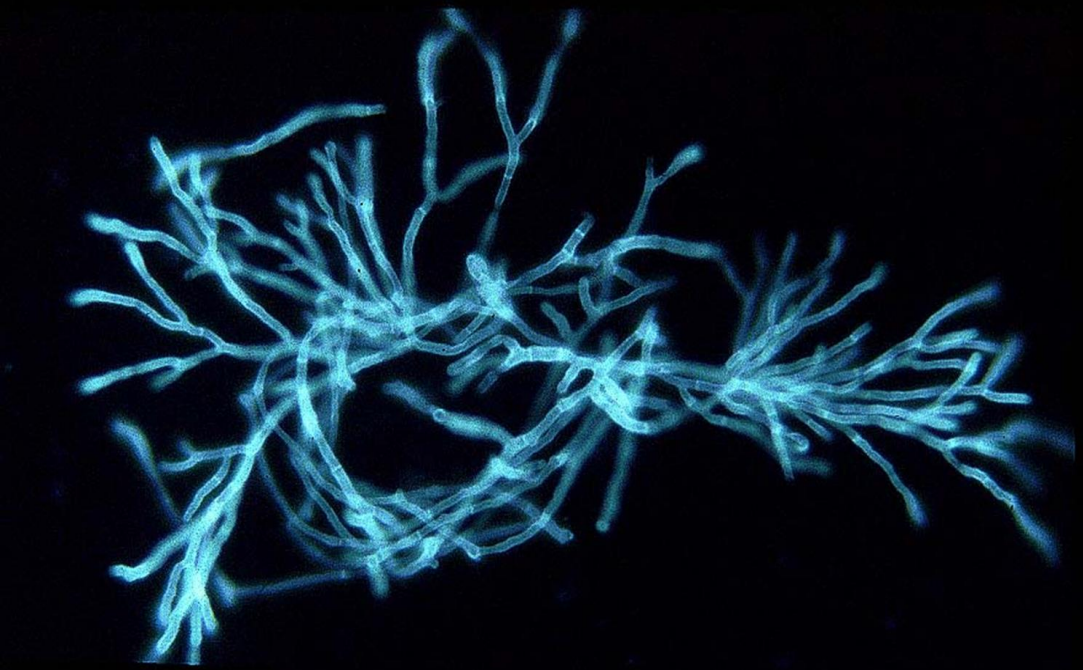
UNIVERSITÄTSMEDIZIN GÖTTINGEN UMG

Update zur Azolresistenz bei *Aspergillus fumigatus*



PEG-Tagung 2018
Bonn

Oliver Bader

Institut für Medizinische Mikrobiologie, Universitätsmedizin Göttingen

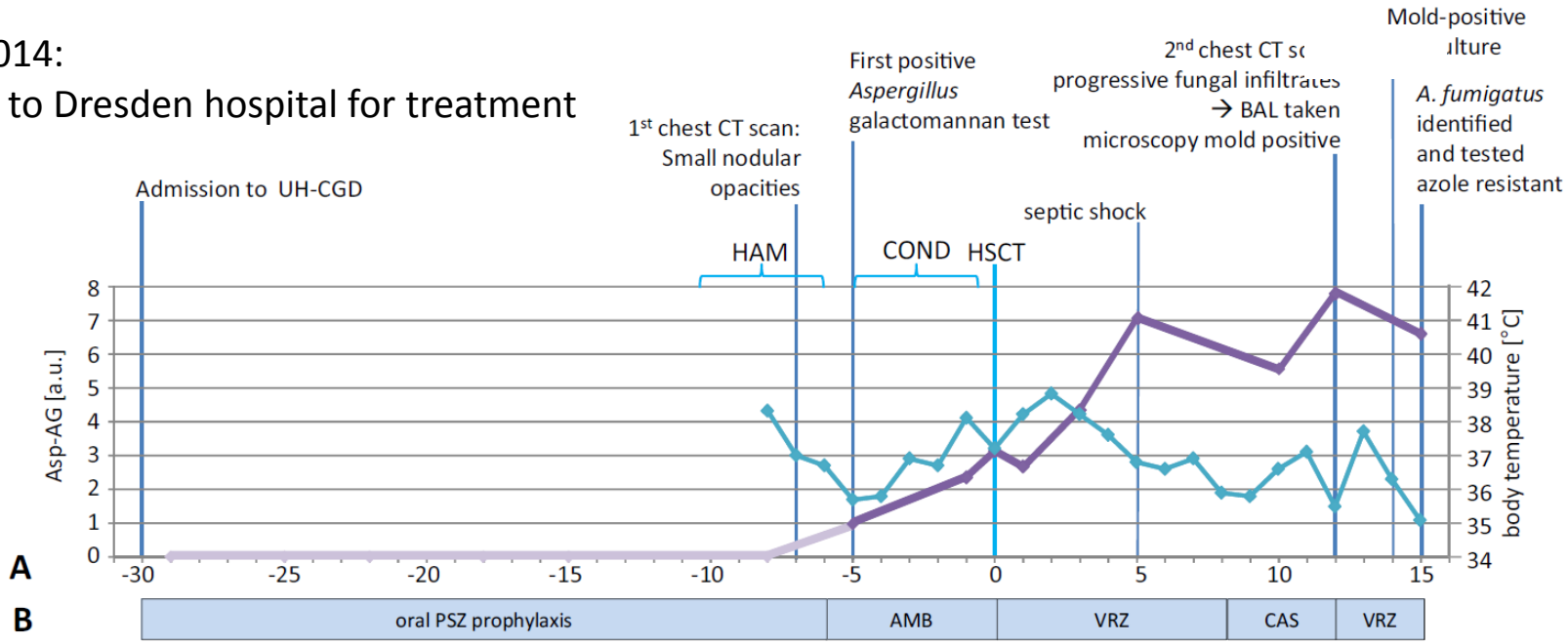
AML Case

Up to June 2014:

- 71-year old male patient
- treated in a general hospital for AML with adverse-risk cytogenetic features.
- received induction chemotherapy and oral posaconazole for antifungal prophylaxis
- Eventually, a new bone marrow aspirate revealed residual AML
- because of progressive AML, decitabine therapy was initiated

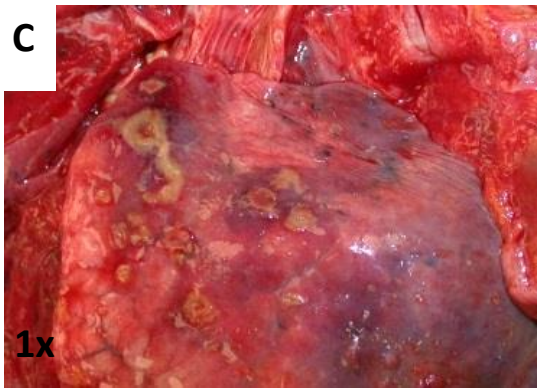
In August 2014:

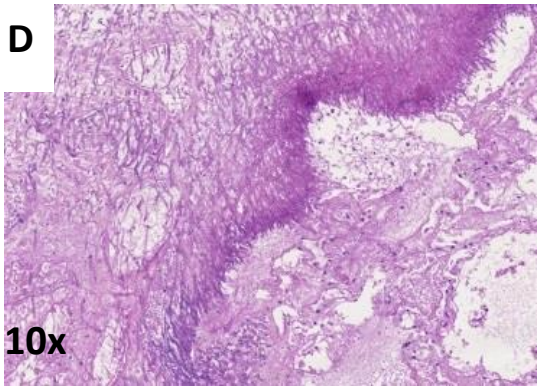
- referred to Dresden hospital for treatment

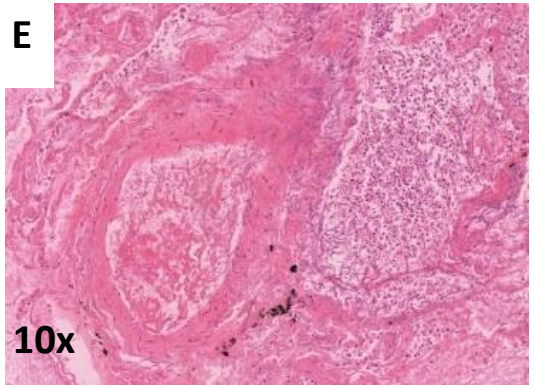


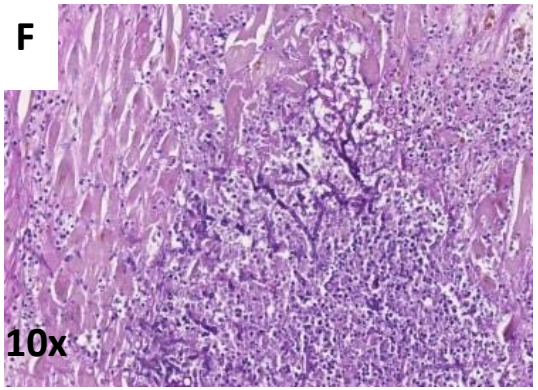
Rößler S, Bader O, *et al.*, AAC 2017

AML Case









(C) multiple fungal lesions on the surface of the lung,
(D) fungal abscess in lung parenchyma (PAS reaction),
(E) vascular invasive growth and detection of dichotomously branched and septated fungi in the lumen of a lung vessel
(F) fungal septipyemic focus in the anterior wall of the heart with surrounding granulocytic reaction (PAS reaction)

Rößler S, Bader O, *et al.*, AAC 2017

AML Case

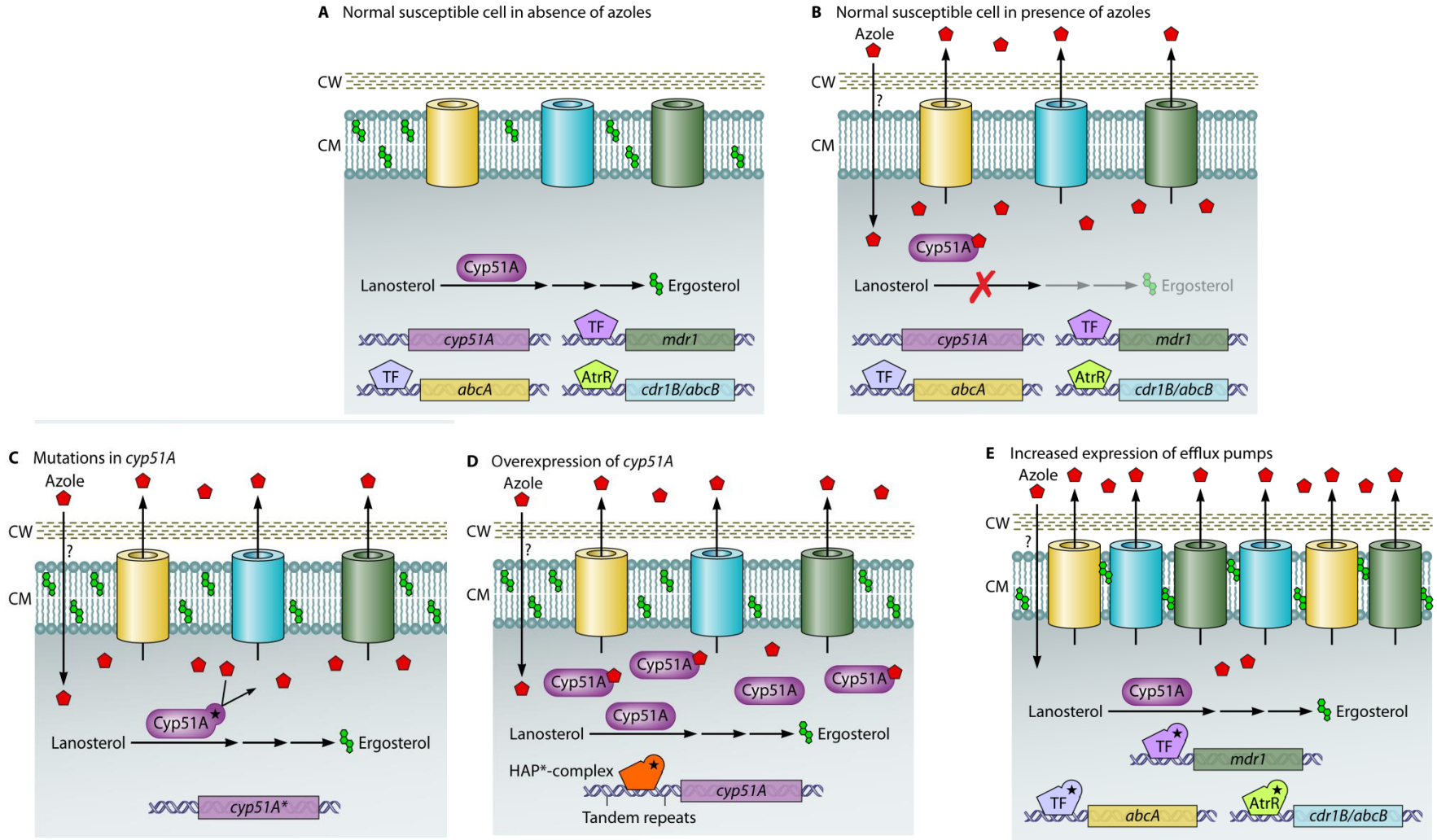
antifungal susceptibility testing of the culture isolate (BAL)

Antifungal agent	E-test		EUCAST Microdilution		
	MIC	interpretation	MIC	breakpoints	Interpretation
posaconazole	0.5	resistant	0.5	S <= 0.12; R > 0.25	resistant
itraconazole	2	sensitive	1	S <= 1; R > 2	sensitive
voriconazole	>32	resistant	> 32	S <= 1; R > 2	resistant
amphotericin B	0.25	sensitive	< 0.125	S <= 1; R > 2	sensitive

MIC = minimal inhibitory concentration [µg/ml]
EUCAST = European Committee on Antimicrobial Susceptibility Testing

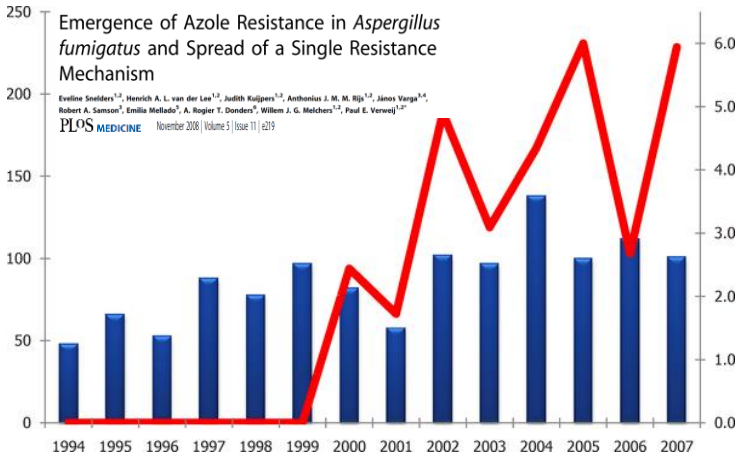
Rößler S, Bader O, *et al.*, AAC 2017

Azole resistance mechanisms in A.f.



Dudakova *et al.*, CMR 2017

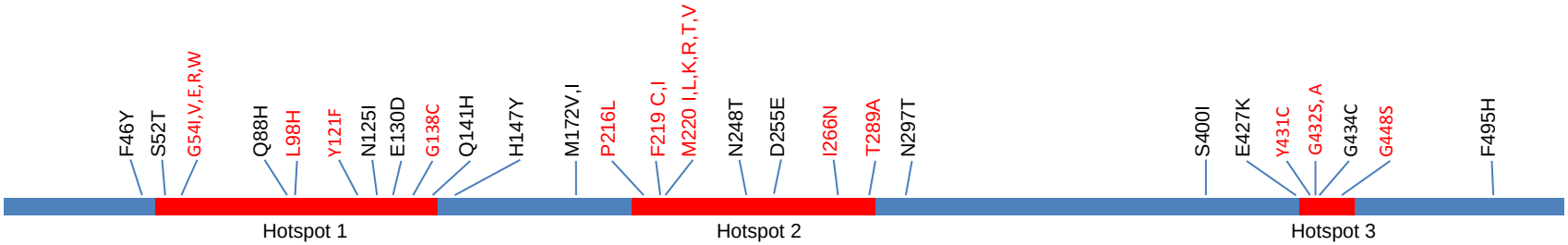
Mechanisms of azole resistance: Cyp51A

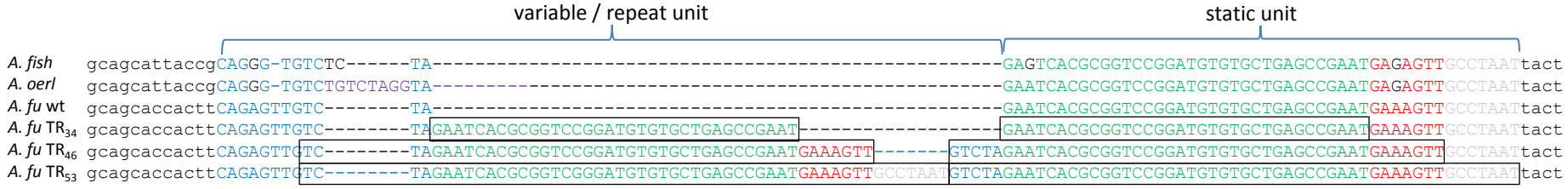


“pandemic” alleles:

TR$_{34}$/L98H

TR$_{46}$/Y121F/T289A





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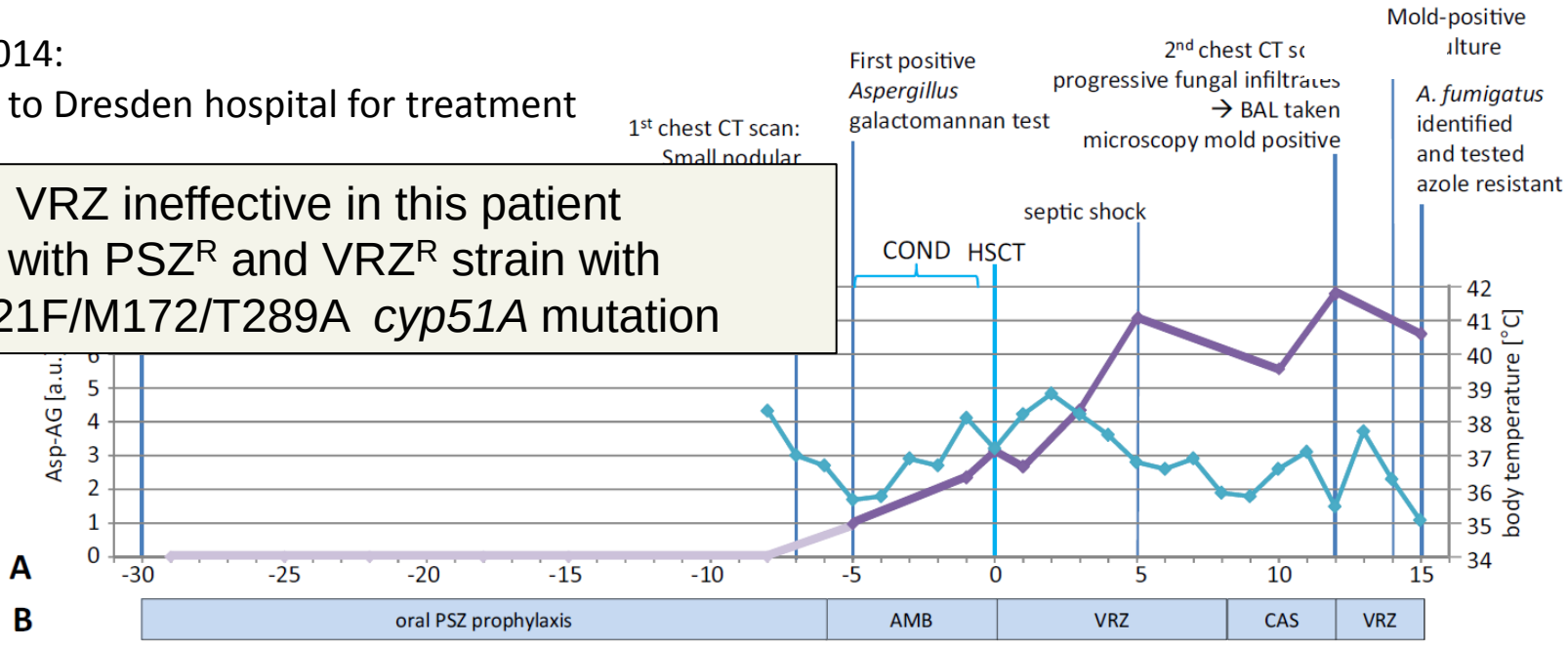
PSZ and VRZ ineffective in this patient infection with PSZ^R and VRZ^R strain with TR_{46}/Y121F/M172/T289A *cyp51A* mutation



Rößler S, Bader O, *et al.*, AAC 2017

Other mechanisms of azole resistance

OPEN ACCESS Freely available online

PLOS ONE

Discovery of a *hapE* Mutation That Causes Azole Resistance in *Aspergillus fumigatus* through Whole Genome Sequencing and Sexual Crossing

Simone M. T. Camps[1,2]*, Bas E. Dutilh[3], Maiken C. Arendrup[4], Antonius J. M. M. Rijs[1,2], Eveline Snelders[1,2], Martijn A. Huynen[3], Paul E. Verweij[1,2], Willem J. G. Melchers[1,2]

1 Department of Medical Microbiology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands, **2** Nijmegen Institute for Infection, Inflammation and Immunity (N4i), Nijmegen, The Netherlands, **3** Centre for Molecular and Biomolecular Informatics, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands, **4** Unit of Mycology, Department of Microbiological Surveillance and Research, Statens Serum Institut, Copenhagen, Denmark

→ Regulation of sterol biosynthesis pathway

PLOS PATHOGENS

RESEARCH ARTICLE

Sterol Biosynthesis and Azole Tolerance Is Governed by the Opposing Actions of SrbA and the CCAAT Binding Complex

Fabio Gsaller[1], Peter Hortschansky[2], Takanori Furukawa[1], Paul D. Carr[1], Bharat Rash[1], Javier Capilla[3], Christoph Müller[4], Franz Bracher[4], Paul Bowyer[1], Hubertus Haas[5], Axel A. Brakhage[2,6], Michael J. Bromley[1]*

1 Manchester Fungal Infection Group, Institute of Inflammation and Repair, University of Manchester, Manchester, United Kingdom, **2** Department of Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany, **3** Microbiology Unit, Medical School, Universitat Rovira i Virgili, Reus, Spain, **4** Department of Pharmacy, Center for Drug Research, Ludwig-Maximilians University of Munich, Munich, Germany, **5** Division of Molecular Biology, Biocentre, Medical University of Innsbruck, Innsbruck, Austria, **6** Institute for Microbiology, Friedrich Schiller University Jena, Jena, Germany

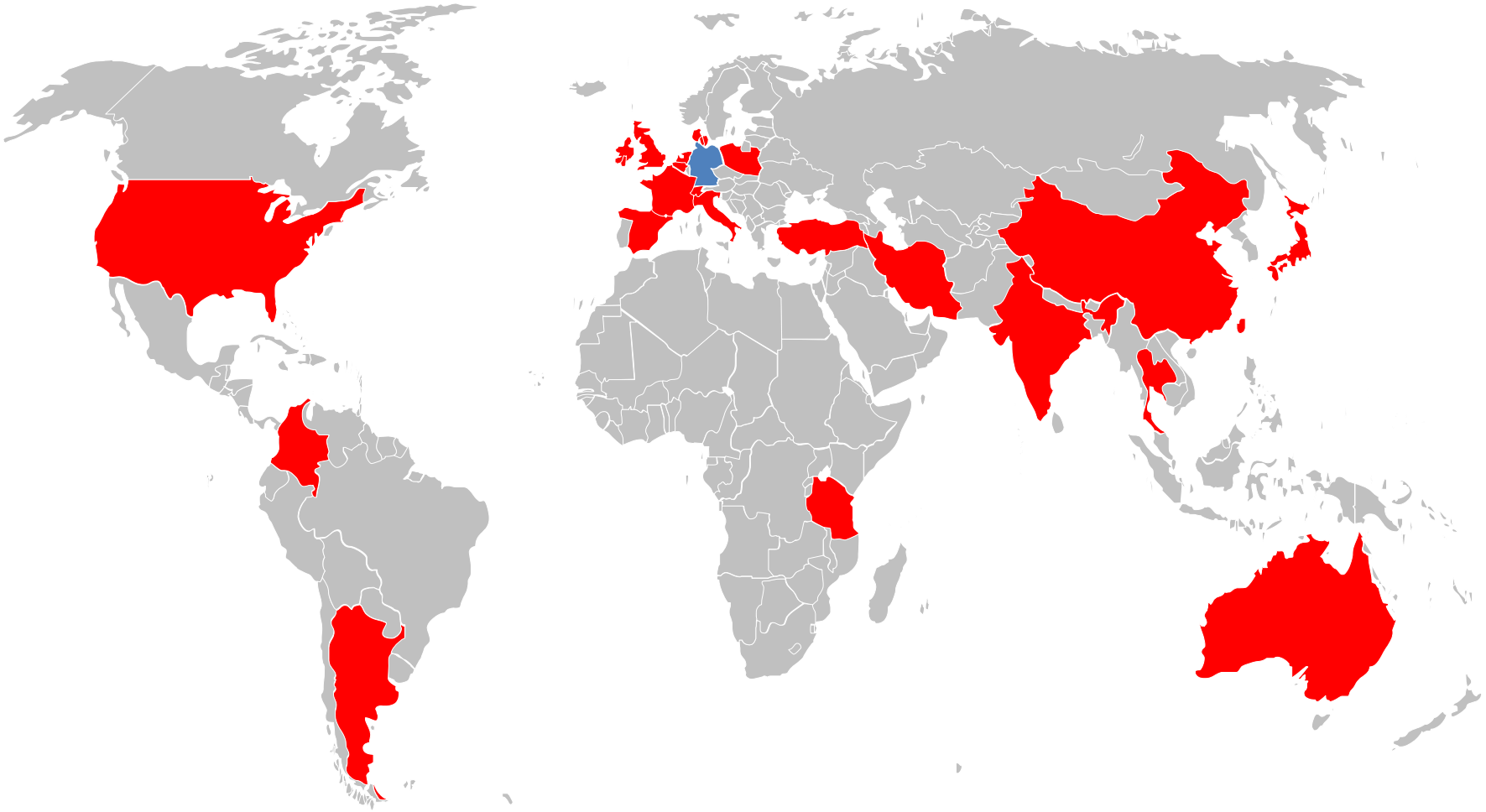
J Antimicrob Chemother 2013; **68**: 1486–1496
doi:10.1093/jac/dkt075 Advance Access publication 10 April 2013

Journal of Antimicrobial Chemotherapy

The *cdr1B* efflux transporter is associated with non-*cyp51a*-mediated itraconazole resistance in *Aspergillus fumigatus*

Marcin G. Fraczek[1], Michael Bromley[1], Ahmed Buied[1], Caroline B. Moore[1], Ranjith Rajendran[2], Riina Rautemaa[1], Gordon Ramage[2], David W. Denning[1] and Paul Bowyer[1]*

ARAf findings by country



→ if people look for ARAf, they will find it !

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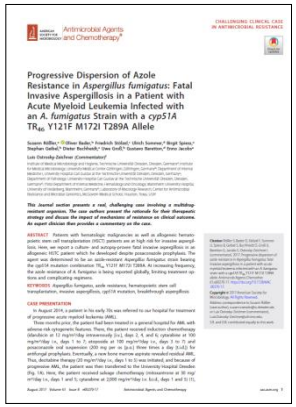
Reported isolates in Germany

clinical

environmental

2012 2012 2013 2014 2015



2017

2017

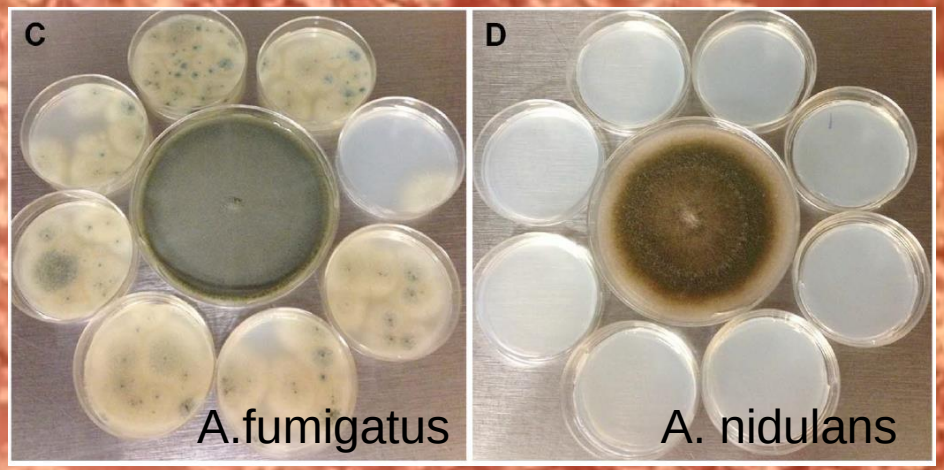
2015

35 reported clinical isolates

55 reported environ-
mental isolates

Current working hypothesis

A. fumigatus is one of the best-adapted fungi to dispersal, and its condida are found world-wide incl. the upper atmosphere, and both polar regions

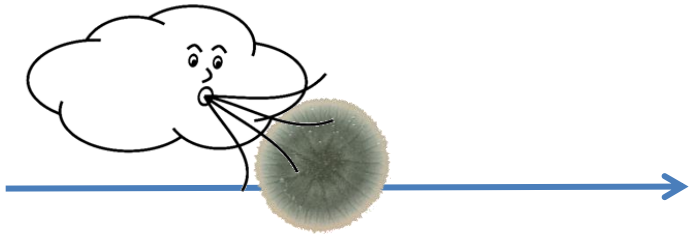


Kwon-Chung and Segui, PPath, 2013

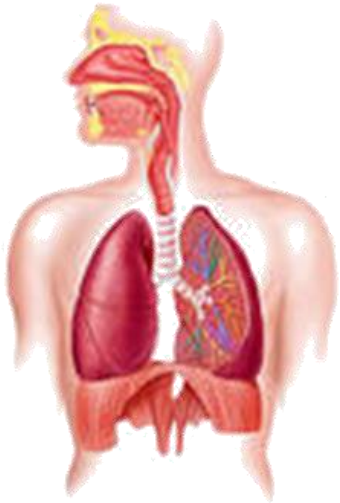
Current working hypothesis

Unintentional induction of resistance in *A. fumigatus* e.g. TR_{34}/L98H or TR_{46}/Y121F/T289A (G54?)

dispersal by wind



selection over susceptible strains through agricultural azoles?



- presence in hosts
- opportunistic infection
- (selection through azole prophylaxis ???)
- not only humans but also wild animals!

conidia also travel the land route…

Intercountry Transfer of Triazole-Resistant *Aspergillus fumigatus* on Plant Bulbs

Katie Dunne,[1] Ferry Hagen,[2,3] Niamh Pomeroy,[1] Jacques F. Meis,[2,3] and Thomas R. Rogers[1]

[1]Department of Clinical Microbiology, Trinity College Dublin, Ireland; [2]Department of Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital, and [3]Centre of Expertise in Mycology, Radboud University Medical Center/Canisius Wilhelmina Hospital, Nijmegen, The Netherlands

Sample No.[a]	Date of Sample	Type of Sample	Origin	No. of Triazole-Resistant/Total *A. fumigatus* Colonies/Plant Bulb Pack	MIC, mg/L[b] Itraconazole (ECV = 1) [9]	Voriconazole (ECV = 1) [9]	Posaconazole (ECV = 0.5) [9]	Resistance Mechanism
P1	Jan 2016	Double mixed tulip bulbs (30[c])	Lisse, the Netherlands	1/5	0.5	>8	0.5	TR_{46}/Y121F/T289A
P2, P3	Jan 2016	Bastogne tulip bulbs (6[a])	Lisse, the Netherlands	2/3	1	4	1	TR_{46}/Y121F/T289A
					8	>8	0.5	TR_{34}/L98H
P4	Jan 2016	Triumph tulip bulbs (6[c])	Breezand, the Netherlands	1/4	8	4	0.5	TR_{34}/L98H
P5, P6	Jan 2016	Narcissus bulbs (8[c])	Breezand, the Netherlands	2/5	0.5	>8	1	TR_{46}/Y121F/T289A
					8	4	0.5	TR_{34}/L98H
P7	Jan 2016	Tall Triumph mixed tulip bulbs (10[c])	The Netherlands (region not specified)	1/2	1	>8	1	TR_{46}/Y121F/T289A
D6	Feb 2016	Soil (2 g)	Hospital campus, Dublin, Ireland	…	8	4	0.5	TR_{34}/L98H

"International expert opinion"

(in the absence of clinical guidelines)

International expert opinion on the management of infection caused by azole-resistant *Aspergillus fumigatus*

Paul E. Verweij[a,*], Michelle Ananda-Rajah[b], David Andes[c], Maiken C. Arendrup[d], Roger J. Brüggemann[e], Anuradha Chowdhary[f], Oliver A. Cornely[g], David W. Denning[h], Andreas H. Groll[i], Koichi Izumikawa[j], Bart Jan Kullberg[k], Katrien Lagrou[l], Johan Maertens[m], Jacques F. Meis[a,n], Pippa Newton[h], Iain Page[h], Seyedmojtaba Seyedmousavi[a], Donald C. Sheppard[o], Claudio Viscoli[p], Adilia Warris[q], J. Peter Donnelly[r]

Verweij et al, (2015) Drug Res Updates 21:30-40

- microbiological diagnostics critical to guiding therapy
- if azole resistant isolates are obtained,
  - the underlying mechanism should be identified for epidemiological reasons,
  - but neither therapy initiation/modification nor MIC determination delayed

- 2-10% environmental prevalence →alternative therapies must be considered
- 10 % environmental prevalence → definitely re-evaluate azole therapy

- at least 5 independent colonies should tested for AST

culture

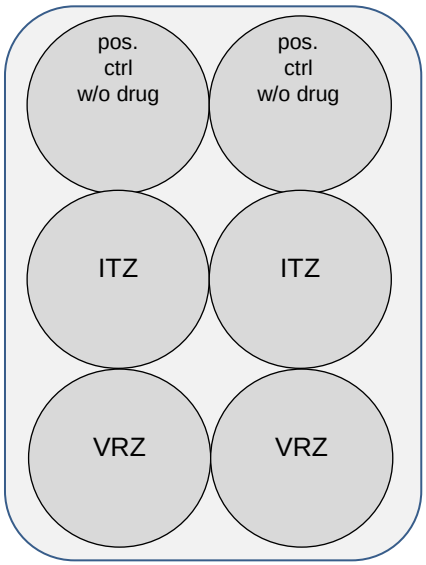
cave: susceptible / resistant – mixed cultures !!! → screening agar

J Antimicrob Chemother 2015; **70**: 412–415
doi:10.1093/jac/dku410 Advance Access publication 17 October 2014

Journal of Antimicrobial Chemotherapy

Concomitant occurrence of itraconazole-resistant and -susceptible strains of *Aspergillus fumigatus* in routine cultures

Ahmad et al, 2015 JAC 70:412-415



CORRESPONDENCE

Voriconazole-Susceptible and Voriconazole-Resistant *Aspergillus fumigatus* Coinfection

To the Editor:

Azole resistance is an increasing problem in *Aspergillus fumigatus* infection (1). Two mutations, TR_{34}/L98H and TR_{46}/Y121F/T289A, are frequently recovered from isolates of patients with azole-resistant invasive aspergillosis and are believed to originate from the environment (2, 3). In regions with these environmental mutations, azole-resistant *Aspergillus* diseases may develop in patients not previously treated with azoles, and mortality rates are very high (4–6). We review the clinical course of three patients with proven invasive aspergillosis resulting from voriconazole-susceptible and voriconazole-resistant *A. fumigatus* strains. We hypothesized that in regions with TR_{34}/L98H and TR_{46}/Y121F/T289A environmental mutations, individual pulmonary lesions may arise from *A. fumigatus* strains with different azole resistance profiles.

unit because of pulmonary deterioration requiring mechanical ventilation. The BAL showed heavy growth of *A. fumigatus*. Resistance screening of four colonies indicated voriconazole-susceptible infection, and voriconazole was added to the regimen. Ten days after voriconazole was started, routine follow-up bronchial aspirate yielded a voriconazol-resistant *A. fumigatus* colony (Table 1). Because the patient was improving clinically with adequate voriconazole plasma levels, voriconazole was continued. However, a bronchial aspirate taken on day 13 showed heavy growth of voriconazole-resistant *A. fumigatus*. Despite continued clinical improvement, anidulafungin was added. Almost 2 weeks after intubation, ventilator support could be withdrawn, and immunosuppressive drugs were reintroduced. A follow-up computed tomography scan of the thorax showed cavity formation in the right upper lobe lesion, and multiple nodular lesions. The patient suddenly died after having received voriconazole and anidulafungin combination therapy for 34 days. At autopsy, multiple pulmonary fungal lesions were found, as well as one fungal lesion in the kidney transplant. *A. fumigatus* colonies cultured from the cavitating

Kolwijck et al, 2016
Am J Respir Crit Care Med 193

large-scale screening

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- molecular mechanisms
- a scheme to screen large scale collections
- tools to locally analyse sequencing data

Molecular Tools for the Detection and Deduction of Azole Antifungal Drug Resistance Phenotypes in *Aspergillus* Species

Anna Dudakova,[a] Birgit Spiess,[b] Marut Tangwattanachuleeporn,[a,c] Christoph Sasse,[d] Dieter Buchheidt,[b] Michael Weig,[a] Uwe Groß,[a] Oliver Bader[a]

Institute for Medical Microbiology, University Medical Center Göttingen, Göttingen, Germany[a]; 3rd Department of Internal Medicine, Hematology and Oncology, Mannheim University Hospital, University of Heidelberg, Mannheim, Germany[b]; Unit of Medical Technology, Faculty of Allied Health Sciences, Burapha University, Chon Buri, Thailand[c]; Institute of Microbiology and Genetics, Department of Molecular Microbiology & Genetics, Georg August Universität Göttingen, Göttingen, Germany[d]

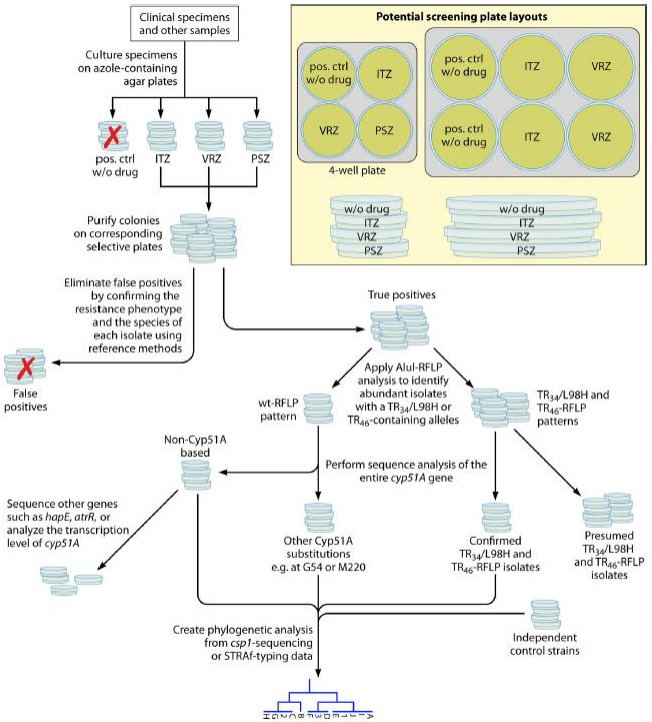
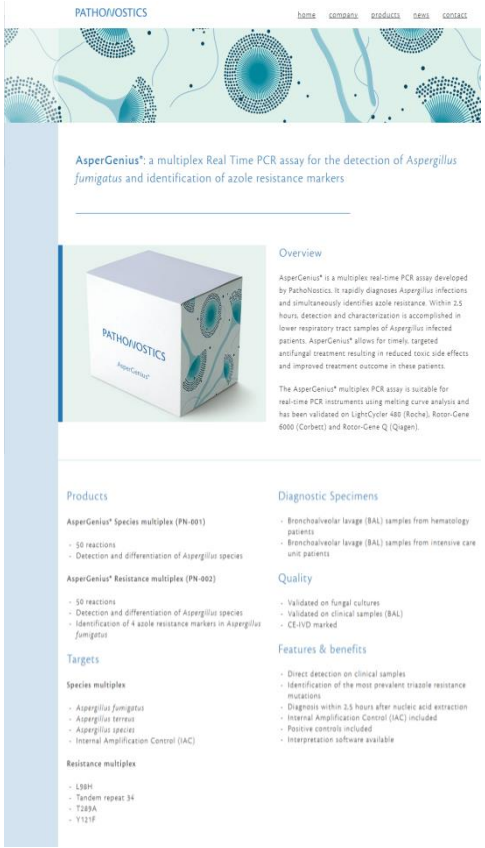


FIG 3 Potential sampling workflow for ARAf screening studies. No standardized scheme for conducting screening studies is established yet, but combining several approaches proposed in the literature gives rise to an efficient workflow that eliminates false-positive results and yields robust numbers on the prevalence and phylogenetic cohesion of resistant isolates.

Multiplex -PCR approach

(AsperGenious Assay, PathoNostics, NL)



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AsperGenius®: a multiplex Real Time PCR assay for the detection of *Aspergillus fumigatus* and identification of azole resistance markers

Overview

AsperGenius® is a multiplex real-time PCR assay developed by PathoNostics. It rapidly diagnoses *Aspergillus* infections and simultaneously identifies azole resistance. Within 2.5 hours, detection and characterization is accomplished in lower respiratory tract samples of *Aspergillus* infected patients. AsperGenius® allows for timely, targeted antifungal treatment resulting in reduced toxic side effects and improved treatment outcome in these patients.

The AsperGenius® multiplex PCR assay is suitable for real-time PCR instruments using melting curve analysis and has been validated on LightCycler 480 (Roche), Rotor-Gene 6000 (Corbett) and Rotor-Gene Q (Qiagen).

Products

AsperGenius® Species multiplex (PN-001)

- 50 reactions
- Detection and differentiation of *Aspergillus* species

AsperGenius® Resistance multiplex (PN-002)

- 50 reactions
- Detection and differentiation of *Aspergillus* species
- Identification of 4 azole resistance markers in *Aspergillus fumigatus*

Targets

Species multiplex

- *Aspergillus fumigatus*
- *Aspergillus terreus*
- *Aspergillus species*
- Internal Amplification Control (IAC)

Resistance multiplex

- L98H
- Tandem repeat 34
- T289A
- Y121F

Diagnostic Specimens

- Bronchoalveolar lavage (BAL) samples from hematology patients
- Bronchoalveolar lavage (BAL) samples from intensive care unit patients

Quality

- Validated on fungal cultures
- Validated on clinical samples (BAL)
- CE-IVD marked

Features & benefits

- Direct detection on clinical samples
- Identification of the most prevalent triazole resistance mutations
- Diagnosis within 2.5 hours after nucleic acid extraction
- Internal Amplification Control (IAC) included
- Positive controls included
- Interpretation software available

<u>company statement</u>: multi-platform RT-PCR
Detects *A. fumigatus*, *A. terreus*, *A. spec*. from BAL samples
From *A. fumigatus*: L98H, TR34, T289A, Y121F

Analytical and Clinical Evaluation of the PathoNostics AsperGenius Assay for Detection of Invasive Aspergillosis and Resistance to Azole Antifungal Drugs during Testing of Serum Samples

P. Lewis White,[a] Raquel B. Posso,[b] Rosemary A. Barnes[b]

Public Health Wales Microbiology Cardiff, Cardiff, United Kingdom[a]; Infection, Immunity and Biochemistry, School of Medicine, Cardiff University, Cardiff, United Kingdom[b]

The commercially developed PathoNostics AsperGenius species assay is a multiplex real-time PCR capable of detecting aspergillosis and genetic markers associated with azole resistance. The assay is validated for testing bronchoalveolar lavage fluids, replacing the requirement for culture and benefiting patient management. Application of this assay to less invasive, easily obtainable samples (e.g., serum) might be advantageous. The aim of this study was to determine the analytical and clinical performance of the AsperGenius species and resistance assays for testing serum samples. For the analytical evaluations, serum samples were spiked with various concentrations of *Aspergillus* genomic DNA for extraction, following international recommendations. For the clinical study, 124 DNA extracts from 14 proven/probable invasive aspergillosis (IA) cases, 2 possible IA cases, and 33 controls were tested. The resistance assay was performed on *Aspergillus fumigatus* PCR-positive samples when a sufficient fungal burden was evident. The limits of detection of the species and resistance assays for *A. fumigatus* DNA were 10 and ≥75 genomes/sample, respectively. Nonreproducible detection at lower burdens was achievable for all markers. With a positivity threshold of 39 cycles, the sensitivity and specificity of the species assay were 78.6% and 100%, respectively. For 7 IA cases, at least one genetic region potentially associated with azole resistance was successfully amplified, although no resistance markers were detected in this small cohort. The AsperGenius assay provides good clinical performance with the added ability to detect azole resistance directly from noninvasive samples. While the available burden will limit application, it remains a significant advancement in the diagnosis and management of aspergillosis.

White, Posso, Barnes (2015) JCM 53:2155-21

Multiplex -PCR approach

(AsperGenius Assay, PathoNostics, NL)

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company statement: multi-platform RT-PCR
Detects *A. fumigatus*, *A. terreus*, *A. spec*. from BAL samples
From *A. fumigatus*: L98H, TR34, T289A, Y121F

Journal of Antimicrobial Chemotherapy Advance Access published August 15, 2016

J Antimicrob Chemother
doi:10.1093/jac/dkw323

Journal of Antimicrobial Chemotherapy

PCR-based detection of *Aspergillus fumigatus Cyp51A* mutations on bronchoalveolar lavage: a multicentre validation of the AsperGenius assay® in 201 patients with haematological disease suspected for invasive aspergillosis

G. M. Chong[1]*, M. T. van der Beek[2], P. A. von dem Borne[3], J. Boelens[4], E. Steel[5], G. A. Kampinga[6], L. F. R. Span[7], K. Lagrou[8], J. A. Maertens[9], G. J. H. Dingemans[10], G. R. Gaajetaan[10], D. W. E. van Tegelen[10], J. J. Cornelissen[11], A. G. Vonk[12] and B. J. A. Rijnders[1]

Results: Two hundred and one patients each contributed one BAL sample, of which 88 were positive controls and 113 were negative controls. The optimal cycle threshold cut-off value for the *Aspergillus* species PCR was <38. With this cut-off, the PCR was positive in 74/88 positive controls. The sensitivity, specificity, positive predictive value and negative predictive value were 84%, 80%, 76% and 87%, respectively. 32/74 BAL samples were culture negative. Azole treatment failure was observed in 6/8 patients with a RAM compared with 12/45 patients without RAMs ($P=0.01$). Six week mortality was 2.7 times higher in patients with RAMs (50.0% versus 18.6%; $P=0.07$).

nested-PCR approach / AML case

(Spieß & Buchheidt; Mannheim)

Development of Novel PCR Assays To Detect Azole Resistance-Mediating Mutations of the *Aspergillus fumigatus cyp51A* Gene in Primary Clinical Samples from Neutropenic Patients

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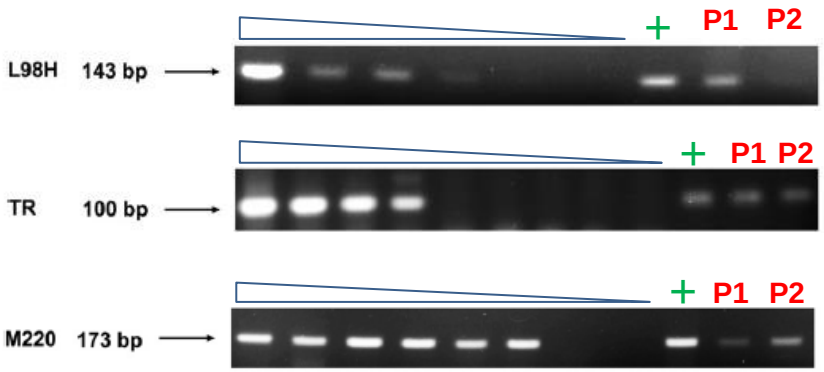

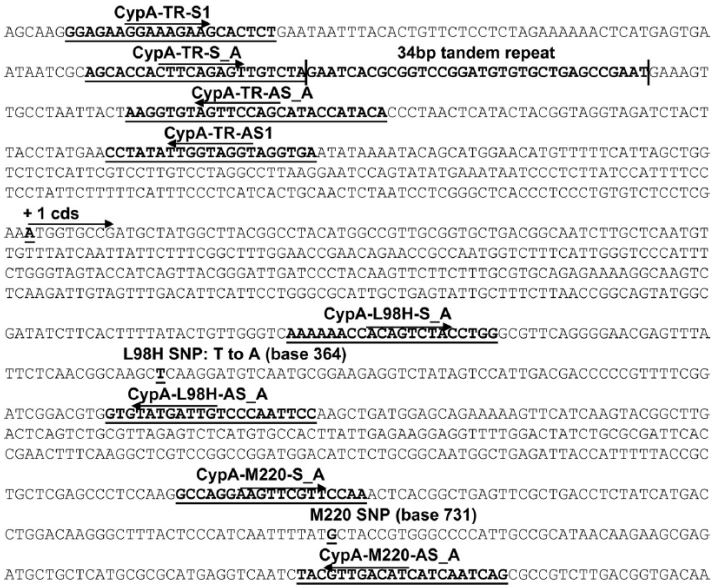

sequencing!

Amplification of specific *cyp51*A fragments from autopsy material (lung, heart) of the patient shown at this talk's beginning

→ confirms TR_{46}, Y121F, and T289A (region with M172 not covered)

Rößler S, Bader O, *et al.*, AAC 2017

summary

- Emergence of azole resistance is not an academic, but a clinical problem.
- It is not restricted to The Netherlands, but of global concern
- Environmental Screening makes sense to estimate the prevalence in local clinics / wards
- Methods for analysis are available, and they are not complicated

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Bundesministerium für Gesundheit

Pfizer

MSD

(Posaconazol)

Current EUCAST breakpoints

Antifungal agent	MIC breakpoint (mg/L)												Notes
	A. flavus		*A. fumigatus*		*A. nidulans*		*A. niger*		*A. terreus*		Non-species related breakpoints[1]		
	S ≤	R >	S ≤	R >	S ≤	R >	S ≤	R >	S ≤	R >	S ≤	R >	
													1. Non-species related breakpoints have been determined mainly on the basis of PK/PD data and are independent of MIC distributions of specific species. They are for use only for organisms that do not have specific breakpoints.
Amphotericin B	IE[2]	IE[2]	1	2	Note[3]	Note[3]	1	2	-	-	IE	IE	**2.** The ECOFFs for these species are in general one step higher than for *A. fumigatus*. **3.** There are too few MIC data to establish ECOFFs and hence to suggest any breakpoints.
Anidulafungin	IE	IE	IE	IE	IE	IE	IE	IE	IE	IE	IE	IE	
Caspofungin	IE	IE	IE	IE	IE	IE	IE	IE	IE	IE	IE	IE	
Fluconazole	-	-	-	-	-	-	-	-	-	-	-	-	
Itraconazole[4]	1	2	1	2	1	2	IE[2,5]	IE[2,5]	1	2	IE[5]	IE[5]	**4.** Monitoring of azole trough concentrations in patients treated for fungal infection is recommended. **5.** The MIC values for isolates of *A. niger* and *A. versicolor* are in general higher than those for *A. fumigatus*. Whether this translates into a poorer clinical response is unknown.
Micafungin	IE	IE	IE	IE	IE	IE	IE	IE	IE	IE	IE	IE	
Posaconazole[4]	IE[2]	IE[2]	0.12[6]	0.25[6]	IE[2]	IE[2]	IE[2]	IE[2]	0.12[6]	0.25[6]	IE	IE	**6.** Provided adequate drug exposure has been confirmed using therapeutic drug monitoring (TDM). There remains some uncertainty regarding cut-off values for posaconazole concentrations that separate patients with a high probability of clinical success from those with a low probability of clinical success. In some circumstances (e.g. patients with persistent and profound neutropenia, large lesions, or those with other features associated with a poor clinical outcome) a relatively high trough concentration should be sought. **Preclinical and clinical data suggest this value should be >1 mg/L at steady state. For other patient groups a lower trough concentration may be acceptable. For prophylaxis a target concentration of >0.7 mg/L has been suggested.**
Voriconazole[4]	IE[2]	IE[2]	1	2	IE	IE	IE[2]	IE[2]	IE[2]	IE[2]	IE	IE	

MIC value distribution across the literature

EUCAST	Itraconazole								Voriconazole								Posaconazole							
	S	I	R						S				I	R			S	I	R					
Mutation	1	2	4	>4	8	>8	16	>16	0,125	0,25	0,5	1	2	4	8	>8	0,06	0,13	0,25	0,5	1	2	4	>4
TR34/L98H				9		11		8				1	5	18	4			1	3	9	10	5		
TR34/L98H+S297T+F495I				8							1	4	2	1						3	4	1		
TR46/Y121F+T289A						1										1						1		
G54E								2			1	1									1	1		
G54R								1					1									1		
G54R+M220T						1				1												1		
G54W								1		1														
H147Y						1										1				1				
M172V						4							4						4					
P216L						1		1				2									2			
F219C								1		1								1						
F219I								6				1		2	3					1				
M220I								1		1										1				
M220K				2									1	1										1
M220L						2					1	1									1			
M220T						3		1			3			1			1		2	1				
M220V								1						1								1		
M220I+V101F				8								4	3	1							1		7	
N248T						1							1						1					
D255E						1							1							1				
Y431C					2									2							2			
E427G/K						5							5						5					
G434C						1								1							1			
G448S						1								1							1			
G448S						1										1								