

# Online Groenendael Sammy Huygens



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Geen (potentiële) belangenverstrengeling	
Voor bijeenkomst mogelijk relevante relaties <sup>1</sup>	Gilead
<ul style="list-style-type: none"><li>• Sponsoring of onderzoeksgeld<sup>2</sup></li><li>• Honorarium of andere (financiële) vergoeding<sup>3</sup></li><li>• Aandeelhouder<sup>4</sup></li><li>• Andere relatie, namelijk ...<sup>5</sup></li></ul>	<ul style="list-style-type: none"><li>• Travel grants</li><li>• Speakers fee</li></ul>



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# Aspergillosis

- *Aspergillus* spp.
  - 80 - 90% *Aspergillus fumigatus*<sup>1</sup>
  - *A. niger*; *A. flavus*; *A. terreus*; other cryptic species
- *A. fumigatus*
  - Saprotroph, widespread in nature: >100 spores inhaled/day
  - Grows within 12 - 56°C (sexual reproduction at 30°C)
- Multiple clinical entities

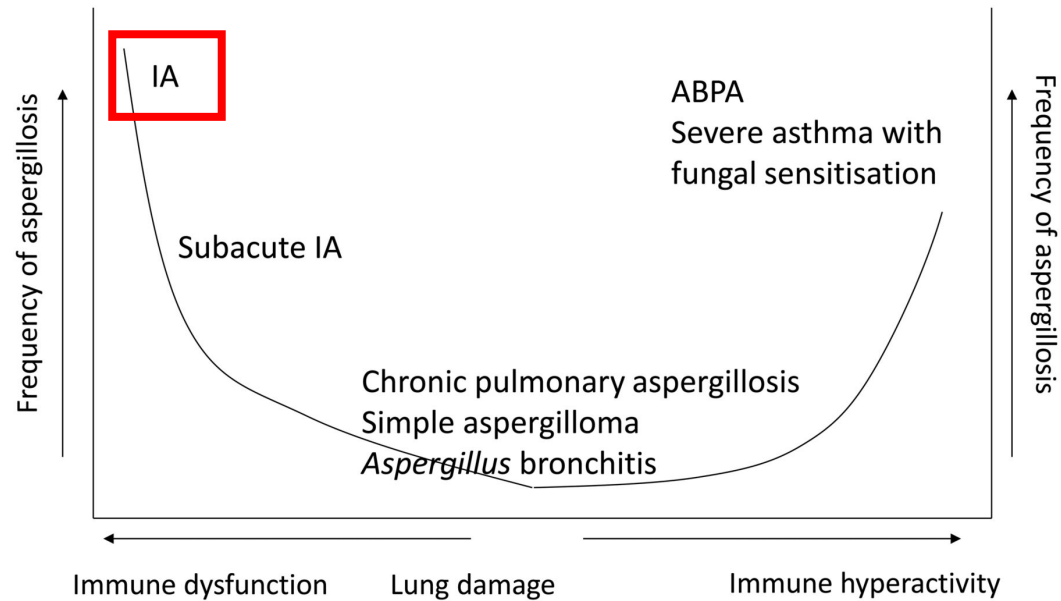


Conidiophores of *Aspergillus*. It may be *Aspergillus fumigatus*. Source: US Department of Health and Human Services, Center for disease control [\[1\]](#).

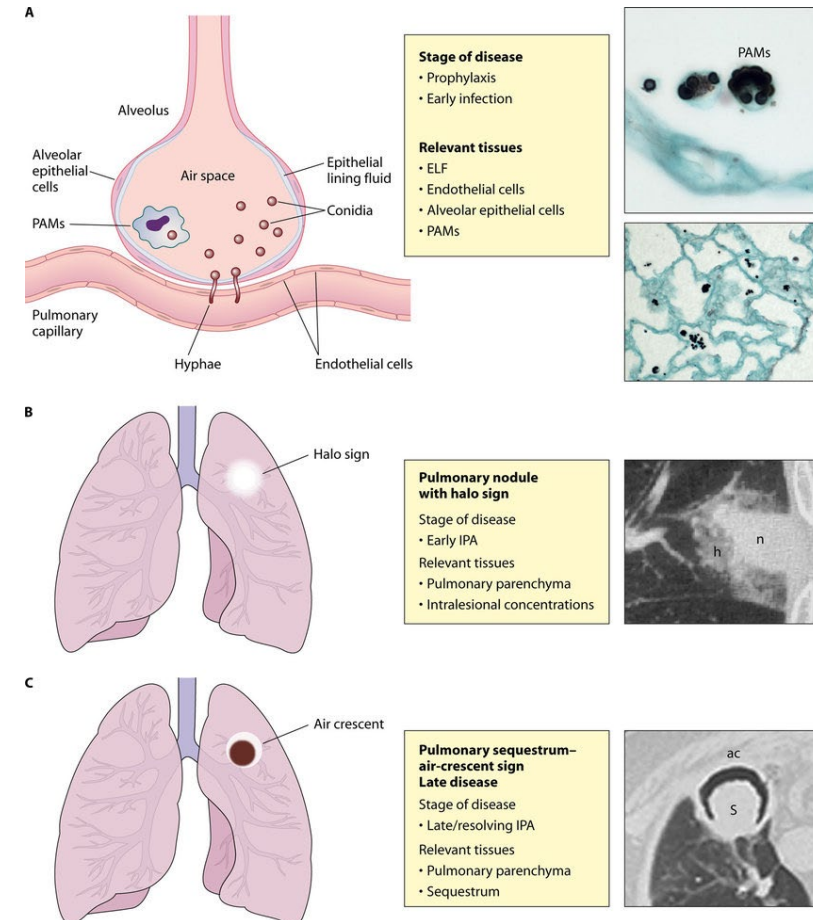
1: Latge, J. P. and G. Chamilos (2019). "Aspergillus fumigatus and Aspergillosis in 2019." *Clin Microbiol Rev* **33(1)**: e00140-00118.



# Invasive aspergillosis



Kosmidis C, Denning DW. The clinical spectrum of pulmonary aspergillosis. *Thorax* 2015;**70**:270-277.



Felton, Timothy & Troke, Peter & Hope, William. (2014). Tissue Penetration of Antifungal Agents. *Clinical microbiology reviews*. 27. 68-88. 10.1128/CMR.00046-13.

# Treatment

- Invasive aspergillosis (IA)
- Most frequent fungal infection in AML patients
- IA treatment started in 10-30%<sup>1</sup>
- First-line treatment:
  - Voriconazole
  - Isavuconazole
  - Posaconazole<sup>2</sup>

Targeted therapy of pulmonary disease—first line

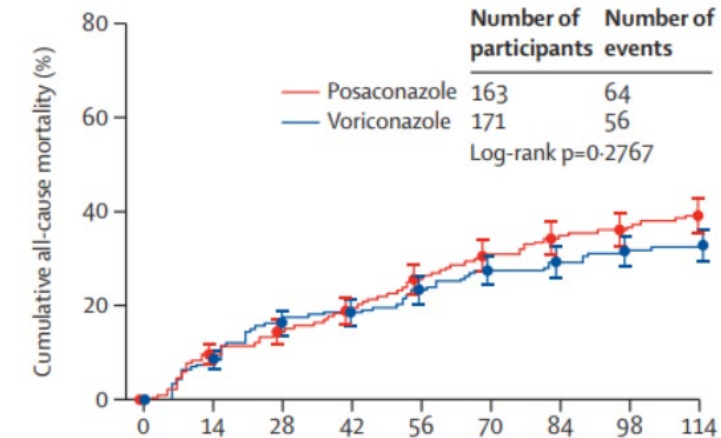
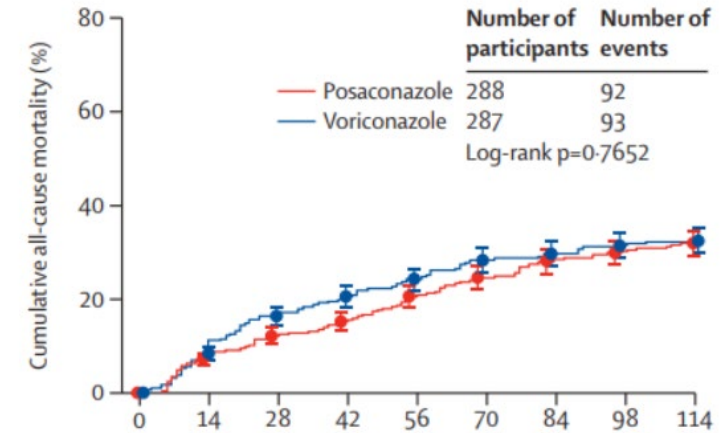
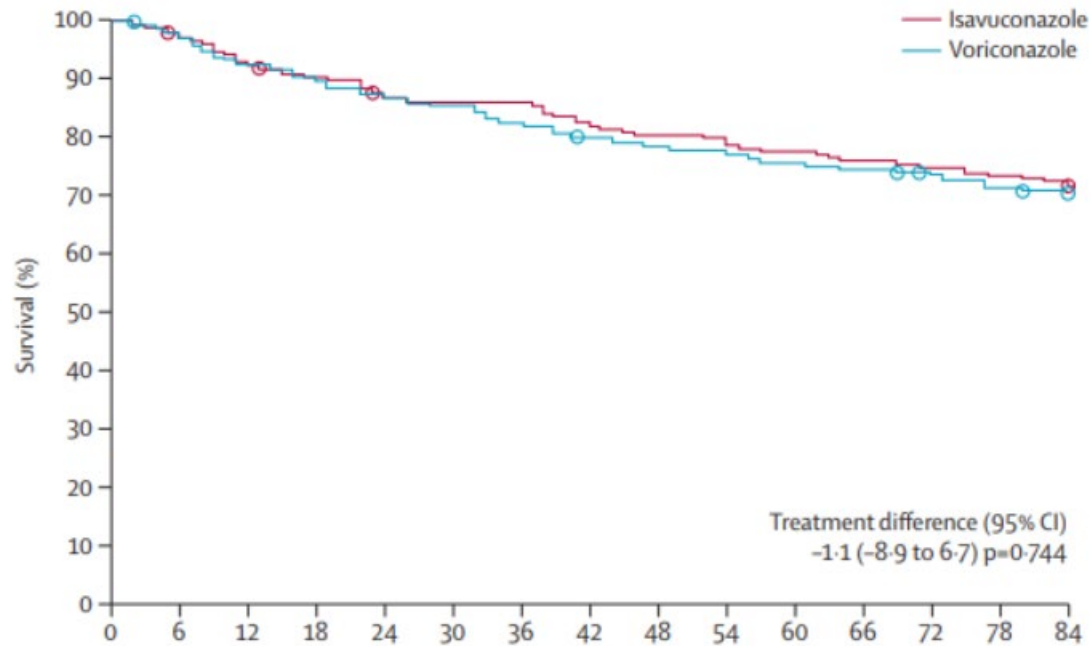
Population	Intention	Intervention	SoR	QoE <sup>1</sup>	QoE <sup>2</sup>	QoE <sup>3</sup>	Comment	Ref.
1] Neutropenia (non-allo HSCT recipients)	To increase response and survival rate	Isavuconazole 200 mg IV tid day 1–2, then 200 mg qd oral	A	I	II <sub>t</sub>	II <sub>t</sub>	D III, if mould active azole prophylaxis fewer adverse effects than voriconazole	[173,507,564,565]
2] Allo-HSCT (during neutropenia)		Voriconazole 2 × 6 mg/kg IV (oral 400 mg bid) on day 1, then 2–4 mg/kg IV (oral 200–300 mg bid)	A	I	II <sub>t</sub>	II <sub>t</sub>	C III for start with oral; D III, if prior mould active azole prophylaxis; TDM	[170,172,507,566]
3] Allo-HSCT (w/o neutropenia) or other non-neutropenic patients		L-AmB 3 mg/kg	B	II	II <sub>t</sub>	II <sub>t</sub>		[171]
		Combination of voriconazole 6/4 mg/kg bid (after 1 week oral possible (300 mg bid)) + anidulafungin 200/100 mg	C	I	II <sub>t</sub>	II <sub>t</sub>	No significant difference compared to voriconazole, in GM-positive (subgroup) better survival; TDM	[172,566]
		Caspofungin 70 mg qd day 1, followed by 50 mg qd (if body weight <80 kg)	C	II	II	II		[567–569]
		Itraconazole 200 mg q12 h IV on day 1, then 200 mg/qd	C	III	II <sub>t,a</sub>	II <sub>t,a</sub>	D III for start with oral, TDM	[507,537]
		AmB lipid complex (ABLC) 5 mg/kg	C	III	III	III		[570]
		Micafungin 100 mg	C	III	III	III		[571–573]
		AmB colloidal dispersion (ABCD) 4–6 mg/kg	D	I	II <sub>t</sub>	II <sub>t</sub>		[142]
		Conventional AmB 1–1.5 mg/kg	D	I	II <sub>t</sub>	II <sub>t</sub>		[170]
		Other combinations	D	III	III	III		[574]
Life-threatening haemoptysis	Bridging until neutrophil recovery	Arterial embolization, emergency surgical intervention	B	III	III	III	Efficacy unproven	[575]

A.J. Ullmann et al. / *Clinical Microbiology and Infection* 24 (2018) e1–e38

1: *New Engl J Med* 2002, Herbrecht R et al. [10.1056/NEJMoa020191](https://doi.org/10.1056/NEJMoa020191)

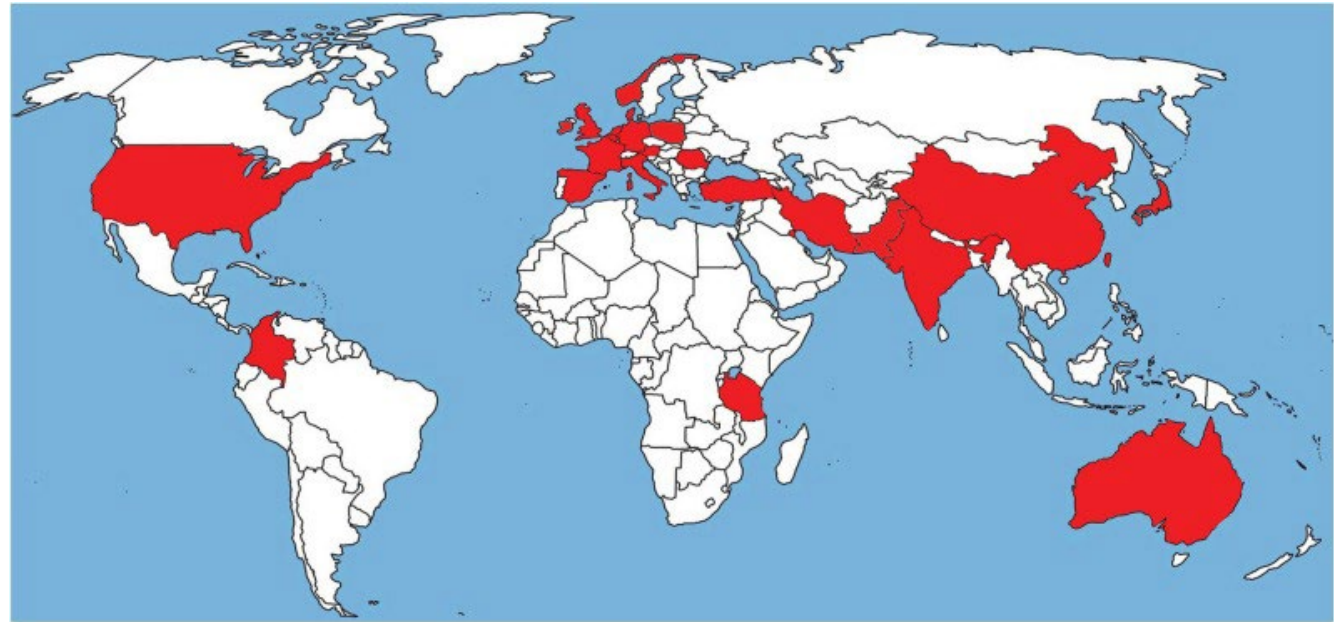
2: J et al. *Lancet* 2021;397:499-509

# Azole = Voriconazole = Isavuconazole = Posaconazole



# Antifungal resistance in *A. fumigatus*

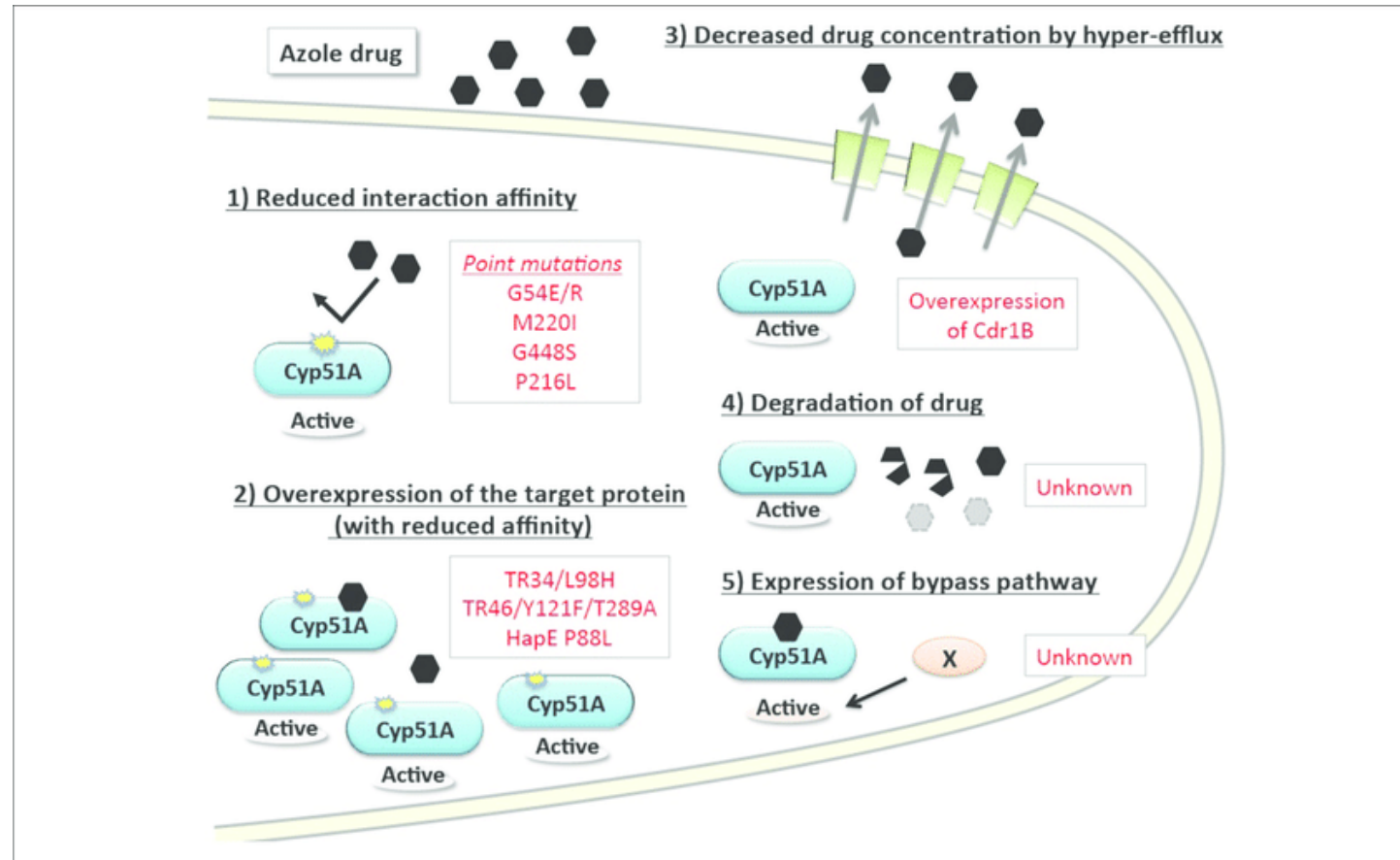
- Early 2000s: First azole-resistant *A. fumigatus* described in the Netherlands<sup>1</sup>
- Currently: Widespread across all continents
- < extensive use of agricultural fungicides



Meis JF. Clinical implications of globally emerging azole resistance in *Aspergillus fumigatus*. *Philos Trans R Soc Lond B Biol Sci*. 2016



# Antifungal resistance in *A. fumigatus*



Berger. Azole Resistance in *Aspergillus fumigatus*: A Consequence of Antifungal Use in Agriculture. *Front Microbiol.* 2017

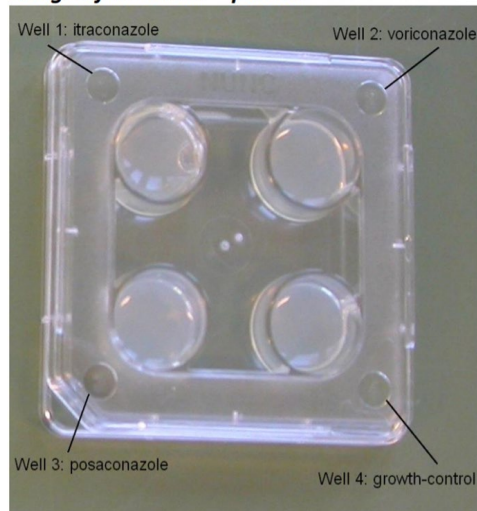
# Antifungal resistance in *A. fumigatus*

- *Yearly Nethmap report*
- *Methods: 5 UMC + 5 TH → screening all clinical *A. fumigatus* isolates*

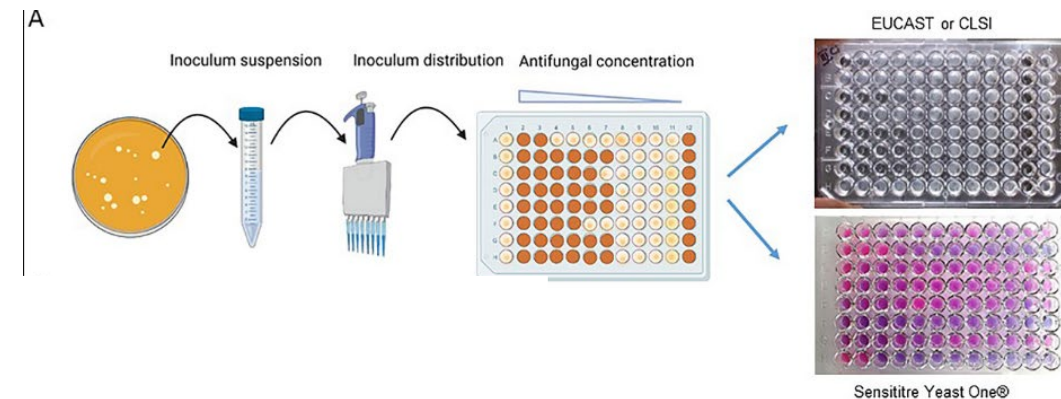
## Phenotypic resistance checking

### ■ VIPcheck™

#### Design of VIPcheck™ plates:



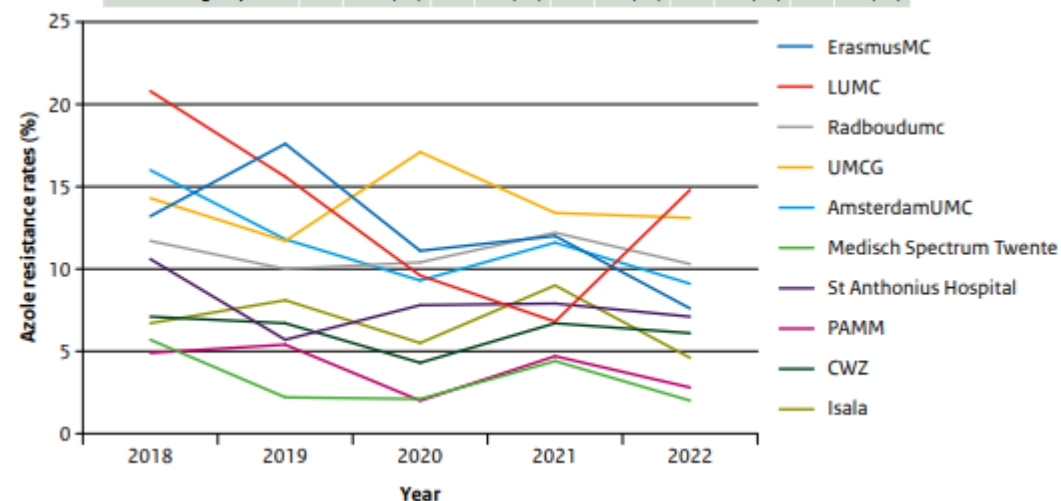
## EUCAST: Broth microdilution in 96-well microplate:



# Antifungal resistance in *A. fumigatus*

- 2022: Triazole resistance 10.6% in UMCs and 4.8% in teaching hospitals
- 82.5% of azole-resistant isolates harbored a TR-mediated resistance mechanism (TR34/L98H or TR46/Y121F/T289A)

	2018		2019		2020		2021		2022	
	Screened	Azole R (%)	Screened	Azole R (%)	Screened	Azole R (%)	Screened	Azole R (%)	Screened	Azole R (%)
<b>UMCs</b>										
ErasmusMC	129	17 (13.2)	102	18 (17.6)	108	12 (11.1)	142	17 (12)	119	7 (7.6)
LUMC	120	25 (20.8)	90	14 (15.6)	83	8 (9.6)	103	7 (6.8)	81	12 (14.8)
Radboudumc	196	23 (11.7)	230	23 (10)	193	20 (10.4)	205	25 (12.2)	175	18 (10.3)
UMCG	238	34 (14.3)	230	27 (11.7)	181	31 (17.1)	209	28 (13.4)	206	27 (13.1)
AmsterdamUMC	81	13 (16)	51	6 (11.8)	172 <sup>a</sup>	16 (9.3)	173	20 (11.6)	175	16 (9.1)
<b>Total UMCs</b>	<b>764</b>	<b>112 (14.7)</b>	<b>703</b>	<b>88 (12.5)</b>	<b>737</b>	<b>87 (11.8)</b>	<b>832</b>	<b>97 (11.7)</b>	<b>756</b>	<b>80 (10.6)</b>
<b>Teaching hospitals</b>										
Medisch Spectrum Twente	88	5 (5.7)	90	2 (2.2)	95	2 (2.1)	182	8 (4.4)	98	2 (2.0)
St Antonius Hospital	265	28 (10.6)	177	10 (5.7)	193	15 (7.8)	151	12 (7.9)	211	15 (7.1)
PAMM	81	4 (4.9)	147	8 (5.4)	150	3 (2)	129	6 (4.7)	141	4 (2.8)
CWZ	155	11 (7.1)	90	6 (6.7)	163	7 (4.3)	120	8 (6.7)	99	6 (6.1)
Isala	195	13 (6.7)	222	18 (8.1)	183	10 (5.5)	222	20 (9)	237	11 (4.6)
<b>Total teaching hospitals</b>	<b>784</b>	<b>50 (7.8)</b>	<b>726</b>	<b>42 (6.1)</b>	<b>784</b>	<b>37 (4.7)</b>	<b>804</b>	<b>54 (6.7)</b>	<b>786</b>	<b>38 (4.8)</b>

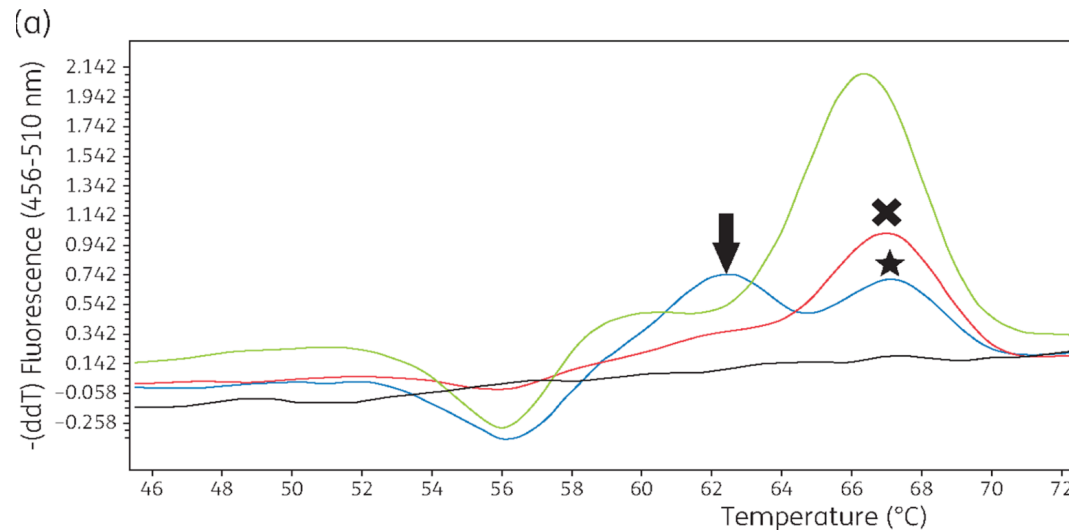


Dutch Working Party on Antibiotic Policy. NethMap 2023: Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands in 2022, National Institute for Public Health and the Environment: 190.

# Antifungal resistance in *A. fumigatus*

- Genotypic resistance checking
  - PCR AsperGenius®
  - Detecting certain mutations that are known to result in resistance
  - Mutations in the *A. fumigatus* Cyp51A gene

Benefit: results within 24 - 48h



TR34/L98H

TR46/Y121F/T289A

(+80% of resistant *A. Fumigatus* in BE and NL)

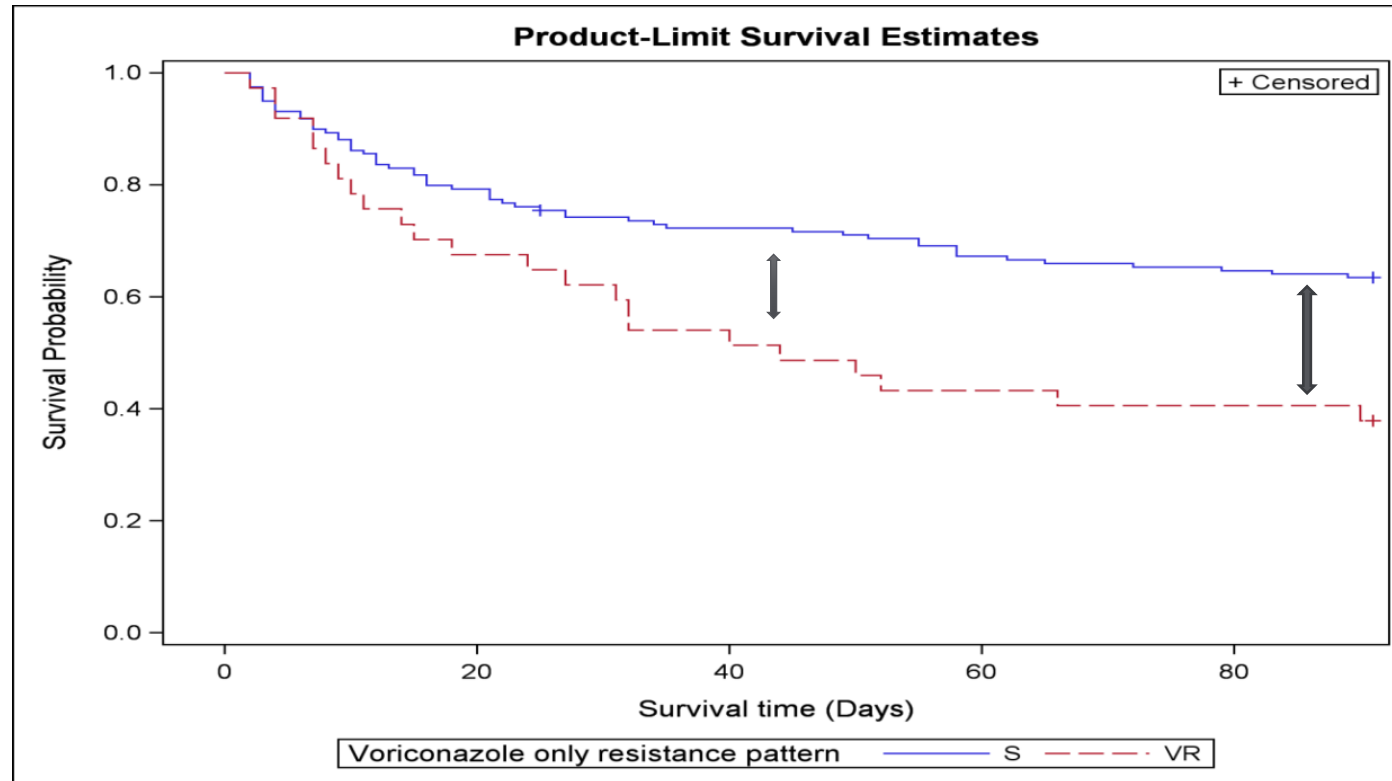
# Fungal priority pathogen list (WHO)

- Global public health concern
  - WHO: MCDA for fungi (~ bacterial list)
- ↕
- Rapid increase of antifungal resistance
  - Only four classes of antifungals



WHO fungal priority pathogens list to guide research, development and public health action. Geneva: World Health Organization; 2022. Licence: CC BY-NC-SA 3.0 IGO.

# Antifungal resistance in *A. fumigatus*



Escalation strategy in this cohort:

Culture-based resistance testing = time consuming

→ Azole-resistant cases were switched after median of 10 days.

# Antifungal resistance?? in *A. fumigatus*

Dilemma: Mortality of azole resistant IA when upfront azole monotherapy is initiated : approx. 50%

- Compared with voriconazole-susceptible cases, voriconazole resistance was associated with an increased overall mortality of 21% on day 42 (49% vs 28%)<sup>1</sup>
- Mortality in patients who received appropriate initial voriconazole therapy **24%** compared with **47%** with inappropriate initial therapy (P = .016), despite switching to appropriate antifungal therapy after a median of 10 days.<sup>1</sup>



Liposomal Amphotericine- B when given for no reason in approx. 90%    Costs + IV only + toxicity<sup>2</sup>

1: Lestrade PP et al. Voriconazole Resistance and Mortality in Invasive Aspergillosis: A Multicenter Retrospective Cohort Study. Clin Infect Dis. 2019 Apr 24;68(9):1463-1471. doi: 10.1093/cid/ciy859. PMID: 30307492.

2: NethMap 2023: Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands in 2022, National Institute for Public Health and the Environment: 190

# Antifungal resistance?? in *A. fumigatus*

Dilemma: Mortality of azole resistant IA when upfront azole monotherapy is initiated : 50%

- Compared with voriconazole-susceptible cases, voriconazole resistance was associated with an increased overall mortality of 21% on day 42 (49% vs 28%)



But...

Data from culture positive patients (high fungal load) probably not representative of culture negative patients in whom resistance PCR is also unsuccessful (low fungal load)

Anno 2024 : Improved diagnostics, use of PCR on BAL, earlier initiation of antifungal therapy  
=> 75% of cases of IA are culture negative !



# Antifungal resistance in *A. fumigatus*

SWAB guidelines (2017)

A. Therapy for isolates with unknown susceptibility to azoles		
Antifungal agent	Loading dose	Maintenance dose
<b>1<sup>st</sup> choice*</b>		
<b>Azole+Echinocandin combination:</b>		
Voriconazole OR	bid 6 mg/kg iv or bid 400 mg po	4 mg/kg bid iv <sup>†</sup> or 200-300 mg bid po <sup>‡</sup>
Isavuconazole	tid 200 mg iv or po on days 1 +2	200 mg qd iv or po
<b>AND</b>		
Caspofungin OR	70 mg	50 mg qd, (>80kg: 70 mg qd)
Micafungin OR	-	100 mg qd
Anidulafungin	200 mg	100 mg qd
<b>OR</b>		
<b>Azole + L-AmB combination:</b>		
Voriconazole	bid 6 mg/kg iv or bid 400 mg po	4 mg/kg bid iv <sup>†</sup> or 200-300 mg bid po <sup>‡</sup>
OR		
Isavuconazole	tid 200 mg iv or po on days 1 +2	200 mg qd iv or po
<b>AND</b>		
L-AmB	-	3 mg/kg/d
<b>2<sup>nd</sup> choice*</b>		
Liposomal AmB	-	3 mg/kg/d
<b>3<sup>rd</sup> choice*</b>		
Caspofungin	70 mg	50 mg qd, (>80kg: 70 mg qd)
Micafungin	-	100 mg qd
Anidulafungin	200 mg	100 mg qd
B. Therapy for isolates with confirmed susceptibility to azoles		
Antifungal agent	Loading dose	Maintenance dose
<b>1<sup>st</sup> choice*</b>		
Voriconazole	bid 6 mg/kg iv or bid 400 mg po	4 mg/kg bid iv <sup>†</sup> or 200 mg bid po <sup>‡</sup>
Isavuconazole	tid 200 mg iv or po on days 1 +2	200 mg qd iv or po
<b>2<sup>nd</sup> choice*</b>		
Liposomal AmB	-	3 mg/kg/d
<b>3<sup>rd</sup> choice*</b>		
<b>Voriconazole+Echinocandin combination:</b>		
Voriconazole	bid 6 mg/kg iv or bid 400 mg po	4 mg/kg bid iv <sup>†</sup> or 200-300 mg bid po <sup>‡</sup>
<b>AND</b>		
Caspofungin OR	70 mg	50 mg qd, (>80kg: 70 mg qd)
Micafungin OR	-	100 mg qd
Anidulafungin	200 mg	100 mg qd
C. Therapy for isolates with confirmed resistance to azoles		
Antifungal agent	Loading dose	Maintenance dose
<b>1<sup>st</sup> choice*</b>		
Liposomal AmB	-	3 mg/kg/d
<b>2<sup>nd</sup> choice*</b>		
Caspofungin	70 mg	50 mg qd, (>80kg: 70 mg qd)
Micafungin	-	100 mg qd
Anidulafungin	200 mg	100 mg qd

\* For specific recommendations, exceptions, and contra-indications, see text.

<sup>†</sup> The dosages in this table are specific for invasive aspergillosis; for other mycoses, different dosages may apply.

<sup>‡</sup> Individual dose based on therapeutic drug monitoring

# Antifungal resistance?? in *A. fumigatus*

Dilemma: Guideline 2017

<b>Recommendation 12</b>	For patients with invasive aspergillosis caused by isolates with unknown susceptibility to azoles, initial combination therapy with voriconazole/isavuconazole plus L-AmB, or voriconazole/isavuconazole plus an echinocandin is recommended. Monotherapy with L-AmB is considered as a second choice in these patients. In case of mixed azole-resistant and azole-susceptible mold infections, or suspected co-infection with mucorales, voriconazole/isavuconazole plus L-AmB is recommended.
<b>Recommendation 13</b>	If susceptibility or BAL Cyp51 resistance PCR results are expected shortly, initial monotherapy with voriconazole may be prescribed to patients with invasive aspergillosis of unknown azole susceptibility. Subsequent PCR results should guide escalation to L-AmB in case of resistance, and to combination therapy if susceptibility results are unavailable. Severely ill patients and patients in the ICU should receive initial combination therapy pending susceptibility results.

In brief:

- ✓ Start azole + drug from another class or
- ✓ Add a drug from another class when resistance testing fails

# AZORMAN trial

- Before: high mortality of azole-R invasive aspergillosis → mainly due to late switch to active antifungal therapy
  - Low sensitivity of fungal culture
  - If culture positive: it takes up to 7 - 10 days for fungi to grow + time for susceptibility testing: 7 - 10 days
- Introduction of PCR test with susceptibility testing (TR34 and TR46): result in 24 - 48h
- Impact on outcome?



# Design of the AzoRMan study

Prospective multicentre observational study

EMC, Rotterdam

UZ Leuven, Leuven

AMC, Amsterdam

UZ Gent, Gent

VUMC, Amsterdam

AZ Sint-Jan, Brugge

RadboudUMC, Nijmegen

Meander MC, Amersfoort

UMCU, Utrecht

MUMC, Maastricht

UMCG, Groningen

LUMC, Leiden



# AIM

## Main objective:

Clinical impact of testing for presence of cyp51A mutations in *A. fumigatus* DNA on BAL fluid



Proportion of patients with probable IA by azole-resistant *A. fumigatus* in whom treatment failure was observed in the 6 weeks following diagnosis  
Mixed infections were excluded

Historical cohort: treatment failure in 75% of patients with IA caused by an azole-resistant *A. fumigatus* treated with azole monotherapy<sup>1</sup>

- Aiming for reduction to 35%
- 15 cases of azole-resistance needed (+/- 120 probable IA)

# AIM

## Main objective:

Clinical impact of testing for presence of cyp51A mutations in *A. fumigatus* DNA on BAL fluid



Proportion of patients with probable IA by azole-resistant *A. fumigatus* in whom treatment failure was observed in the 6 weeks following diagnosis  
Mixed infections were excluded

## Secondary objectives:

- Prevalence of the 2 most frequent resistance associated mutations (TR<sub>34</sub>/L98H and TR<sub>46</sub>/Y121F/T289A)
- To evaluate outcome of patients with an isolated positive *Aspergillus* PCR on BAL fluid

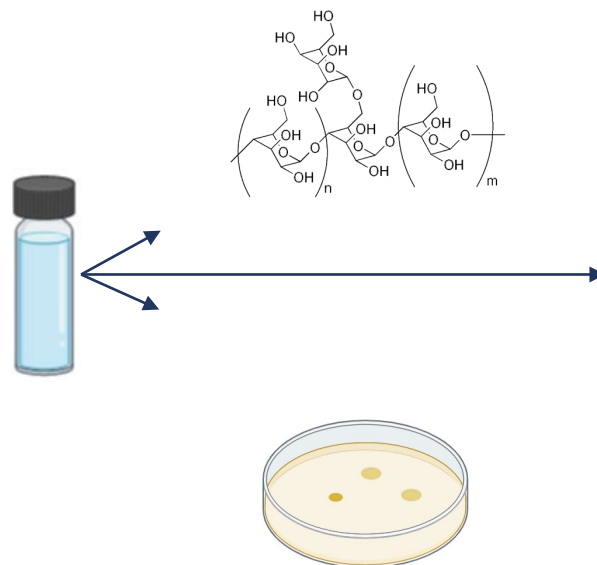
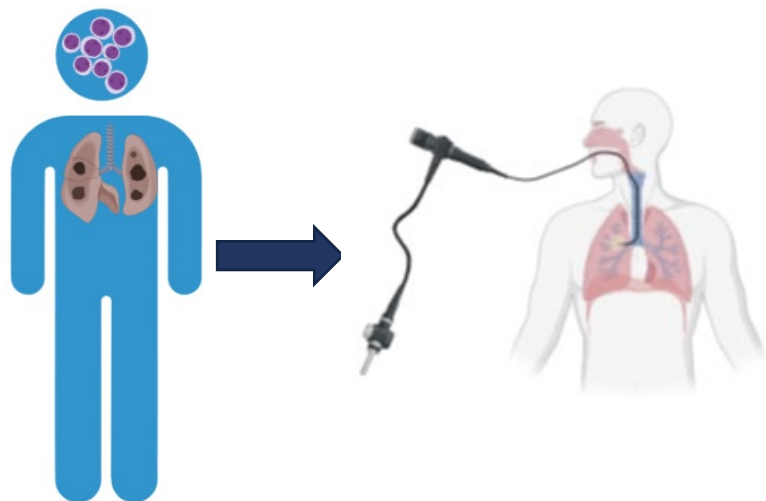


6-week overall mortality of patients without mycological criteria versus isolated positive PCR on BAL

# Material and methods

## Population

- Patients with haematological malignancies
- New pulmonary infiltrate suspect for IFI
- Bronchoalveolar lavage (+/- 48 hours of inclusion)
- <120 hours of azole monotherapy



## PN-002 AsperGenius® Resistance Multiplex real-time PCR

### Species multiplex

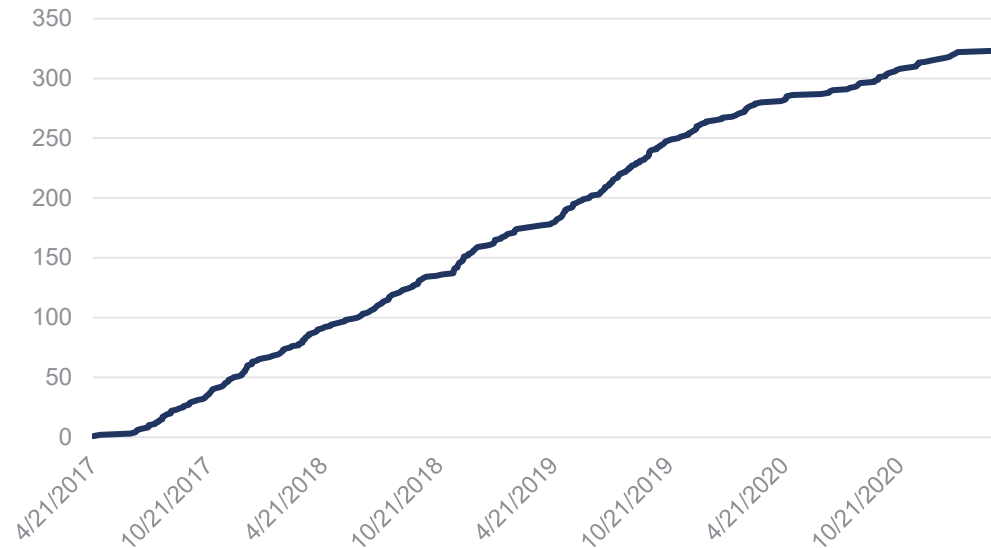
- *Aspergillus fumigatus*
- *Aspergillus terreus*
- *Aspergillus* species
- Internal Control (IC)

### Resistance multiplex

- L98H
- Tandem repeat 34
- T289A
- Y121F

# Results

Recruitment



Recruitment: 4 years

323 patients included

## Baseline Characteristics of the enrolled patients

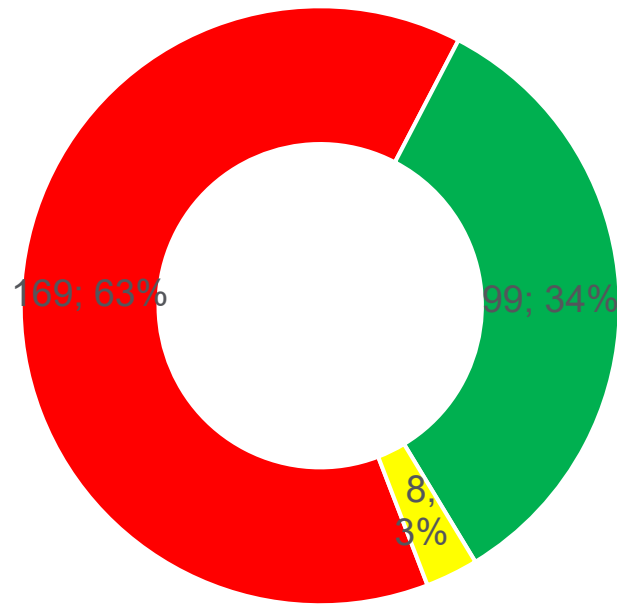
Total  
N=323

Age, median (IQR)	63 (53, 69)
Male sex (%)	219 (68)
Allogeneic stem cell transplant (%)	102 (32)
Autologous stem cell recipient (%)	13 (4)
Underlying haematological disease (%)	
• AML	163 (51)
• MDS	40 (12)
• ALL	20 (6)
• Other	98 (30)
Acute GvHD, grade II-IV, n (%)	23 (7)
Chronic GvHD, n (%)	19 (6)
• Mild	6 (2)
• Moderate	5 (2)
• Severe	8 (3)
Use of prednisolone <sup>a</sup> (%)	
• <0,3mg/kg/day	41 (13)
• >0,3mg/kg/day	51 (17)
Chemotherapy in last 90 days <sup>b</sup> (%)	195 (71)
Neutropenia <sup>c</sup> , Yes (%)	170 (58)



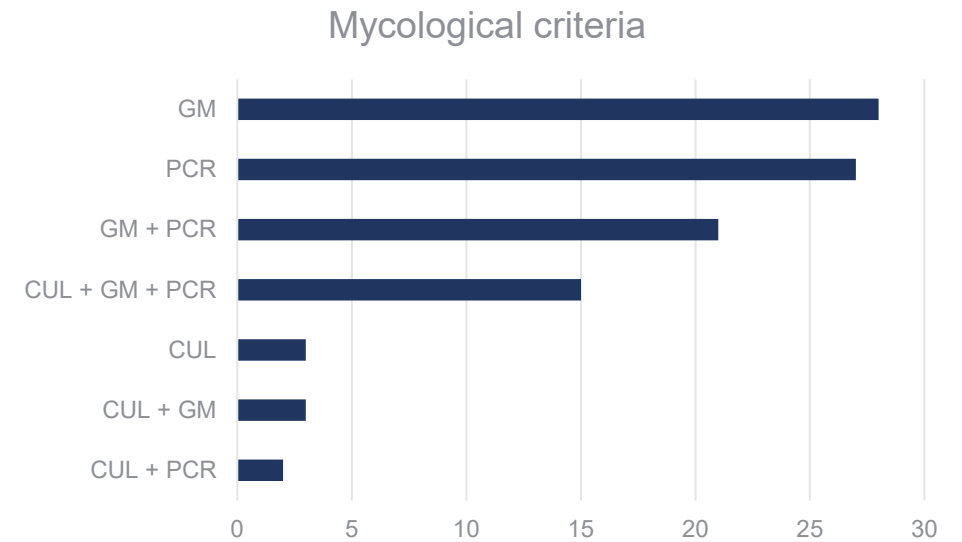
# Results

Complete diagnostic information available in 276 patients (information on CT imaging + BAL PCR + BAL culture + BAL GM)



■ Possible IA   ■ Probable IA   ■ No IA

Based on the 2020 EORTC/MSGERC consensus guidelines



# Results

Aspergillus PCR and culture results based on galactomannan

BALf GM (OD)	<0.5	0.5–0.99	≥1
Aspergenius Performed (n)	193	31	68
PCR Aspergillus species positive (n)	50 (26%)	16 (52%)	50 (74%)
PCR A. fumigatus positive (n)	38 (20%)	12 (39%)	39 (57%)
PCR A. terreus positive (n)	1 (0.5%)	0 (0%)	2 (3%)
TR <sub>34</sub> /L98H PCR successful (n)	19 (50%)	8 (67%)	36 (92%)
TR <sub>46</sub> /T289A/Y121F PCR successful (n)	21 (55%)	5 (42%)	36 (92%)
TR <sub>34</sub> /L98H and TR <sub>46</sub> /T289A/Y121F both WT	16	4	32
TR <sub>34</sub> /L98H Resistant and TR <sub>46</sub> /T289A/Y121F WT (n)	1	0	5 (2#)
TR <sub>34</sub> /L98H WT and TR <sub>46</sub> /T289A/y121F Resistant (n)	1	0	1
Culture positive for Aspergillus species (n)	6	1	17
Culture positive for A. fumigatus (n)	5	0	16
Culture positive for A. niger (n)	1	0	0
Culture positive for A. terreus (n)	0	0	1
Culture positive for A. flavus (n)	0	1	0

Aspergillus DNA in 116/292 (40%)

A. Fumigatus DNA in 89/292 (30%)

Performance of PCR increases with higher GM

Resistance PCR was successful in 58/89

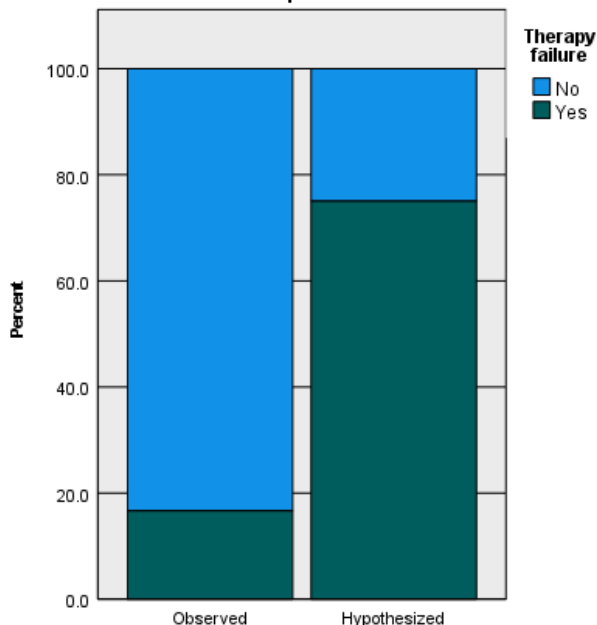
Performance of resistance PCR increases with higher GM

→ *cyp51A* RAMs were found in 8/58 (14%) patients

# Results

Age, y	Sex	Disease	Stem Cell Transplant	Bronchoalveolar Fluid Galactomannan	Culture	Resistance Testing on Culture	AsperGenius Resistance Testing	Initial Therapy	Subsequent Therapy	Time of Antifungal Switch, d	Therapy Failure	6-Week Mortality	12-Week Mortality
66	M	AML	-	1.6	+	Azole resistant	TR <sub>46</sub>	Azole	L-AmB	1	No	No	No
53	F	Non-Hodgkin lymphoma	+	0.3	+	Azole resistant	TR <sub>34</sub>	Azole + L-AmB	L-AmB	4	No	No	No
54	M	Hodgkin lymphoma	+	4.8	+	Azole resistant	TR <sub>34</sub>	Azole + L-AmB	L-AmB <sup>a</sup>	5	Yes on day 42	No	No
48	F	AML	-	5.6	-	-	TR <sub>34</sub>	Azole + echinocandin	L-AmB	2	No	No	Yes
64	F	AML	+	0.07	-	-	TR <sub>46</sub>	Azole		NA	No	No	No
57 <sup>b</sup>	M	Mantle cell lymphoma	-	3.08	+	Not tested	Mixed pattern: WT and TR <sub>34</sub>	Azole		NA	No	No	No
23	M	T-cell acute lymphocytic leukemia	-	8	+	Azole susceptible <sup>c</sup>	TR <sub>34</sub>	Azole	Azole + L-AmB	15 <sup>d</sup>	No	No	No
79	M	MW	-	5.6	+	Not tested	Mixed pattern: WT and TR <sub>34</sub>	Azole		NA	No	Yes	Yes

One-Sample Binomial Test



One-Sample Binomial Test Summary

Total N	6
Test Statistic	5.000
Standard Error	1.061
Standardized Test Statistic	2.828
Asymptotic Sig.(1-sided test)	.002
Exact Sig.(1-sided test)	.005

Only non-mixed infections  
< 323 inclusions

Proportion of observed treatment failure: 16,7%

95% C.I.: 0,4 - 64,1%

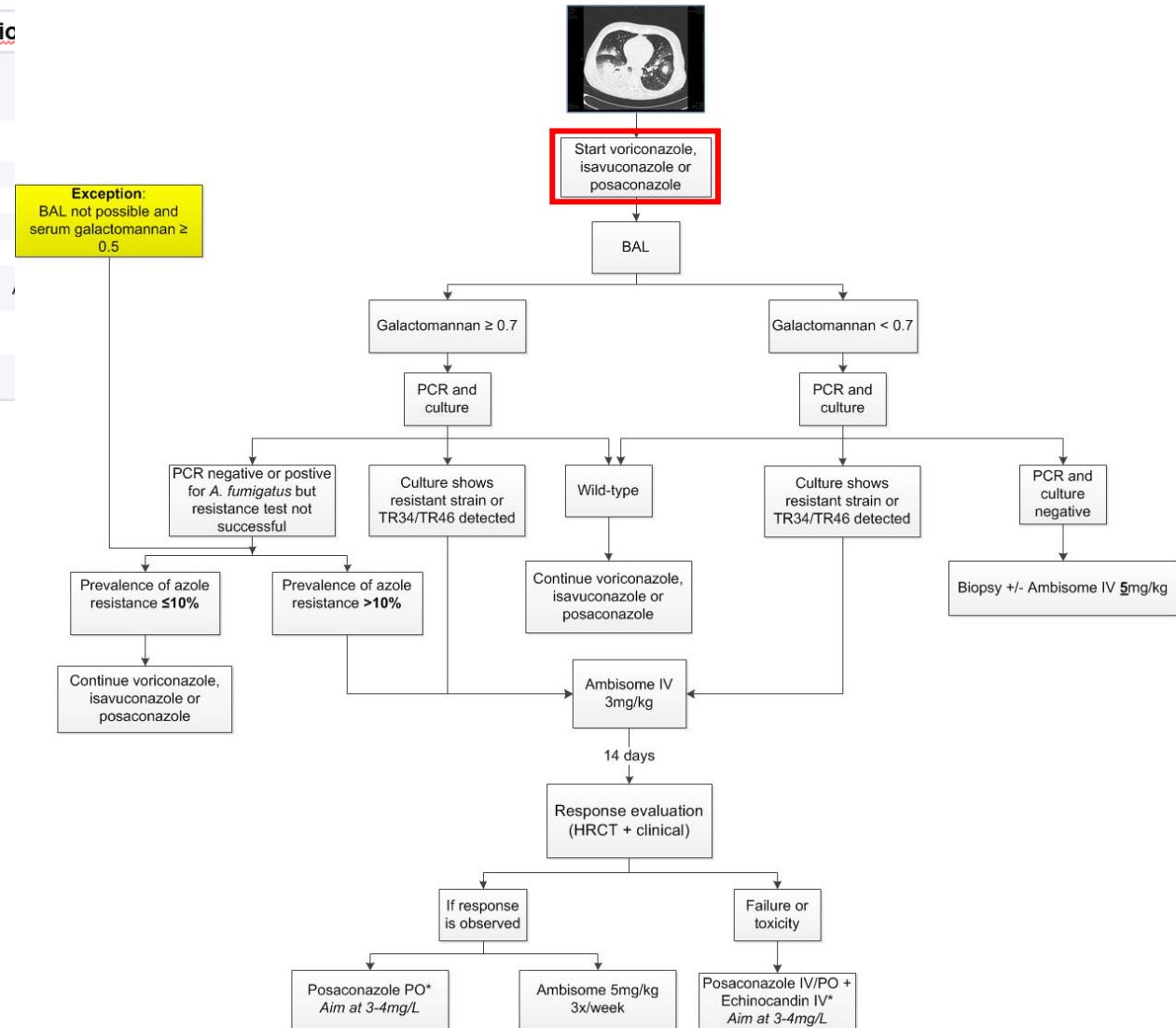
BUT!!!

Smaller number R azole cases → 15 cases expected (with reduction of treatment failure from 75% to 35%)

# Results

Details on patients with presence of resistance associated mutations

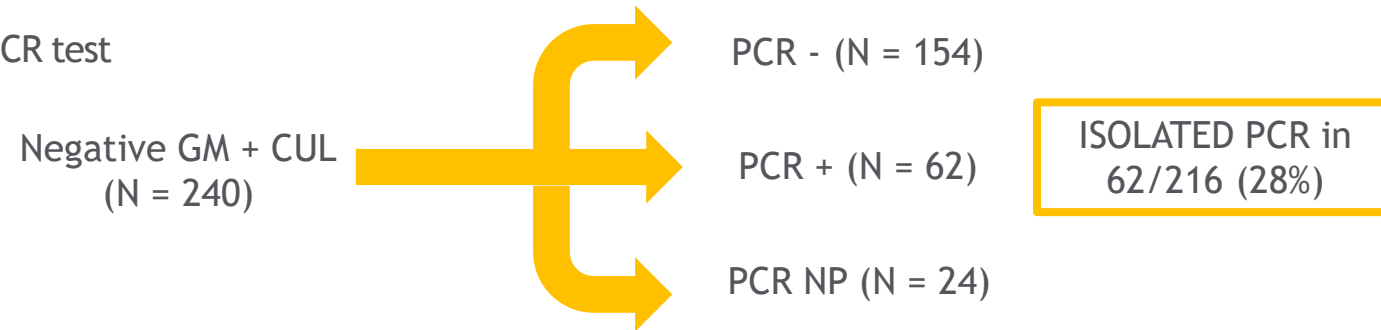
Patient	Age	Underlying disease	Resistance PCR	GM	Culture	Initial therapy
1	54	GVHD	TR <sub>34</sub>	4.8	A. fumigatus, azole resistant	Azole + amphotericin
2	53	GVHD	TR <sub>34</sub>	0.3	A. fumigatus, azole resistant	Azole + amphotericin
3	64	AML	TR <sub>46</sub>	0.07	Negative	Azole
4	66	AML	TR <sub>46</sub>	1.6	A. fumigatus, azole resistant	Azole
5	48	AML	TR <sub>34</sub>	5.6	Negative	Azole + echinocandin
6	23	ALL	TR <sub>34</sub>	8	A. fumigatus, azole susceptible	Azole
7	57	Lymphoma	WT/TR <sub>34</sub>	3.08	A. fumigatus, no resistance testing	Azole
8	79	Waldenström	WT/TR <sub>34</sub>	5.6	A. fumigatus, no resistance testing	Azole



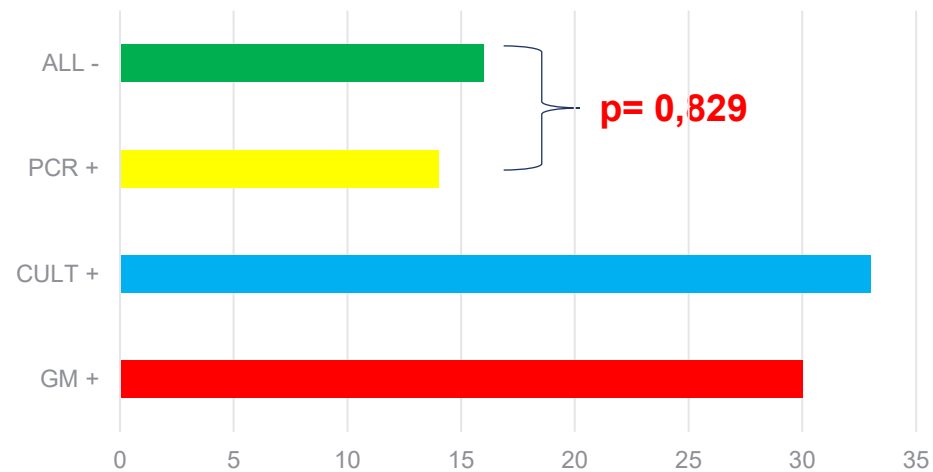
# Results

## SECONDARY ENDPOINTS

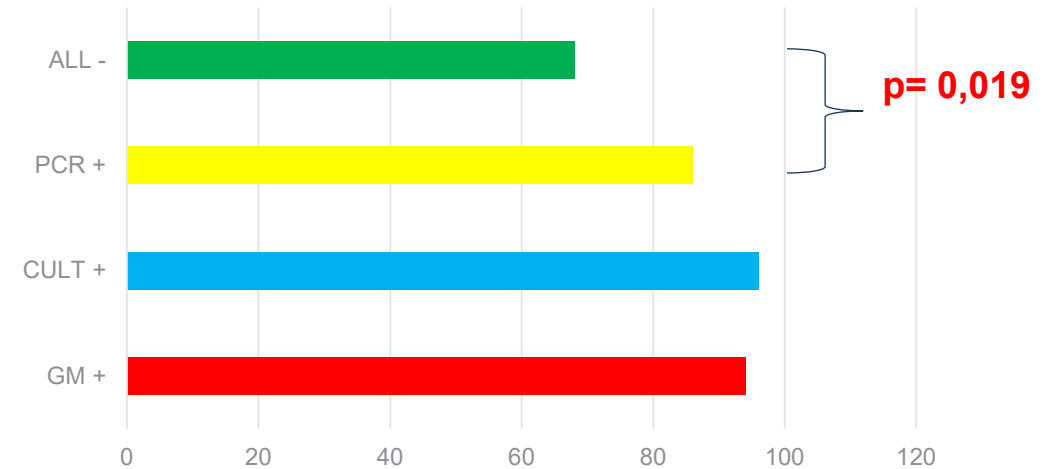
Impact of isolated positive Aspergillus PCR test



6-week mortality



Antifungals started



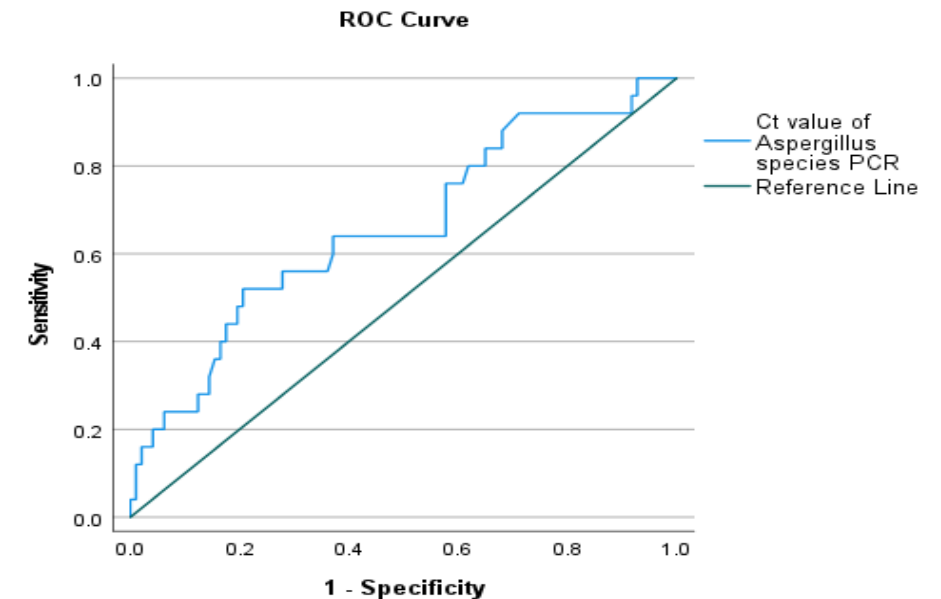
# Results

## SECONDARY ENDPOINTS

Relevance of Aspergillus PCR Ct value: mortality

Patients with isolated positive PCR		
	6-week mortality	
	Yes	No
Ct value $\leq 33.11$	2	4
Ct value $> 33.11$	7	48

	Isolated PCR	GM + PCR positive	Culture + PCR positive
Species PCR Ct value – Median (IQR)	36.4 (35.1 – 37.5)	33.8 (31.8 – 36.1)	33.4 (32.6 – 36.4)
Fumigatus PCR Ct value – Median (IQR)	35.6 (34.1 – 36.3)	33.5 (31.6 – 34.6)	33.7 (32.6 - ...)



**ROC curve: 6-wk mortality ~ A. species Ct value**

**AUC (95%CI): 65,9% (53.4 - 78.3%)**

**Max. Youden index at 31.4: Ct value 33.11**

**(SE 52% SP 80%)**

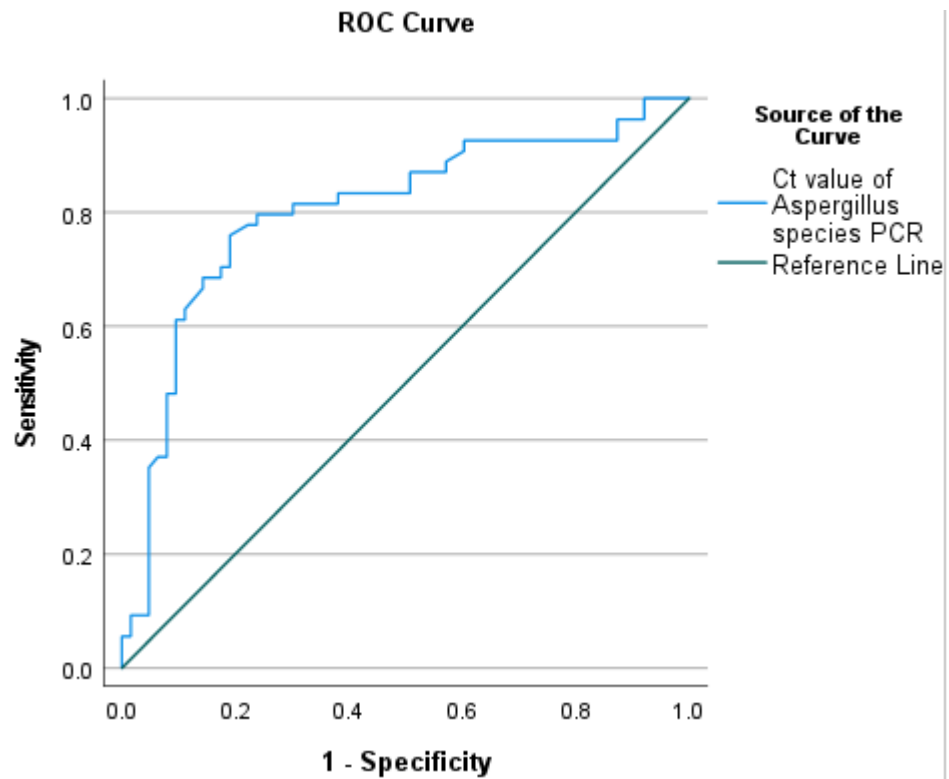
# Results

## SECONDARY ENDPOINTS

Relevance of Aspergillus PCR Ct value

CASES: probable/proven IA (PCR excluded from definition)

CONTROLS: no/possible IA



ROC curve: probable IPA ~ A. species Ct value

AUC (95%CI): 80.3% (71.8 - 88.7%)

Youden index: 56.9 (SE 75.9% SP 81%)

Best discriminative Ct value for diagnosis of IPA:

**35.05**

# Conclusion

1/ Significant impact on treatment failure but:

Major limitations for primary objective

- Smaller number of azole R than anticipated
- 3/6 patients received combination therapy as first-line therapy

2/ It's all about perspective

- Azole resistance in 15% of the 'population' → high prevalence
  - 8/95 probable IPAs (8%)
  - 8/295 performed PCRs in patients at risk (2,7%)
  - 8/323 participants (2,4%)
- Impact of azole resistance is limited on a population level

3/ One in five patients had an isolated positive *Aspergillus* PCR

- Mortality was similar to patients without mycological proof of invasive aspergillosis (14% vs 16%, p value = 0,682)
- Usefulness of PCR in EORTC/MSGERC criteria?
- Ct value as a better predictor for classification of IA?

[Link to manuscript](#)





# Time for adjustments?

B. Therapy for isolates with confirmed suscep	
Antifungal agent	
1 <sup>st</sup> choice*	
Voriconazole	
Isavuconazole	
2 <sup>nd</sup> choice*	
Liposomal AmB	
3 <sup>rd</sup> choice*	
Voriconazole+Echinocandin combination:	
Voriconazole	
AND	
Caspofungin OR	
Micafungin OR	
Anidulafungin	

A. Therapy for isolates with unknown suscep	
Antifungal agent	
1 <sup>st</sup> choice*	
Azole+Echinocandin combination:	
Voriconazole OR	
Isavuconazole	
AND	
Caspofungin OR	
Micafungin OR	
Anidulafungin	
OR	
Azole + L-AmB combination:	
Voriconazole	
OR	
Isavuconazole	
AND	
L-AmB	
2 <sup>nd</sup> choice*	
Liposomal AmB	
3 <sup>rd</sup> choice*	
Caspofungin	
Micafungin	
Anidulafungin	

C. Therapy for isolates with confirmed resiste	
Antifungal agent	
1 <sup>st</sup> choice*	
Liposomal AmB	
2 <sup>nd</sup> choice*	
Caspofungin	
Micafungin	
Anidulafungin	

\* For specific recommendations, exceptions, and con  
 \* The dosages in this table are specific for invasive as  
 \* Individual dose based on therapeutic drug monitori

→ Azole-S IA: superior survival with voriconazole over conv AmB: Herbrecht et al. NEJM 2002  
 12wk survival: 71% vs 58%

→ Isavuco = vorico = posaco

→ Combination therapy?<sup>3</sup>

→ Cohort studies: improved survival with voriconazole compared with L-AmB or echinocandin monotherapy<sup>1</sup>

→ Modeling: best outcome for unknown susceptibility: azole (for S) and echino/L-AmB (for R)<sup>2</sup>

1: Upton A, Kirby KA, Carpenter P, Boeckh M, Marr KA. Invasive aspergillosis following hematopoietic cell transplantation: outcomes and prognostic factors associated with mortality. Clin Infect Dis. 2007 Feb 15;44(4):531–40; Perkhof S, Lass-Flörl C, Hell M, Russ G, Krause R, Hönl M, et al. The Nationwide Austrian Aspergillus Registry: A prospective data collection on epidemiology, therapy and outcome of invasive mould infections in immunocompromised and/or immunosuppressed patients. Int J Antimicrob Agents. 2010;36(6):531–6.; Herbrecht R, Maertens J, Baila L, Aoun M, Heinz W, Martino R, et al. Caspofungin first-line therapy for invasive aspergillosis in allogeneic hematopoietic stem cell transplant pa;  
 2: Marr KA, Schlamm HT, Herbrecht R, Rottinghaus ST, Bow EJ, Cornely OA, et al. Combination Antifungal Therapy for Invasive Aspergillosis. Ann Intern Med. 2015 Jan 20;162(2):81.

# Time for adjustments?

Age, y	Sex	Disease	Stem Cell Transplant	Bronchoalveolar Fluid Galactomannan	Culture	Resistance Testing on Culture	AsperGenius Resistance Testing	Initial Therapy	Subsequent Therapy	Time of Antifungal Switch, d	Therapy Failure	6-Week Mortality	12-Week Mortality
66	M	AML	-	1.6	+	Azole resistant	TR <sub>46</sub>	Azole	L-AmB	1	No	No	No
53	F	Non-Hodgkin lymphoma	+	0.3	+	Azole resistant	TR <sub>34</sub>	Azole + L-AmB	L-AmB	4	No	No	No
54	M	Hodgkin lymphoma	+	4.8	+	Azole resistant	TR <sub>34</sub>	Azole + L-AmB	L-AmB <sup>a</sup>	5	Yes on day 42	No	No
48	F	AML	-	5.6	-	-	TR <sub>34</sub>	Azole + echinocandin	L-AmB	2	No	No	Yes
64	F	AML	+	0.07	-	-	TR <sub>46</sub>	Azole		NA	No	No	No
57 <sup>b</sup>	M	Mantle cell lymphoma	-	3.08	+	Not tested	Mixed pattern: WT and TR <sub>34</sub>	Azole		NA	No	No	No
23	M	T-cell acute lymphocytic leukemia	-	8	+	Azole susceptible <sup>c</sup>	TR <sub>34</sub>	Azole	Azole + L-AmB	15 <sup>d</sup>	No	No	No
79	M	MW	-	5.6	+	Not tested	Mixed pattern: WT and TR <sub>34</sub>	Azole		NA	No	Yes	Yes

To consider:

- Culture is positive in 6/8 patients with a TR34 or TR46 mutation
- Are azole-resistant strains easier to culture?
- ➔ Nethmap results: overestimation of true azole resistance in the Netherlands?
- No patients with resistant colony on culture with negative resistance PCR
- Rapid results using the resistance PCR

# Time for adjustments?

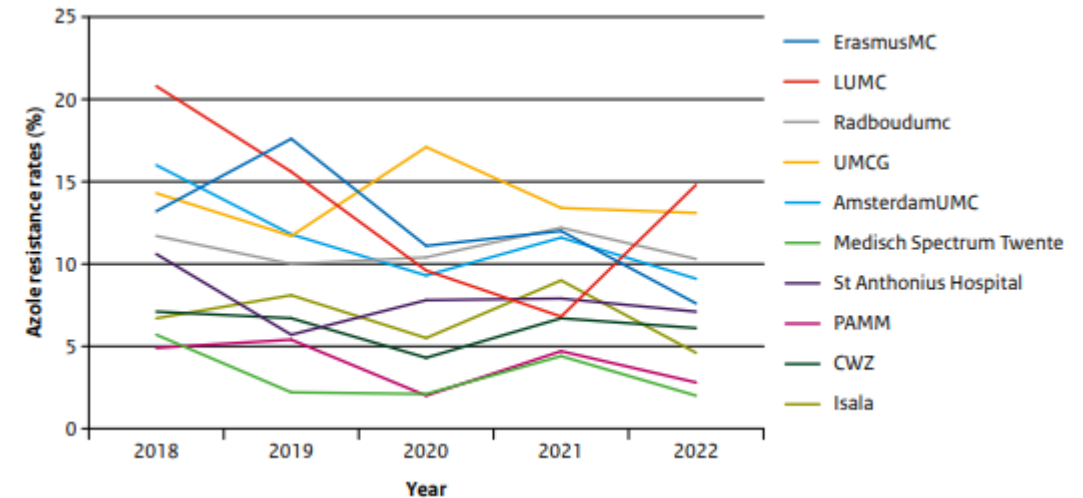
## NETHMAP REPORT 2023:

The azole resistance rates have shown overall a declining trend over the past years, although the frequency in individual centers may vary (Figure 4.8.7.1). In 2022 seven of the 10 surveillance centers had an azole resistance rate below 10%. An expert panel previously recommended that a resistance rate above 10% should prompt reconsideration of azole monotherapy as first line treatment option.<sup>2</sup> In the Netherlands

the 2017 SWAB guideline recommends to start with combination antifungal therapy in order to cover azole resistance empirically, but declining resistance rates may require reconsideration of this recommendation.

Furthermore, PCR-based tests may allow for rapid detection of resistance. A recent prospective multicenter study evaluated the performance of *Aspergillus* PCR and resistance PCR in bronchoalveolar lavage (BAL)-fluid of 323 patients with hematological malignancy.<sup>3</sup> *A. fumigatus* DNA was detected in 89/293 (30%) patients with sufficient DNA in the BAL fluid for PCR testing. The resistance PCR was conclusive in 58/89 (65%) and resistance detected in 8/58 (14%). Although in 35% of patients the resistance PCR was not conclusive, 6 of 8 patients with azole-resistant invasive aspergillosis were culture positive. Improving diagnostic sensitivity of resistance PCR remains an important goal, while resistance detection is also challenged by the emergence of TR-variants. TR-variants are TR<sub>34</sub>/L98H or TR<sub>46</sub>/Y121F/T289A isolates that

contain additional SNPs or variations in the number of TR's. Due to the use of predefined PCR targets, such variations might not be detected with current resistance PCR-tests, while TR-variations may alter the azole phenotype. In 2022, variations were frequent in isolates harboring TR<sub>46</sub>, where 75% of mutations included variations in the TR<sub>46</sub>/Y121F/T289A background.



# Time for adjustments?

Treatment information in Azorman trial:

PATIENTS WITH PROBABLE INVASIVE ASPERGILLOSIS:

- 79/98 (81%) patients started on azole monotherapy → 55/79 (70%) remained on azole monotherapy  
10 switched to L-AmB  
11 switched to azole/L-AmB  
3 switched to azole/echinocandin
- 6/98 (6%) patients started on L-AmB or echinocandin (50/50)
- 4/98 (4%) patients started on azole/L-AmB
- 9/98 (9%) patients started on azole/echinocandin

# Time for adjustments?

Treatment information in Azorman trial:

PATIENTS WITH PROBABLE INVASIVE ASPERGILLOSIS:

- 79/98 (81%) patients started on azole monotherapy → 55/79 (70%) remained on azole monotherapy → 6-week mortality 12/55 (21,8%)  
10 switched to L-AmB  
11 switched to azole/L-AmB  
3 switched to azole/echinocandin
- 6/98 (6%) patients started on L-AmB or echinocandin (50/50)
- 4/98 (4%) patients started on azole/L-AmB
- 9/98 (9%) patients started on azole/echinocandin

6-week mortality

6/13 (46%)

1: Upton A, Kirby KA, Carpenter P, Boeckh M, Marr KA. Invasive aspergillosis following hematopoietic cell transplantation: outcomes and prognostic factors associated with mortality. Clin Infect Dis. 2007 Feb 15;44(4):531–40; Perkhof S, Lass-Flörl C, Hell M, Russ G, Krause R, Hönigl M, et al. The Nationwide Austrian Aspergillus Registry: A prospective data collection on epidemiology, therapy and outcome of invasive mould infections in immunocompromised and/or immunosuppressed patients. Int J Antimicrob Agents. 2010;36(6):531–6.; Herbrecht R, Maertens J, Baila L, Aoun M, Heinz W, Martino R, et al. Caspofungin first-line therapy for invasive aspergillosis in allogeneic hematopoietic stem cell transplant pa;

2: Marr KA, Schlamm HT, Herbrecht R, Rottinghaus ST, Bow EJ, Cornely OA, et al. Combination Antifungal Therapy for Invasive Aspergillosis. Ann Intern Med. 2015 Jan 20;162(2):81.

# Time for adjustments?

## Probable IA, non ICU

In only 1 in 4, azole monotherapy was changed when “culture negative + resistance PCR failed”

## Outcome when monotherapy is continued?

In 28 cases of probable IA with unknown resistance, azole monotherapy was continued (“guideline incompliance”)

⇒ 7 switched from voriconazole to isavu or posaconazole for intolerance (all after day 14)

⇒ 4 switched to second line therapy for non-response/failure

⇒ Day 42 overall mortality 6/28 (21.4%)

# Final suggestions

- With changing epidemiology of azole resistance, it is time to review the SWAB guidelines:
  - It remains important to perform thorough susceptibility testing (phenotypical if possible, PCR)
  - Azole monotherapy may be appropriate therapy, even if susceptibility testing is unsuccessful
    - Unnecessary addition of L-Amb in the majority of patients ~ real world clinical practice
    - Acceptable outcome in patients with guideline incompliance



- Azole monotherapy may be appropriate therapy, even if susceptibility testing is unsuccessful
  - Unnecessary addition of L-Amb in the majority of patients ~ real world clinical practice
  - Acceptable outcome in patients with guideline noncompliance

**Thus: Recommendation 12 and 13 very often not followed in clinical practice**

**Alternative that is considered reasonable by most treating physicians:**

Watchful monitoring under monotherapy under the following conditions

- Not critically ill
- IA limited to the lung / no disseminated disease
- (serum galactomannan <1.0 given the correlation with serum GM positivity and outcome)

What is watchful monitoring under monotherapy?

- Serum galactomannan 2x/week during first 2 weeks as long as patient is hospitalized
- When no decrease in GM from baseline after at least 7d of therapy (or + when initially -) OR When clinical or radiological progression or serum galactomannan becomes positive  
=> Progressive disease => New BAL whenever feasible and add second drug preferably L-AmB



# Final suggestions

- With changing epidemiology of azole resistance, it is time to review the SWAB guidelines:
  - It remains important to perform thorough susceptibility testing (phenotypical if possible, PCR)
  - Azole monotherapy may be appropriate therapy, even if susceptibility testing is unsuccessful
    - Unnecessary addition of L-Amb in the majority of patients ~ real world clinical practice
    - Acceptable outcome in patients with guideline incompliance
  - Should PCR resistance screening be embedded in the NETHMAP reports?
- (In case of high prevalence of azole resistance  
Alternative strategy: Liposomal AmB instead of combination therapy if resistance is unknown?)



# Questions?

