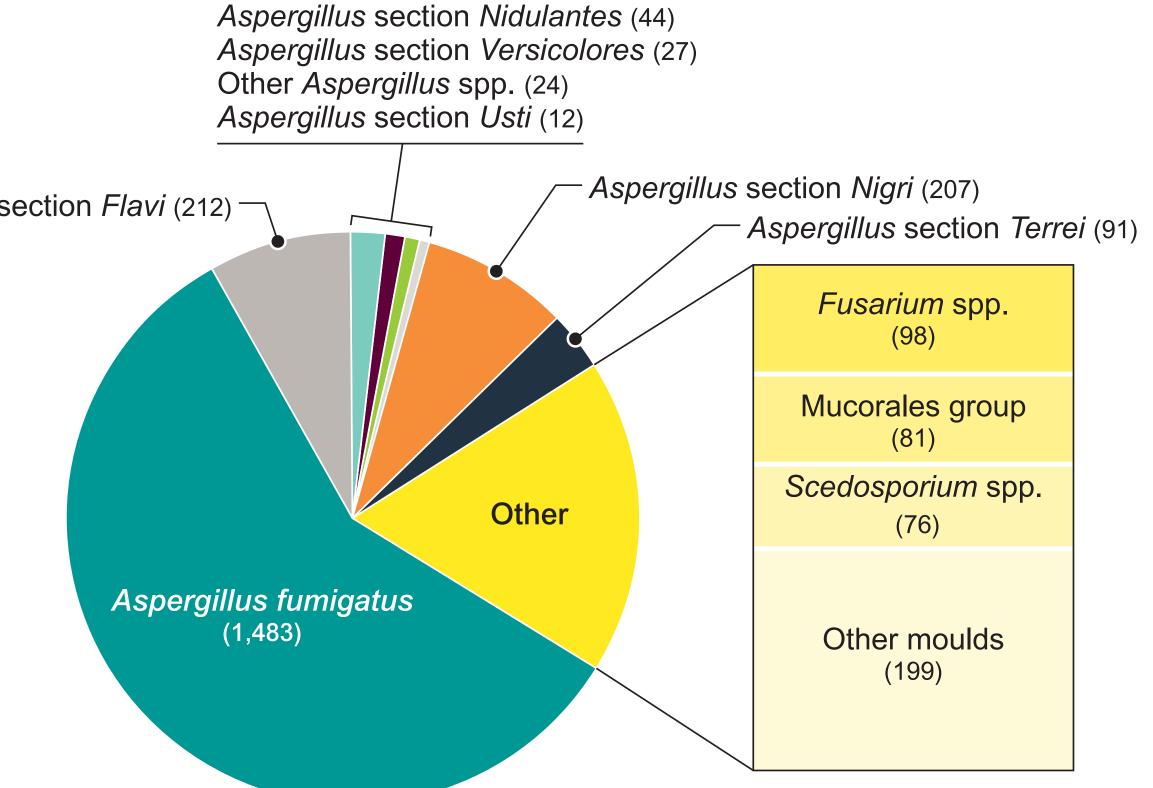
- Organism group and distribution per geographic origin and specimen type are shown in Figures 1 and 2, respectively.
- Posaconazole (MIC<sub>50/90</sub>, 0.25/0.5 mg/L) showed comparable activity to itraconazole (MIC<sub>50/90</sub>, 0.5/1 mg/L) and voriconazole (MIC<sub>50/90</sub>, 0.5/0.5 mg/L) against 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.

#### Conclusions

- alterations.
- Scedosporium species.

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

Aspergillus section Flavi (212)



 Categorical agreement between posaconazole and the other azoles tested against *A. fumigatus* ranged from 98.2–98.7%.

 Posaconazole (MIC<sub>50/90</sub>, 0.5/0.5 mg/L) exhibited similar activity to voriconazole (MIC<sub>50/90</sub>, 0.5/1 mg/L) and itraconazole (MIC<sub>50/90</sub>, 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  $14\alpha$ -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<sub>50/90</sub>, 1/2 mg/L) and amphotericin B (MIC<sub>50/90</sub>, 1/2 mg/L) were the most active agents against the Mucorales isolates (Table 2).

 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

• Azoles and echinocandins have limited activity against *Fusarium* and

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

Antimicrobial agent	MIC <sub>50</sub>	MIC <sub>90</sub>		1
(No. of isolates)			%WT	%NWT
A. fumigatus (n=1,483)		0.5	07 Oh	0.4h
Posaconazole	0.25	0.5	97.9 <sup>b</sup>	2.1 <sup>b</sup>
	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	7)		I	1
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		
<sup>a</sup> ECV criteria published in CL	SI M59 (20	18).		

ECV criteria published in CLSI 1059 (2018). <sup>b</sup> 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 <sup>a</sup> (No. of isolates)	MIC <sub>50</sub>	MIC <sub>90</sub>					
Fusarium solani species con	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	n/S. boydii (n=6	55)					
Posaconazole	1	2					
Itraconazole	2	8					
Voriconazole	0.5	1					
Caspofungin	2	>4					
Anidulafungin	4	4					
Micafungin	0.5	>4					
Amphotericin B	2	>2					
<sup>a</sup> Clinical breakpoint criteria or ECV in	nterpretation unavai	lable.					

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	North America (n=757)		Europe (n=493)		Asia-Pacific (n=171)		Latin America (n=62)	
Antifungal agent	MIC <sub>50</sub> /MIC <sub>90</sub>	(%NWT) <sup>a</sup>						
Posaconazole	0.25/0.5	2.0 <sup>b</sup>	0.25/0.5	2.8 <sup>b</sup>	0.25/0.5	0.6 <sup>b</sup>	0.25/0.5	1.6 <sup>b</sup>
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

<sup>a</sup> ECV criteria published in CLSI M59 (2020) <sup>b</sup> Based on the ECV (at 97.5%) calculated for this study.

Organiam		Decien (ne. of iceletec)	MIC range according to CLSI method (mg/L):			Aminoacid substitutions:	
Organism	no. or isolates	Region (no. of isolates)	Posaconazole	Itraconazole	Voriconazole	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	VVT	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

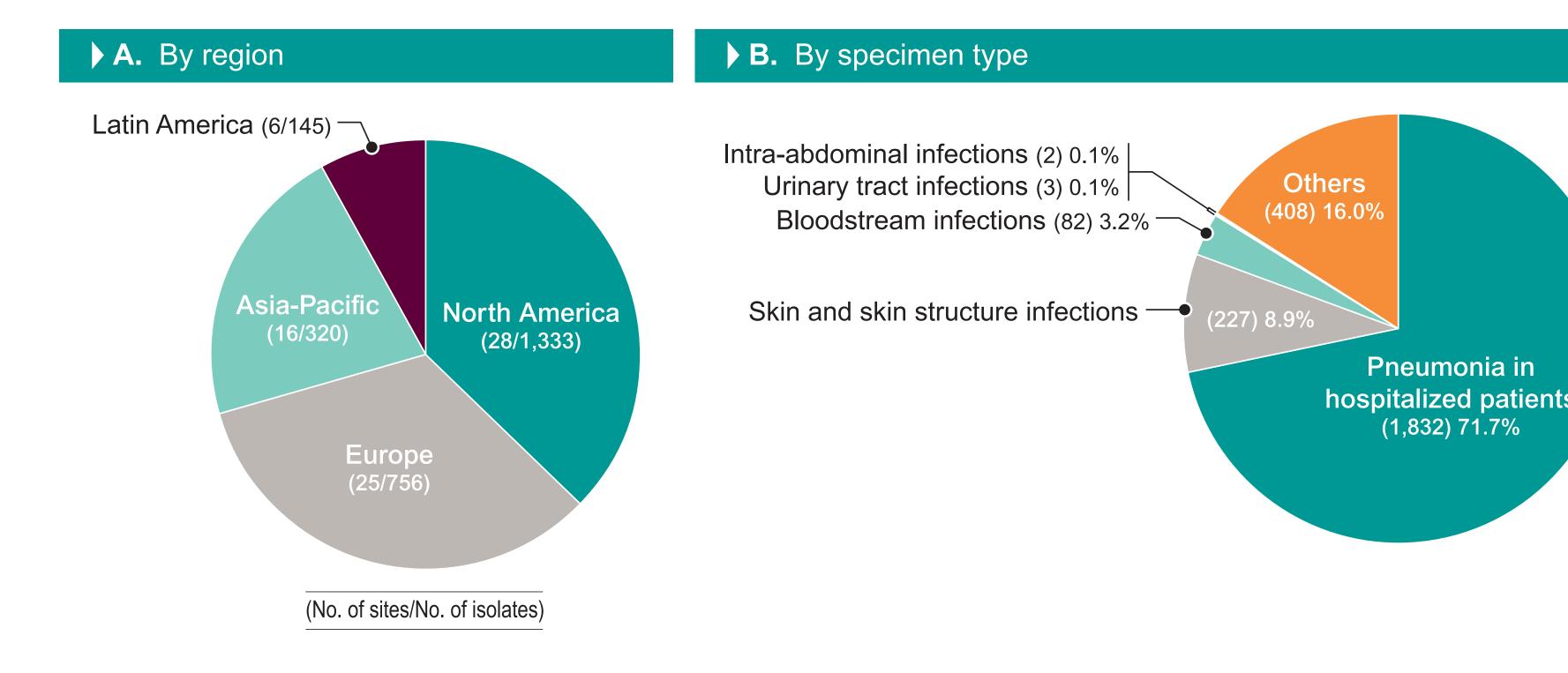
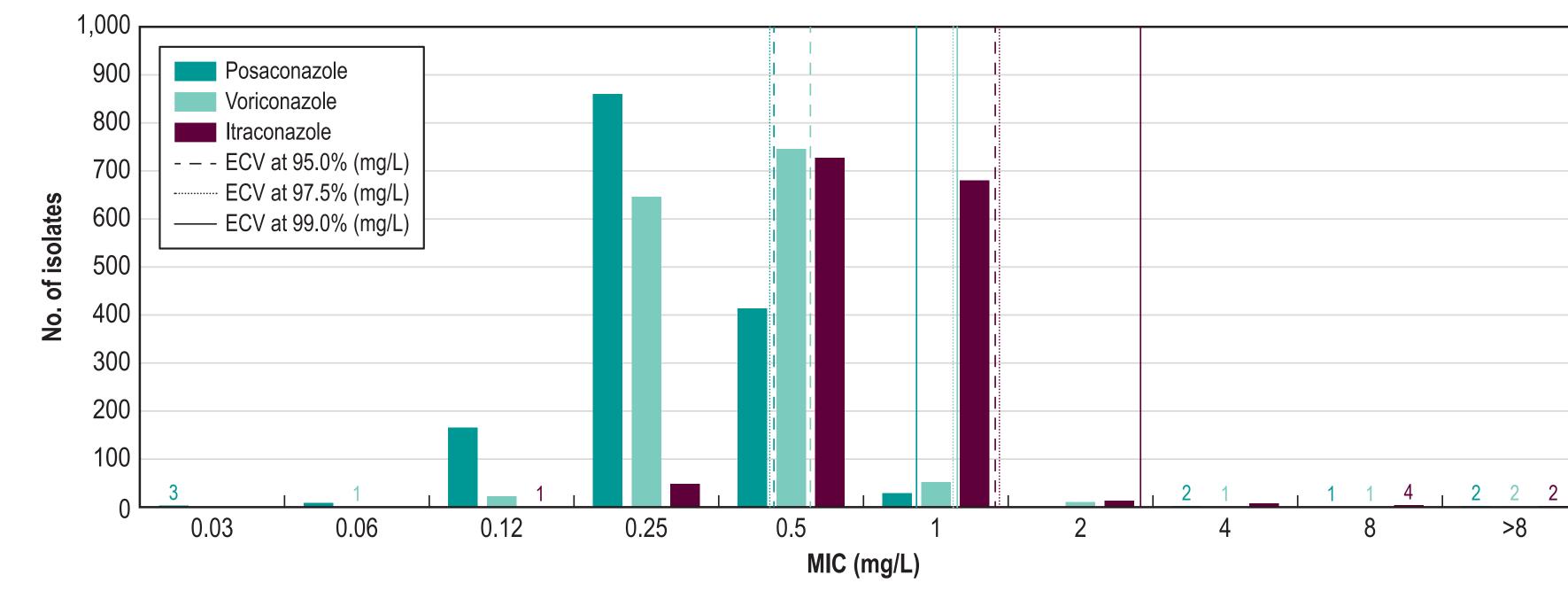


Figure 3 MIC distribution of posaconazole, itraconazole, and voriconazole against Aspergillus fumigatus isolates collected from 2010–2018 and proposed ECV



#### Acknowledgements

Funding for this research was provided by Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA. Merck was involved in the design and decision to present these results. Merck did not contribute to decisions in the collection, analysis, or interpretation of the data.

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