

***In Vitro* Activity of
Isavuconazole and Other
Mould-Active Triazoles
Against *Aspergillus fumigatus*
With and Without *cyp51*
Alterations**

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Disclosure

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Achaogen
Allegra
Amplyx
Antabio
Arietas Corp.
Arixa Pharmaceuticals
Artugen Therapeutics
Astellas Pharma
Athelas
Basilea
Bayer
Boston Pharmaceuticals
Bugworks Research
Cidara
Cipla
Contrafect
Cormedix
Crestone
Curza,
CXC7

DePuy Synthes
Destiny Pharma
Discuva Ltd.
Dr. Falk Pharma GmbH
Emery Pharma
Entasis Therapeutics
F. Hoffmann-La Roche Ltd.
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GenePOC Inc.
Geom Therapeutics, Inc.
GSK
Harvard University
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HiMedia Laboratories
Janssen
ICON plc
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Johnson & Johnson
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The Medicines Co.
Theravance
VenatoRX
Viosera Therapeutics
Vyome Therapeutics
Wockhardt
Yukon Pharmaceuticals
Zai Lab
Zavante Therapeutics

Background

- Invasive aspergillosis (IA) remains a life-threatening infection among high-risk patients.
- *Aspergillus fumigatus* is the most common species causing IA.
- IA mortality ranges from 20% to 30% with first-line azole therapy.
- Azole resistance in *A. fumigatus* is mainly associated with mutations in *cyp51A* and its promoter region or its homologue, *cyp51B*.
 - Reduced drug-target interaction and/or increased target copy number.

Background

- In *A. fumigatus*, there are 2 types of environments that produce resistance selection:
 - Single nucleotide mutations in *cyp51* genes, which are related to long-term azole therapy.
 - Specific amino acid changes in the Cyp51A protein in combination with tandem repeats (TR) in the gene promoter, which are related to environmental selection, such as:
 - TR₃₄/L98H
 - TR₄₆/Y121F/T289A

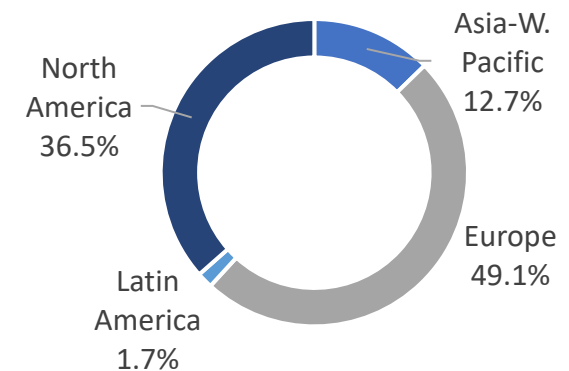
Objective

To evaluate the *in vitro* activity of isavuconazole, itraconazole, posaconazole, and voriconazole against a large contemporaneous collection of *A. fumigatus clinical* isolates and the presence of *cyp51* alterations in NWT isolates.

Methods

Organisms

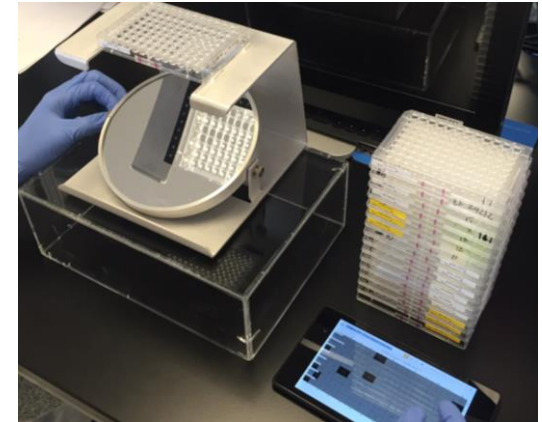
- Collected by the SENTRY Antifungal Surveillance Program 2017–2020.
- 660 *Aspergillus fumigatus* isolates from invasive infections.
- Sent from 40 hospitals in 18 countries.
- All isolates were identified by MALDI-TOF and/or sequencing analysis.



Methods

Antifungal Susceptibility Testing and *cyp51* characterization

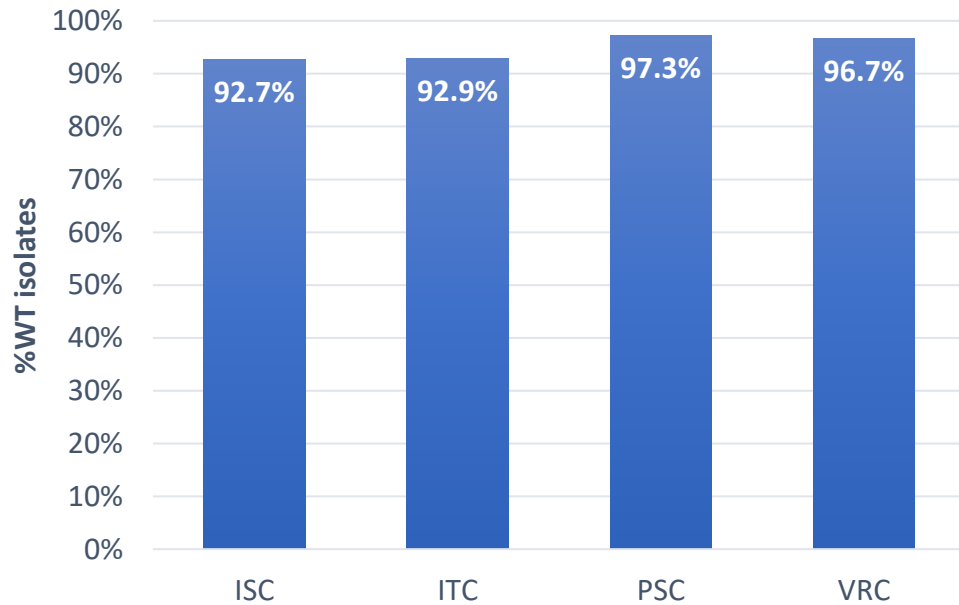
- Used CLSI (M38) broth microdilution method.
- Applied CLSI epidemiological cut-off values (ECV).
 - Posaconazole ECV of 0.5 mg/L.
- Screened isolates that were non-wildtype (NWT) to the azoles for alterations in the *cyp51* genes using whole genome sequencing.



CLSI M38 (2017) and M59 (2020)
Buil et al., *J Fungi (Basel)*. 2018 Aug 29;4(3):103
Castanheira et al., *Mycoses*, 2021 Oct;64(10):1279-1290.

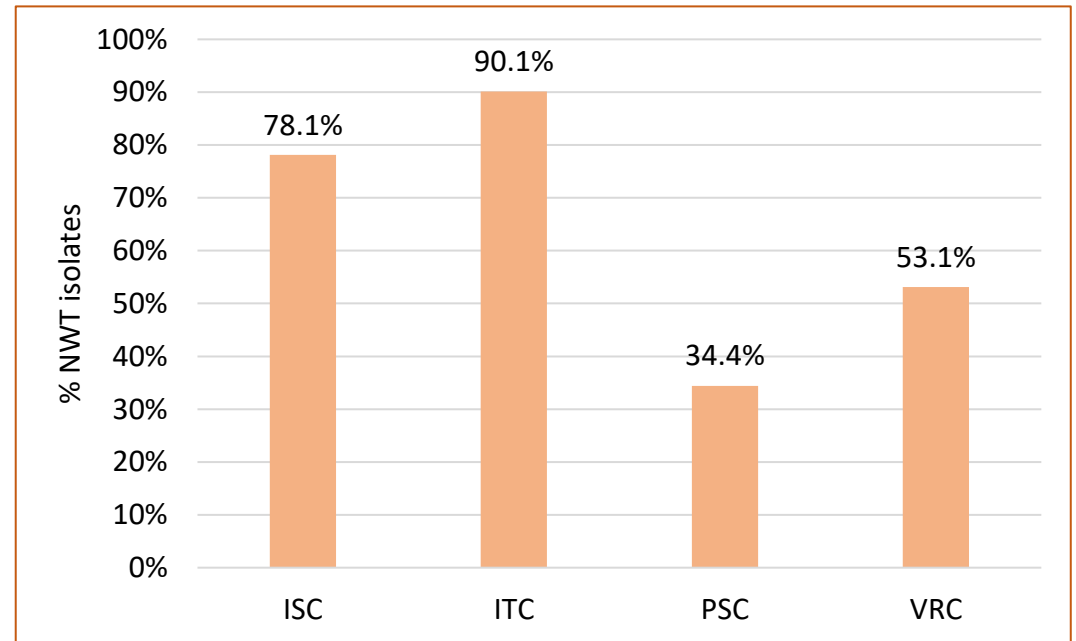
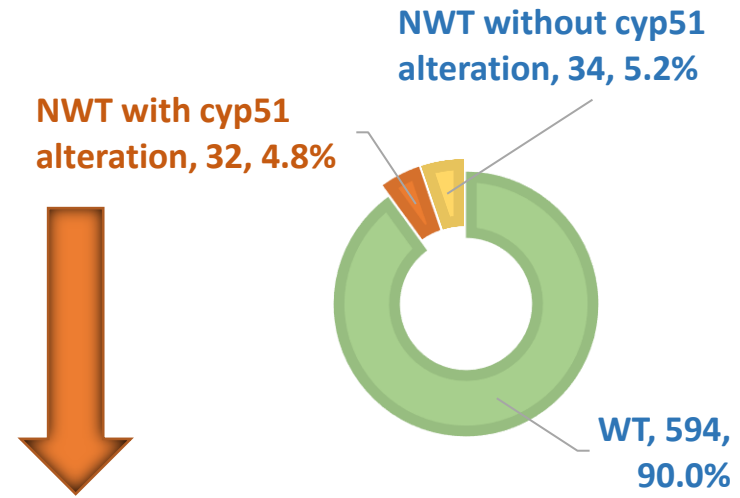
Results

Percentage of WT *A. fumigatus* isolates (n=660)



ISC, isavuconazole; ITC, itraconazole; PSC, posaconazole; VRC, voriconazole

66 *A. fumigatus* isolates were NWT to 1 or more azoles and were further characterized for *cyp51* alterations



Isolates displaying *cyp51* alterations (n=32)

Region	Total # of isolates	<i>cyp51</i> alterations	
		# of isolates	% of isolate
Asia-W. Pacific	84	4	4.8%
Europe	324	17	5.2%
North America	241	11	4.6%
Latin America	11	0	0.0%

TR34/L98H was the most frequent alteration in CYP51A

14 EU isolates

All were NWT to ISC and ITC

Study Year	Continent	Country	MIC (mg/L)				CYP51A	CYP51B
			ISC	ITC	VRC	PSC		
2018	Europe	Czech Republic	2	2	1	0.5	F46Y,M172V,N248T,D255E,E427K	wild-type
2020	Europe	Germany	8	>8	4	1	L98H, TR34	wild-type
2018	Europe	Belgium	4	4	2	1	L98H,TR34	wild-type
2018	Europe	Italy	>8	>8	>8	4	L98H,TR34	wild-type
2018	Europe	Italy	8	8	2	1	L98H,TR34	wild-type
2018	Europe	Italy	4	4	2	1	L98H,TR34	wild-type
2018	Europe	Italy	4	4	2	1	L98H,TR34	wild-type
2019	Europe	Italy	4	2	2	0.5	L98H,TR34	wild-type
2019	Europe	Italy	2	2	2	0.5	L98H,TR34	wild-type
2018	Europe	Italy	4	4	1	0.5	L98H,TR34	wild-type
2019	Europe	Slovenia	4	>8	2	0.5	L98H,TR34	wild-type
2020	Europe	UK	8	8	2	1	L98H,TR34	wild-type
2020	Europe	UK	4	4	2	1	L98H,TR34	wild-type
2019	Europe	UK	4	>8	2	0.5	L98H,TR34	wild-type
2020	Europe	UK	4	4	2	0.5	L98H,TR34	wild-type
2020	Europe	France	4	4	1	1	wild-type	Q42L
2019	Europe	Belgium	>8	8	>8	0.5	Y121F,M172I,T289A,G448S,TR46	wild-type

Isolates displaying *cyp51* alterations (n=32)

Single resistance mutations in *cyp51A*

Study Year	Continent	Country	MIC (mg/L)				CYP51A	CYP51B
			ISC	ITC	VRC	PSC		
2020	Asia-W. Pacific	New Zealand	>8	>8	8	8	G138C	wild-type
2018	North America	USA	2	1	0.5	0.25	A9T	wild-type
2019	North America	USA	>8	>8	4	0.5	G448S	wild-type
2018	North America	Canada	1	2	0.5	0.5	I242V	wild-type
2018	North America	USA	1	2	1	1	I242V	wild-type
2018	North America	USA	1	2	0.5	0.5	I242V	wild-type
2020	North America	USA	1	2	0.5	0.25	I242V	wild-type

1 isolate carried G138C and was NWT to all 4 azoles

4 NA isolates carried I242V
All NWT to ITC
but WT to ISC and VRC

1 isolate carried G448S
and
1 carried A9T

Isolates displaying *cyp51* alterations (n=32)

5 isolates displayed multiple alterations in *cyp51A*

Study Year	Continent	Country	MIC (mg/L)				CYP51A	CYP51B
			ISC	ITC	VRC	PSC		
2017	Asia-W. Pacific	Thailand	2	1	0.5	0.25	F46Y,M172V,N248T,D255E,E427K	Q42L
2018	Europe	Czech Republic	2	2	1	0.5	F46Y,M172V,N248T,D255E,E427K	wild-type
2019	Europe	Belgium	>8	8	>8	0.5	Y121F,M172I,T289A,G448S,TR46	wild-type
2018	North America	USA	2	2	1	0.5	F46Y,M172V,E427K	wild-type
2020	North America	USA	1	2	0.5	0.5	F46Y,M172V,N248T,D255E,E427K	wild-type

4/5 NWT to ISC or ITC

All WT to PSC

Isolates displaying *cyp51* alterations (n=32)

7 isolates showed alterations in *cyp51B*

Study Year	Continent	Country	MIC (mg/L)				CYP51A	CYP51B
			ISC	ITC	VRC	PSC		
2020	Asia-W. Pacific	Australia	2	2	1	0.5	wild-type	K82Q, F149V, P383L
2017	Asia-W. Pacific	Thailand	2	1	0.5	0.25	F46Y,M172V,N248T,D255E,E427K	Q42L
2018	Asia-W. Pacific	Australia	1	1	2	0.25	wild-type	Q42L
2020	Europe	France	4	4	1	1	wild-type	Q42L
2019	North America	USA	2	2	1	0.5	wild-type	Q42L
2020	North America	USA	2	2	1	0.5	wild-type	Q42L
2020	North America	USA	0.5	2	0.5	0.25	wild-type	Q42L

6/7 carried Q42L
and
5/7 NWT to ISC or ITC

Take-Home Points

- The majority of *A. fumigatus* were WT to the azoles.
- Ten different *cyp51* alterations were detected in 32 of 66 NWT isolates.
- Only EU isolates harbored the environmental alteration TR34/L98H that was associated with the NWT phenotype to isavuconazole and itraconazole.
- Alterations in *A. fumigatus cyp51* can have variable effects on the *in vitro* activity of the azoles.
- These effects are best delineated by testing all triazoles.

Acknowledgements



JMI Fungal Team



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