

Azole resistance in *Aspergillus fumigatus*: a forgotten global threat

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November 2019

Disclosures

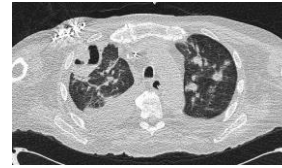


Research grants – advisory boards – speaker

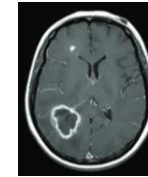
Survival



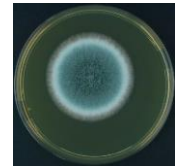
ALL



Invasive
pulmonary
aspergillosis



CNS
aspergillosis



Azole
Resistant

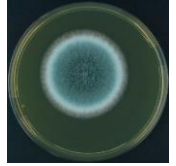
94%

70%

50%

0%

Why?



**Azole
Resistant**

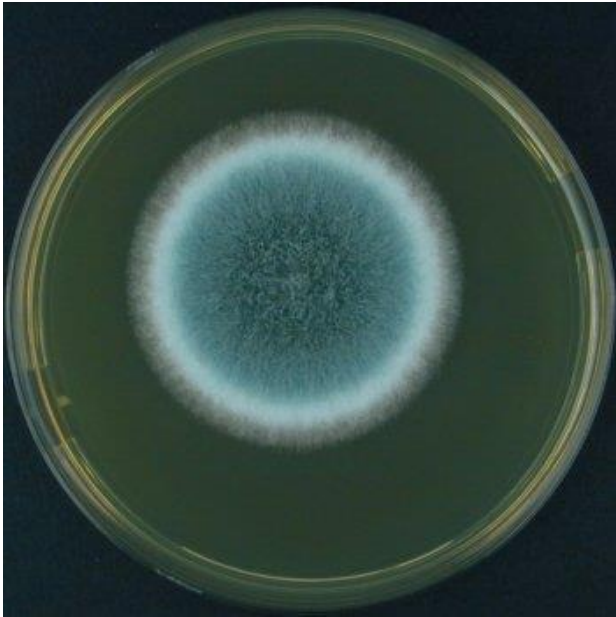
**Lack of sensitive diagnostic tools for
early resistance detection**

**Lack of alternative drugs with similar
efficacy**

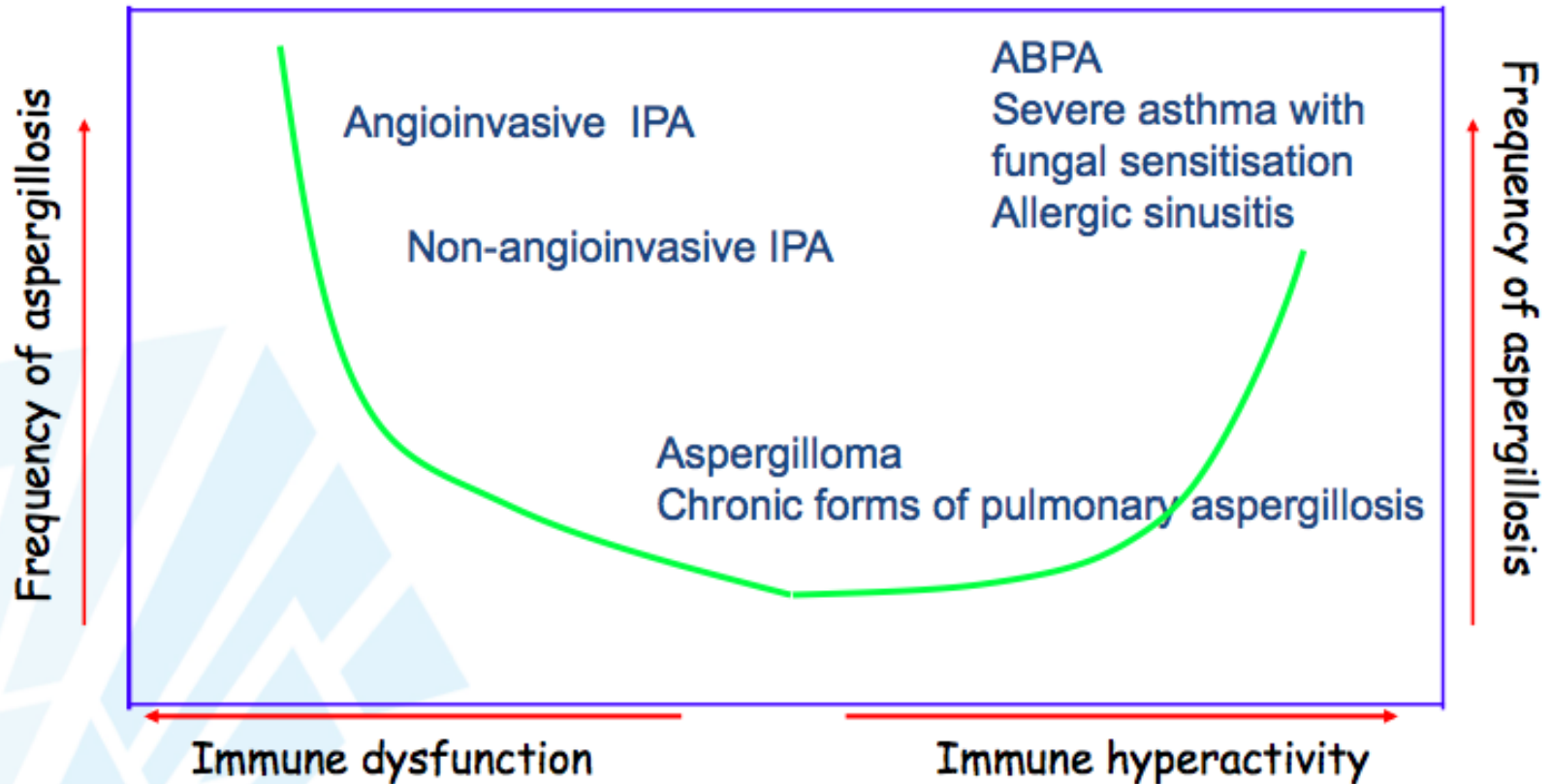
a forgotten global threat?

0%

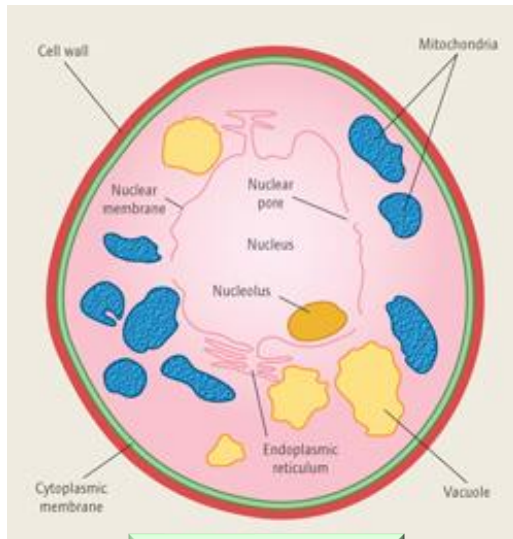
Aspergillus and its habitat



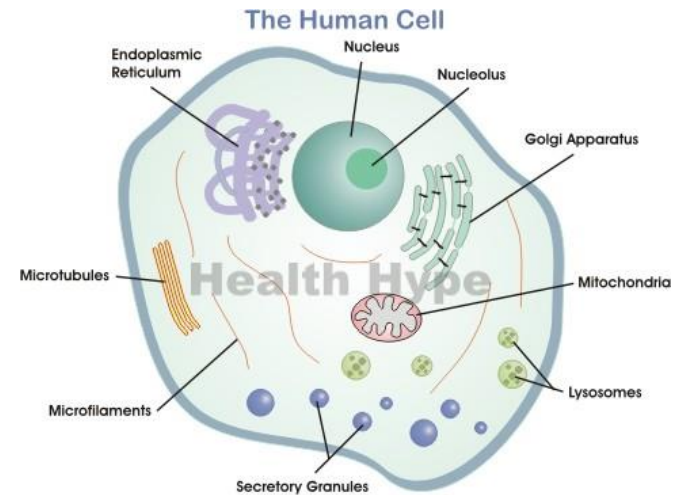
The spectrum of *Aspergillus* diseases



Fungi are eukaryotes just like humans.....



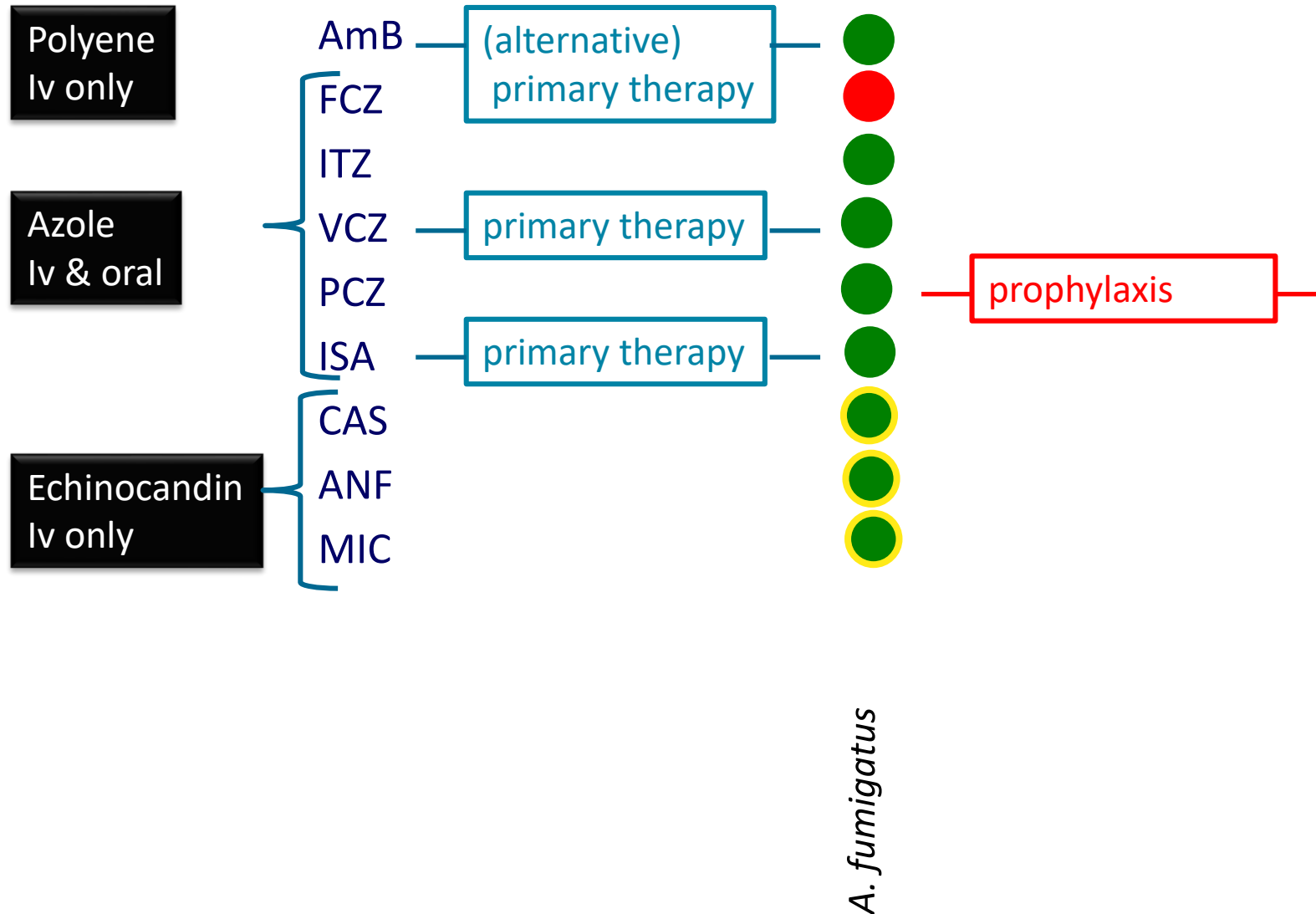
Fungal cell



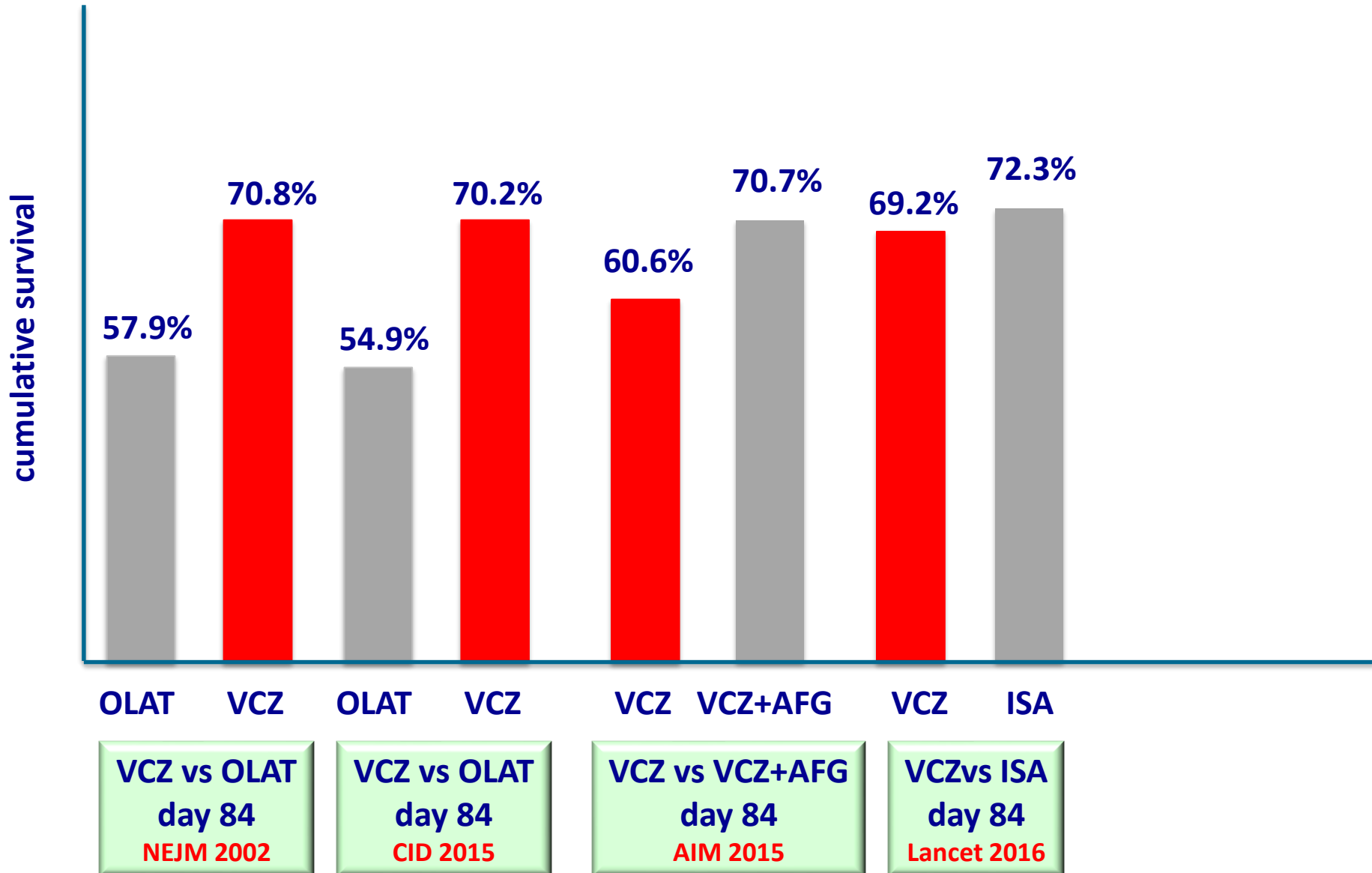
Copyright www.healthhype.com

Human cell

Azoles are the main drug class for management of IA



12-week survival of patients with IA treated with VCZ



Threat?

Mortality of voriconazole R IA > voriconazole S IA?

Radboudumc – LUMC – ErasmusMC

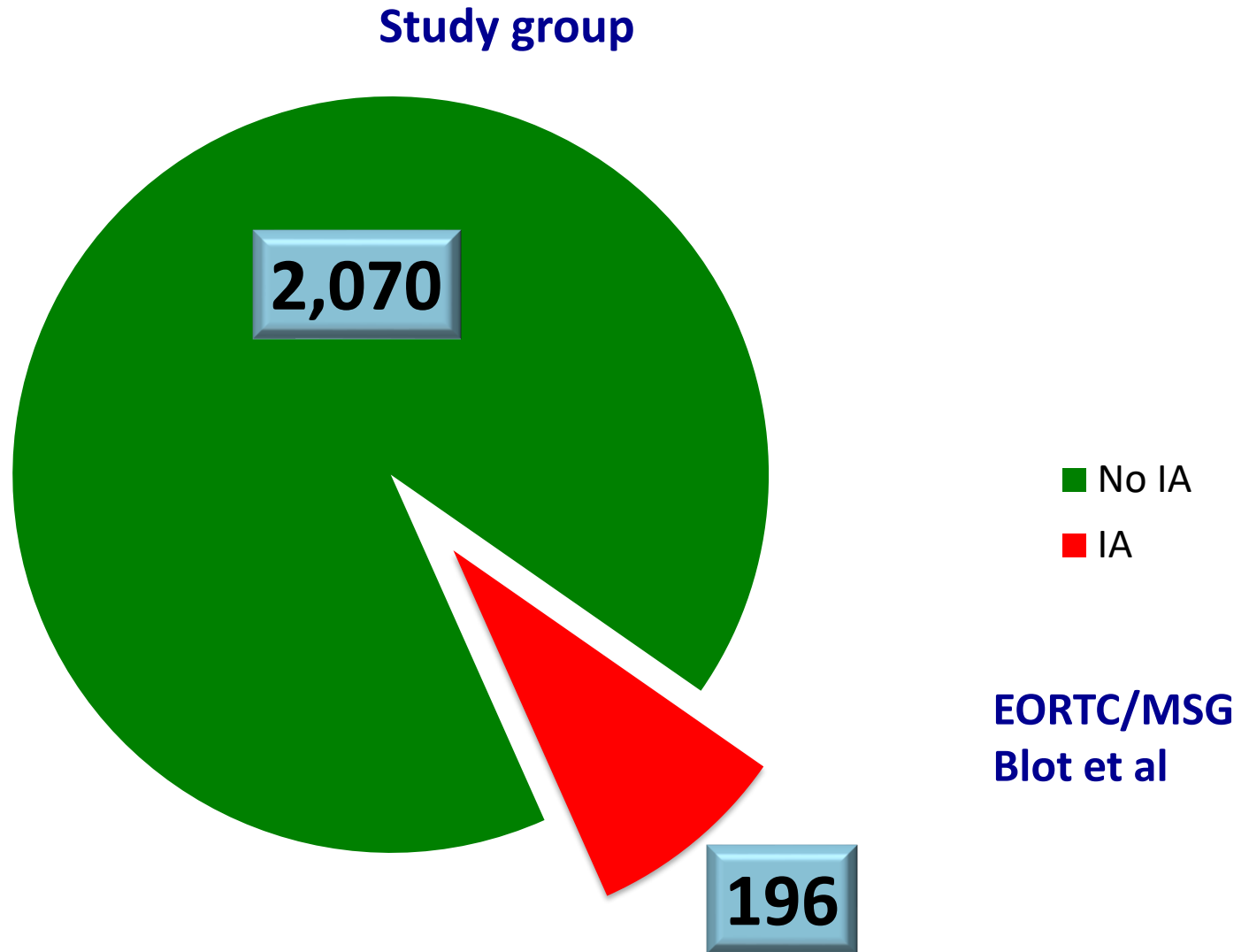
2011 - 2015

All patients with *A. fumigatus* in culture

All isolates screened with VIPcheck™

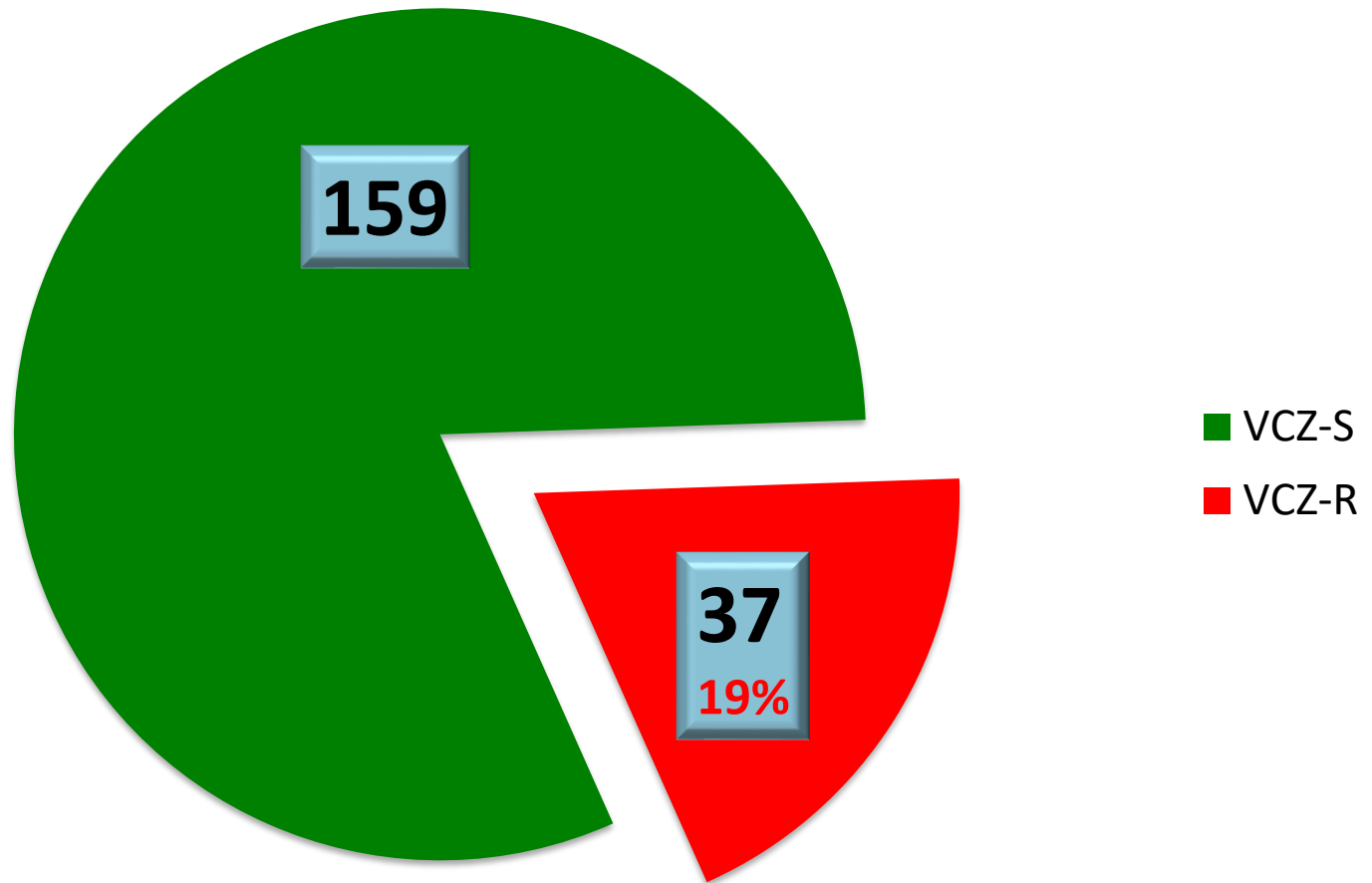
Compare mortality in R versus S

2,266 patients with positive *Aspergillus fumigatus* culture



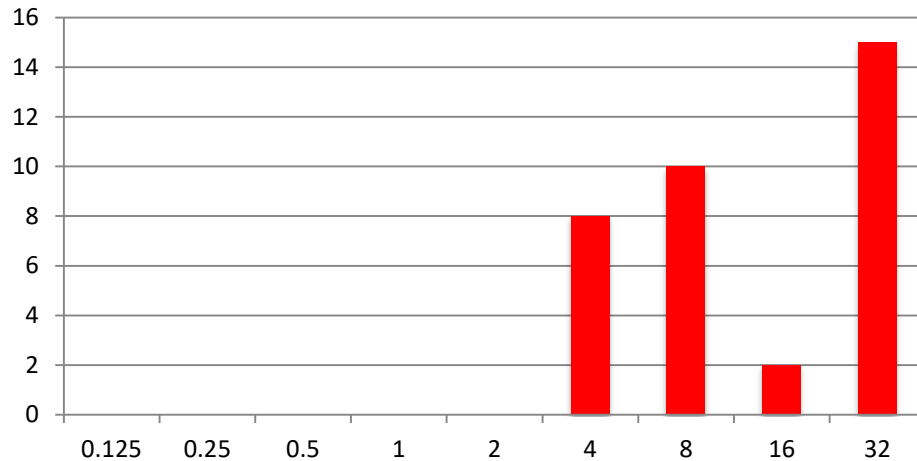
196 patients with invasive aspergillosis

Resistance phenotype



A. fumigatus resistance phenotypes

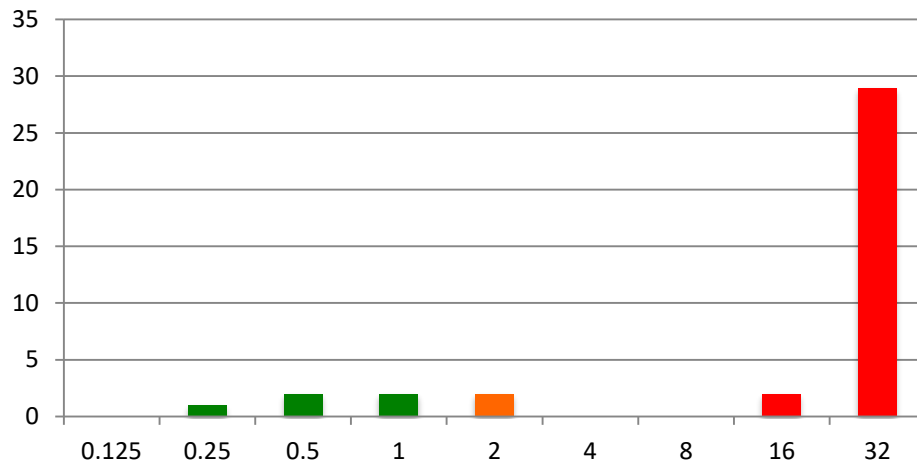
VCZ (>2 mg/l)



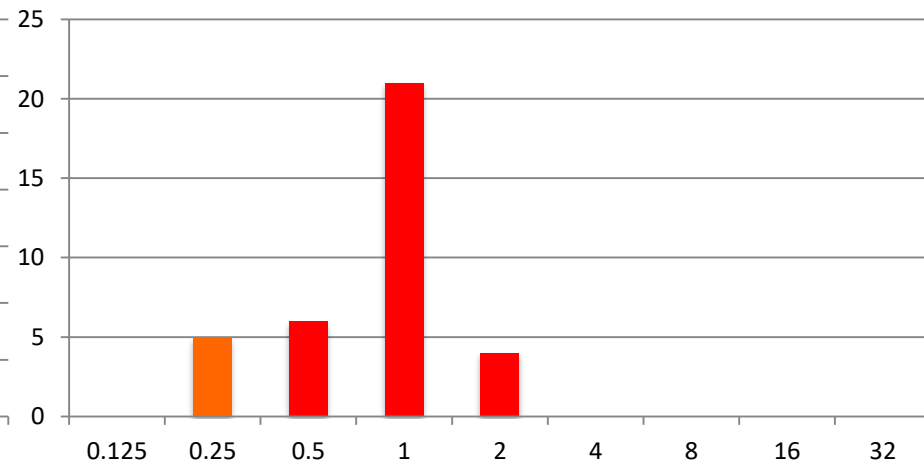
**100% cross-resistance
with isavuconazole**

**All susceptible to
AmB**

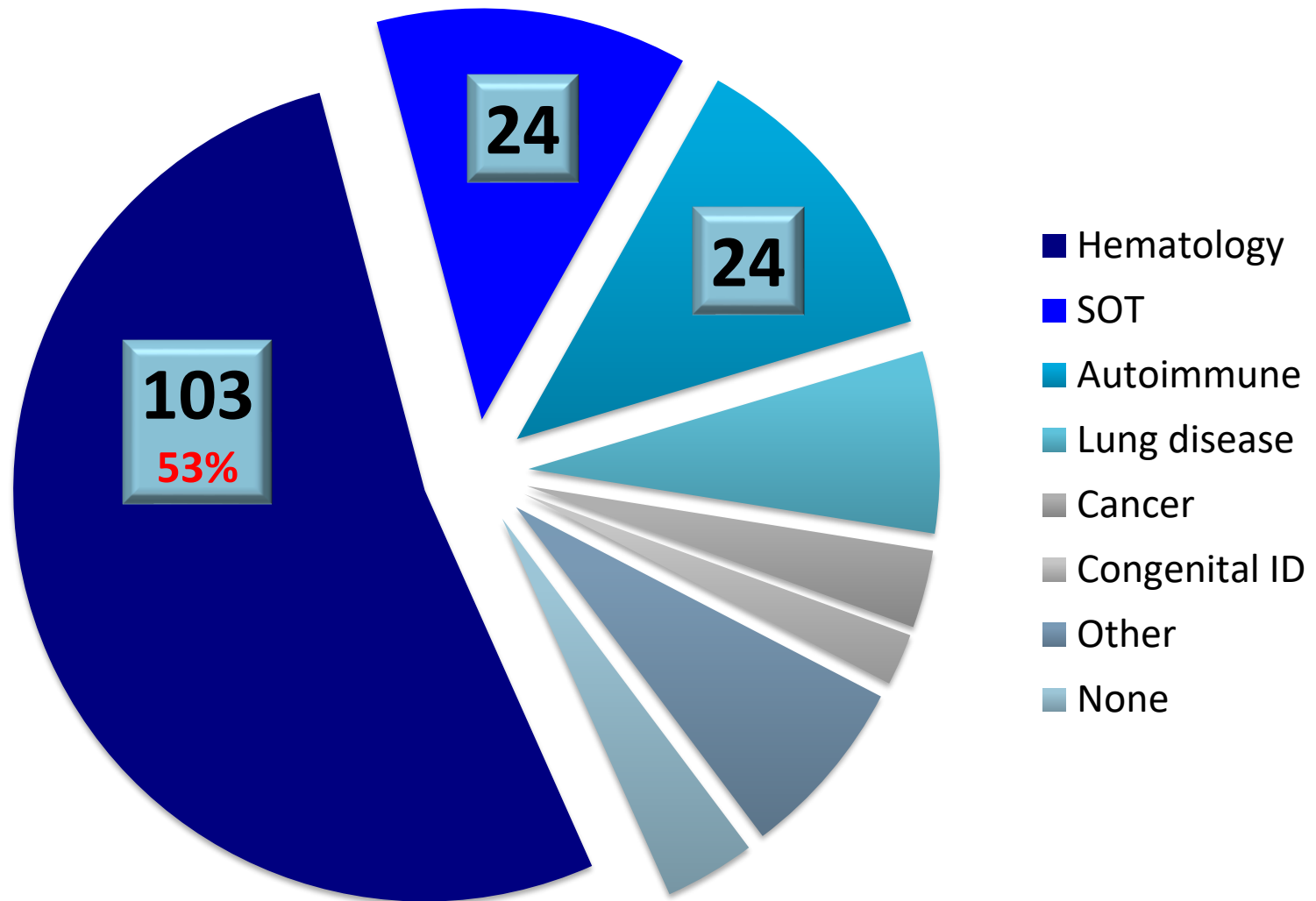
ITZ (> 2 mg/l)



POS (>0.25 mg/l)

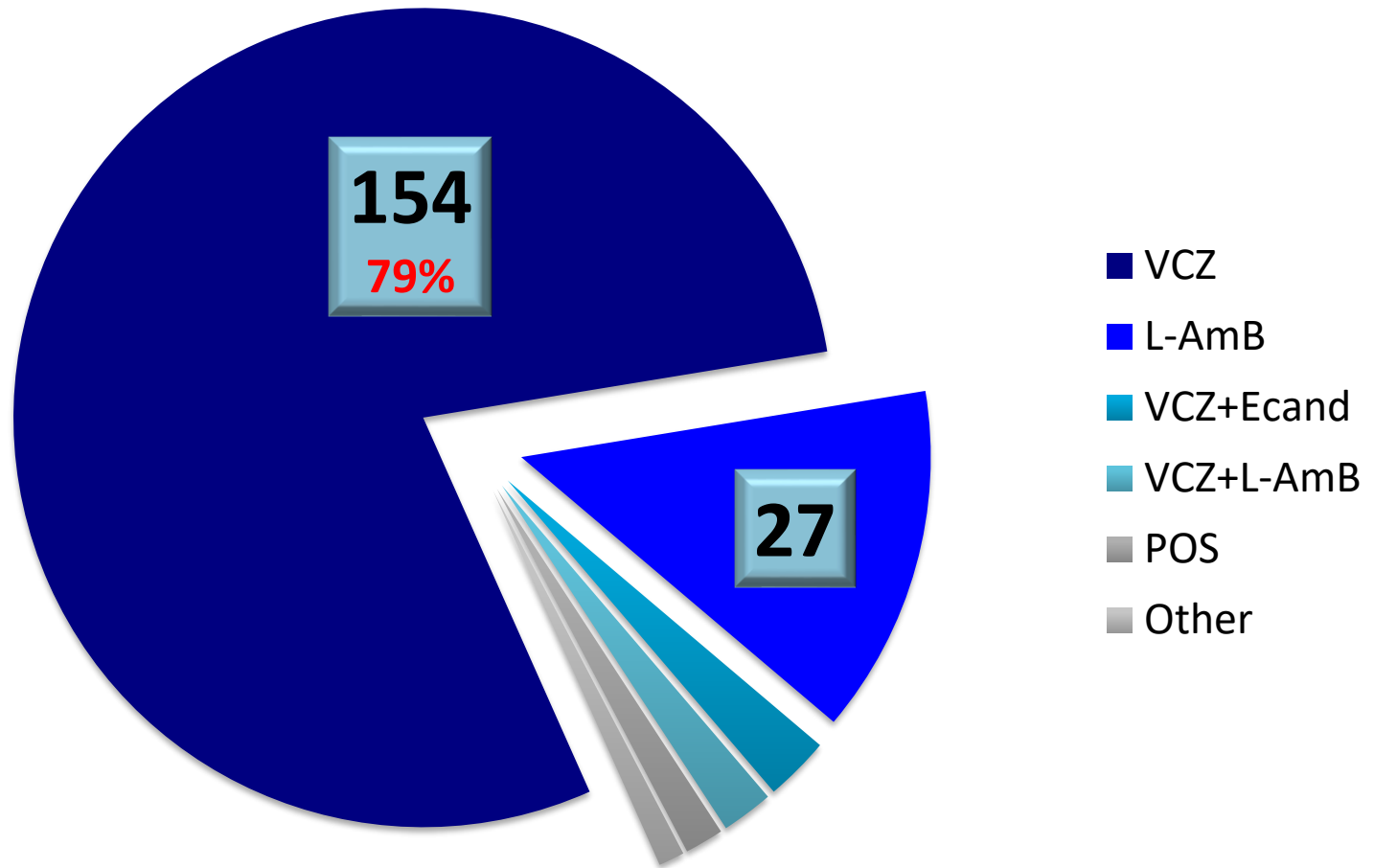


196 patients with invasive aspergillosis: underlying disease

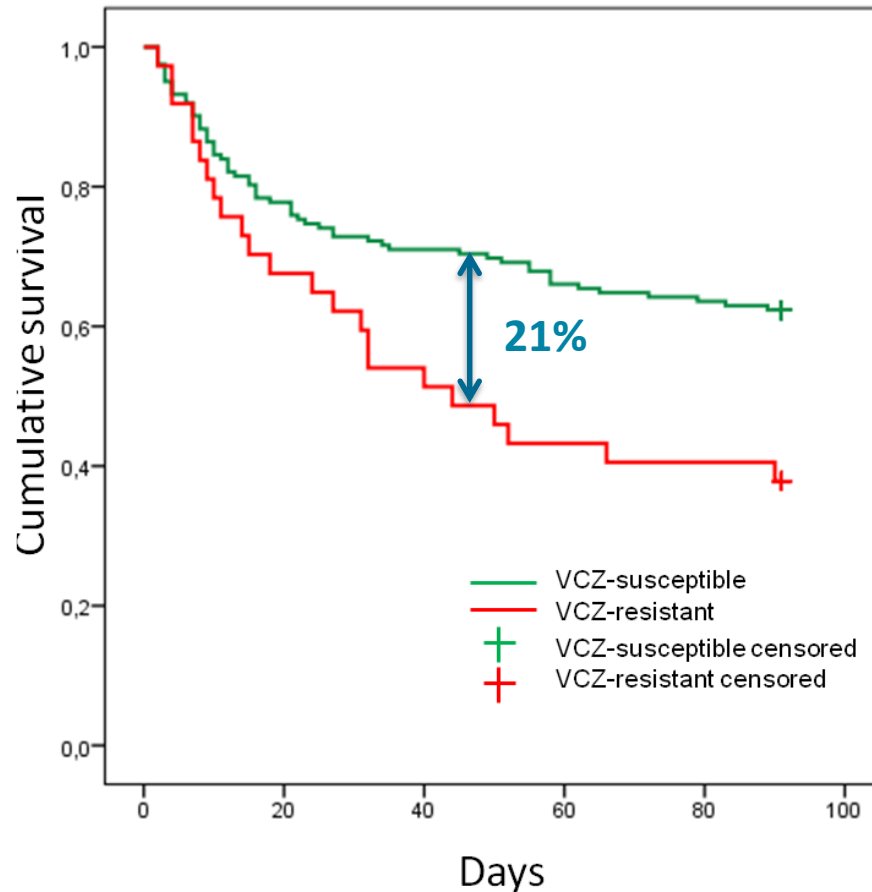


196 patients with invasive aspergillosis

Initial AF therapy



Overall mortality in **vor**i **R** versus **vor**i **S** (hospital wide study)



Mortality

Day 42

VCZ-S 28%

VCZ-R 49%

$p=0.017$

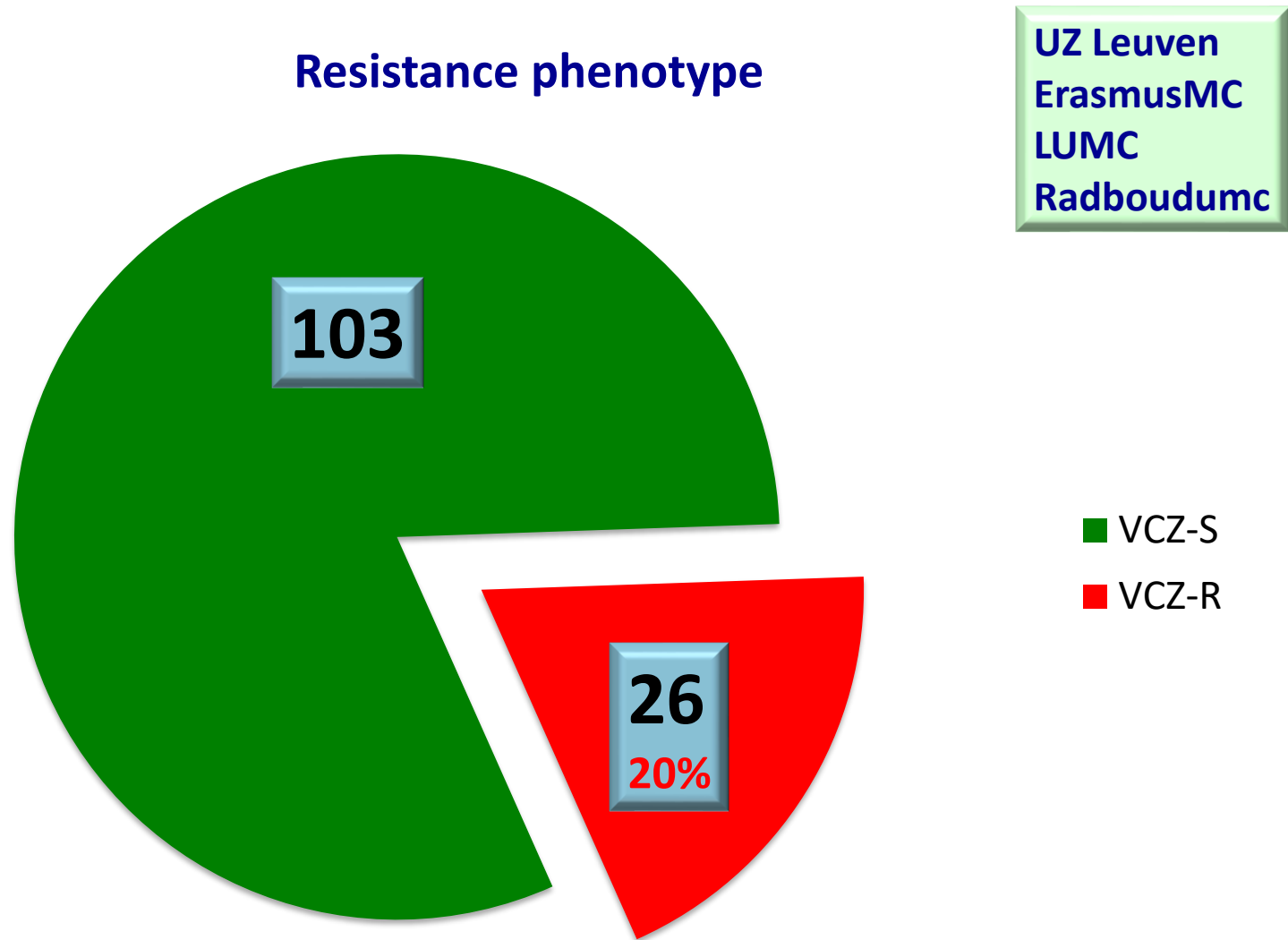
Day 90

VCZ-S 37%

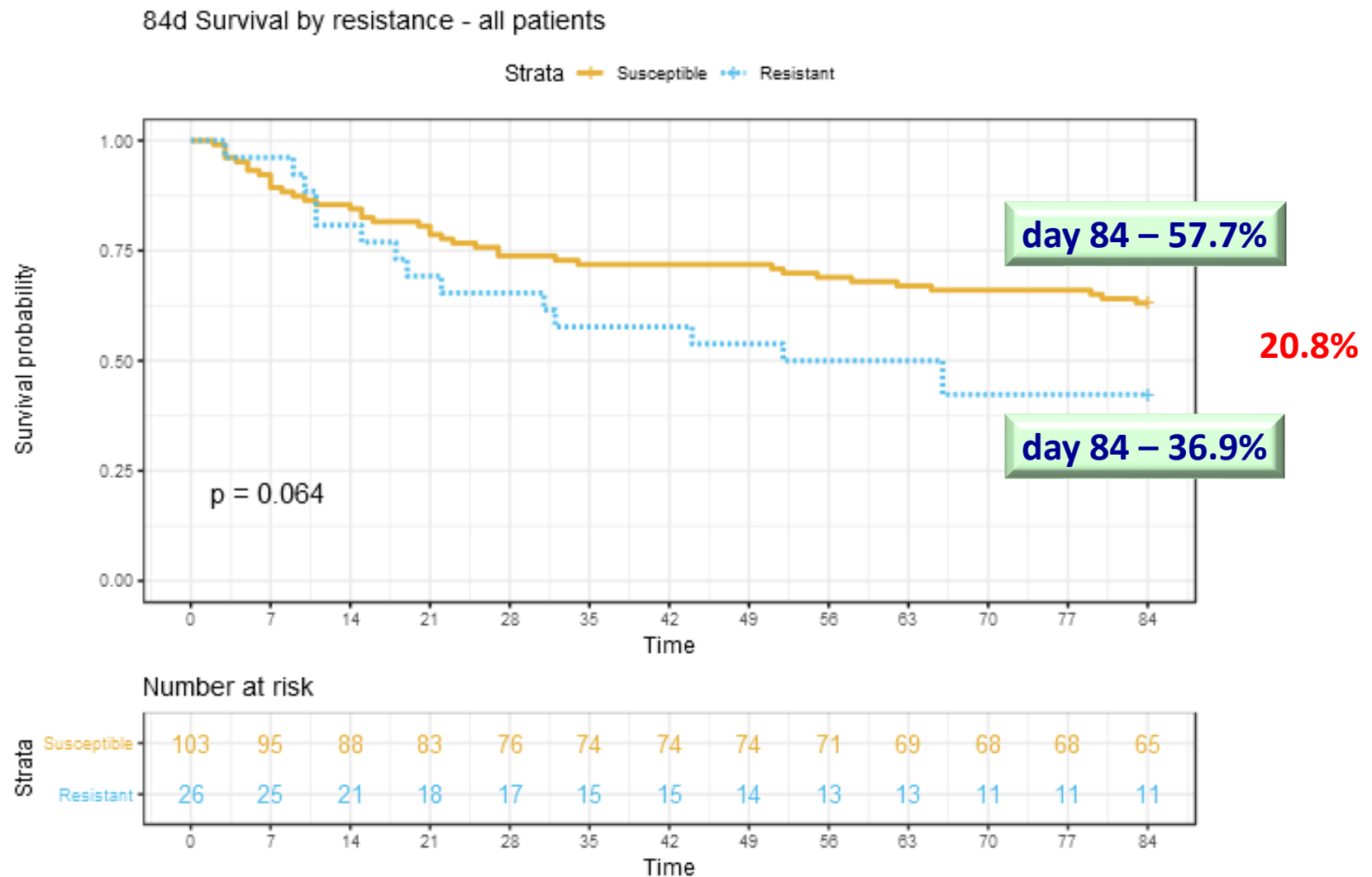
VCZ-R 62%

$p=0.0038$

129 **hematology** patients with invasive aspergillosis

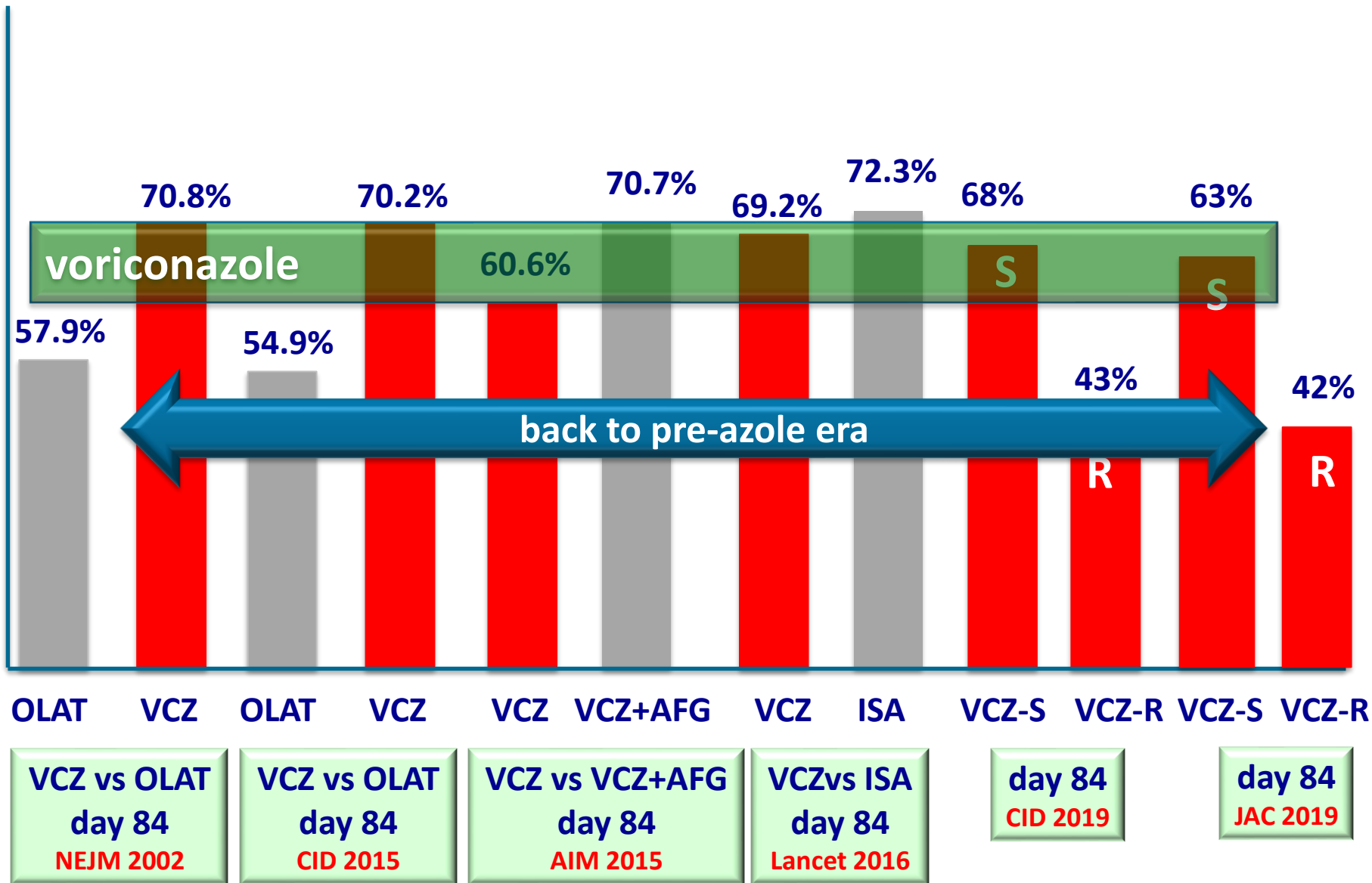


Azole resistance and survival

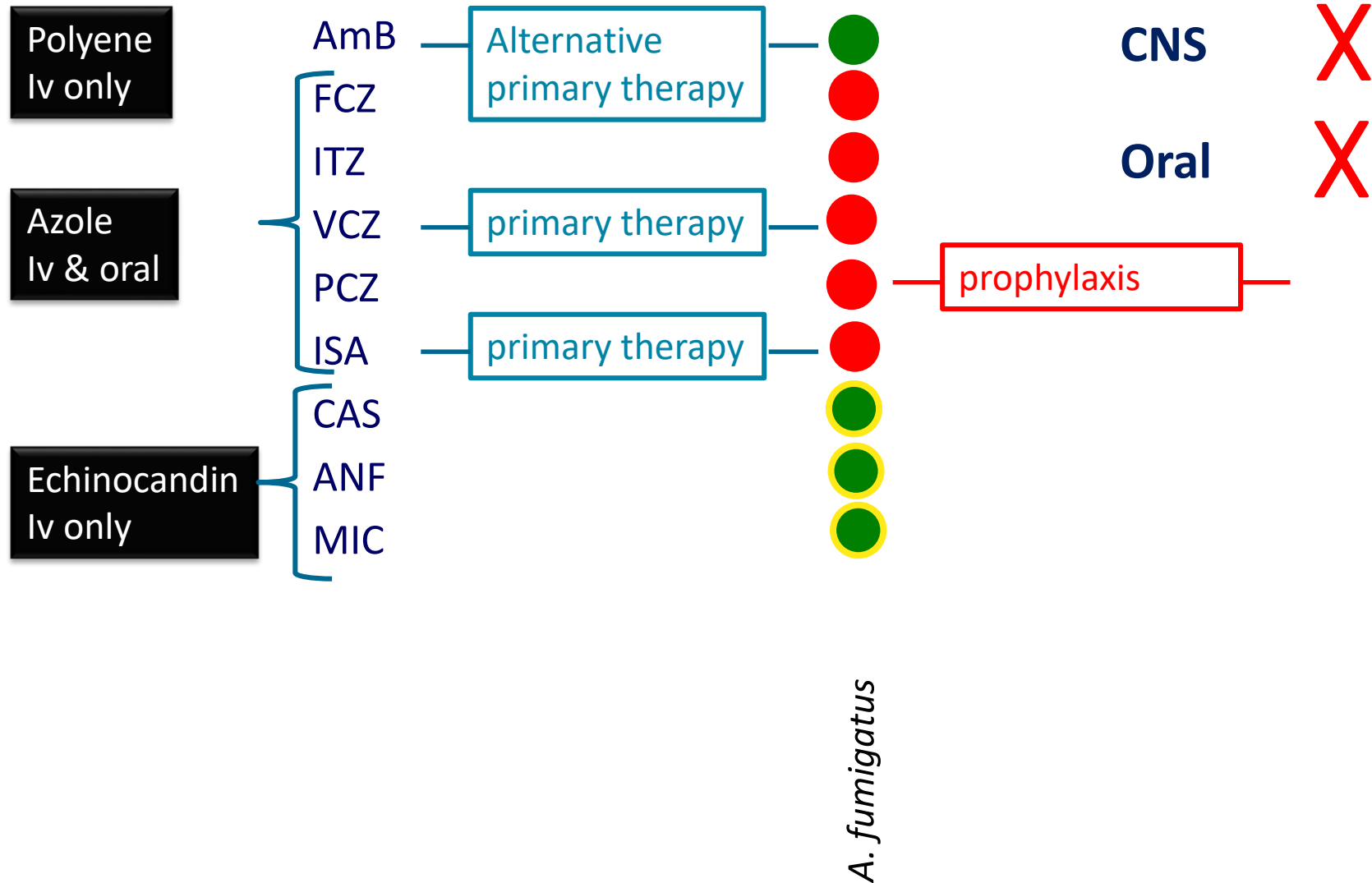


Putting azole-resistant associated 12-week mortality of IA in perspective

cumulative survival



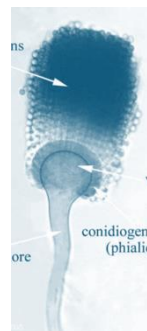
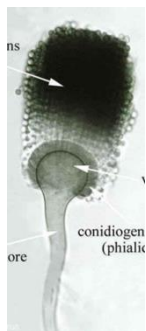
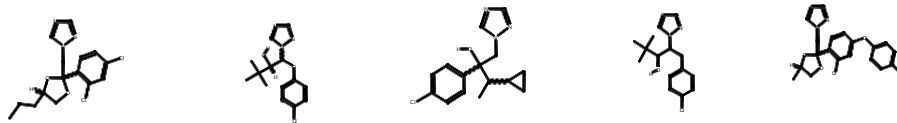
Activity of antifungal agents: azole resistance



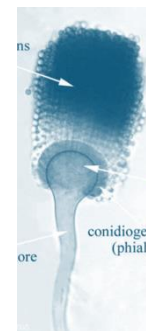
How are patients infected?



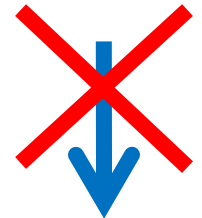
Propiconazole; tebuconazole; epoxiconazole; difenoconazole; bromuconazole



TR₃₄/L98H
TR₅₃
TR₄₆/Y121F/T289A



Medical triazoles

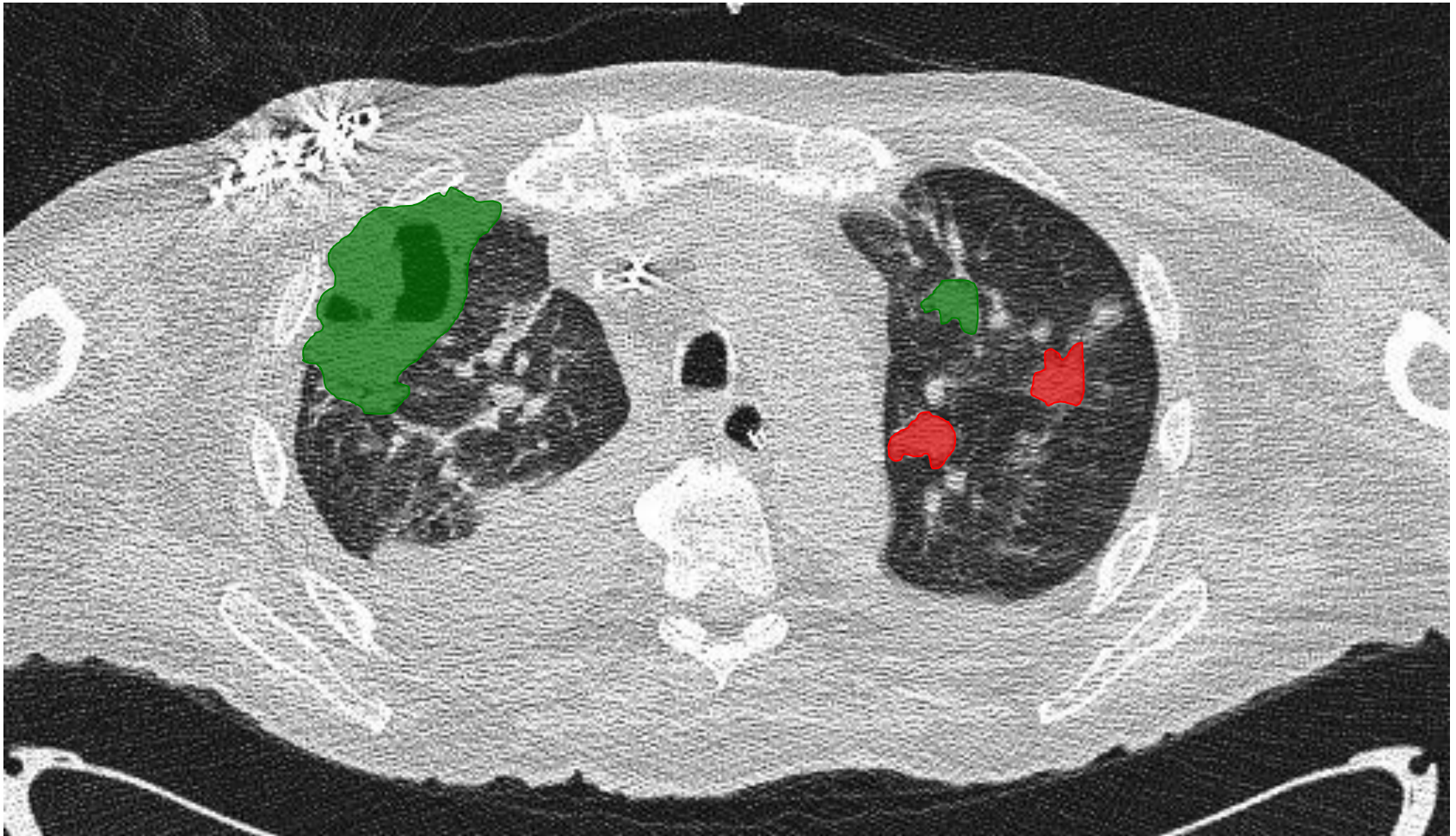


Specific aspects

Disease	Route of resistance	Comments
CF	E > P	
ABPA	?	
Aspergilloma	P >> E	azole therapy, cavity, multiple R-mutations, fitness cost
CPA	P >> E	azole therapy, cavity, multiple R-mutations, fitness cost
IA - pulmonary	E	2/3 no azole therapy, $TR_{34} > TR_{46}$, mixed infections
IAA	E	$TR_{34} > TR_{46}$, mixed infections, tracheobronchitis
CNS-IA	E	TR_{34} & TR_{46} , sanctuary site

P = patient route E = environmental route

Mixed infections

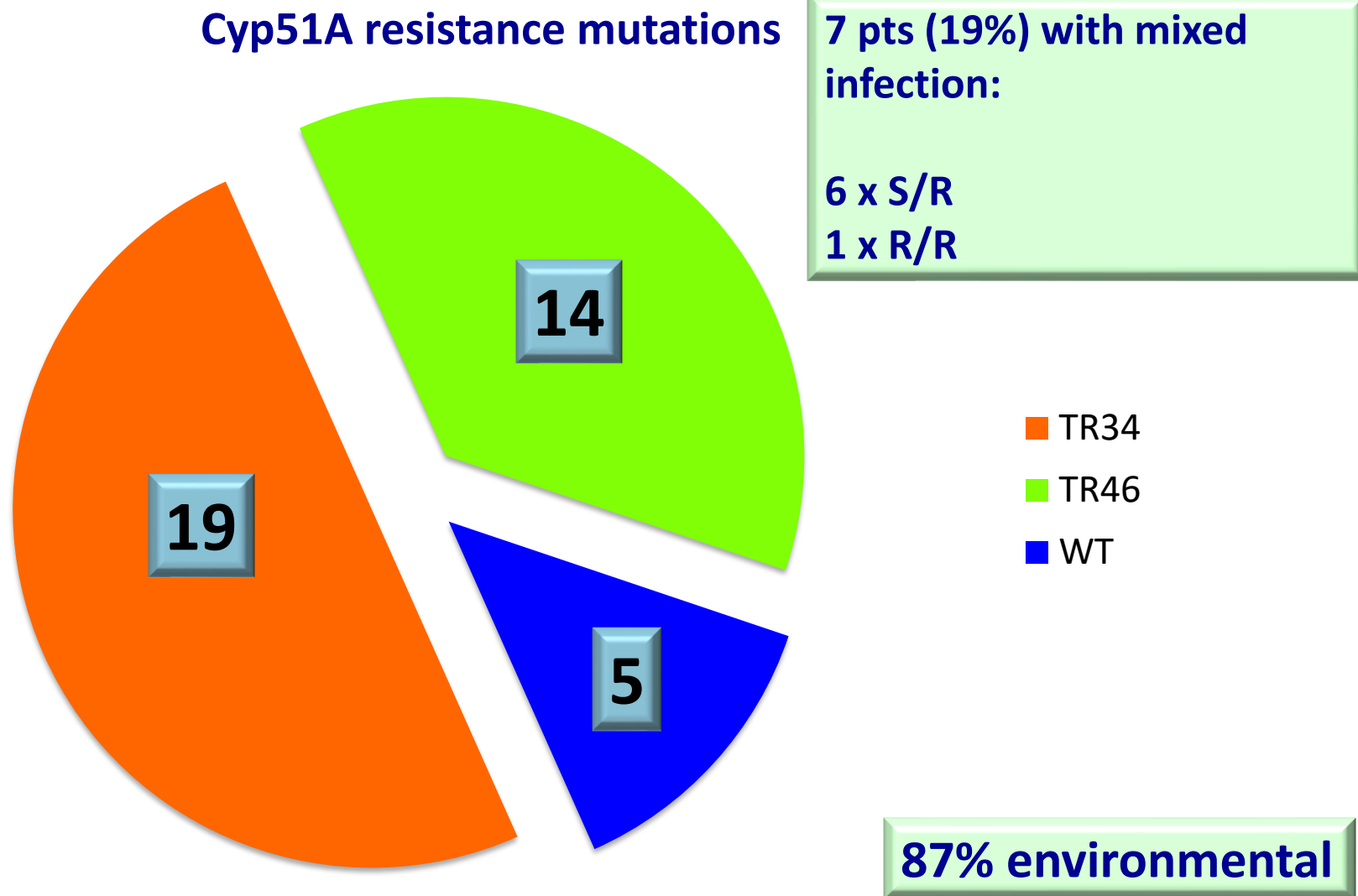


Azole susceptible

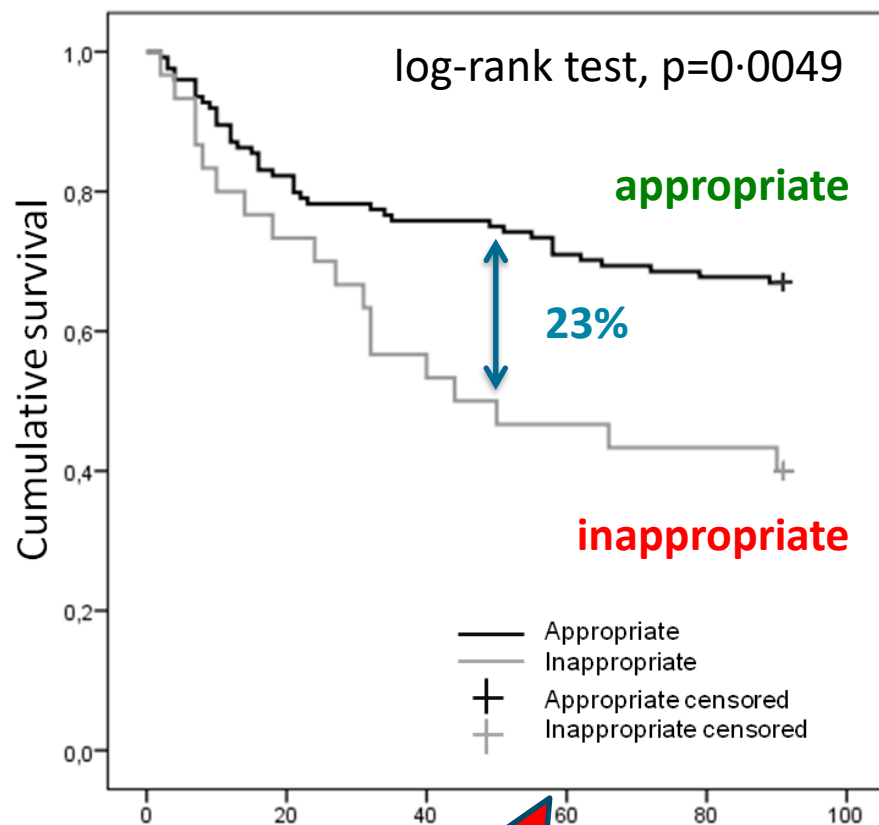


Azole resistant

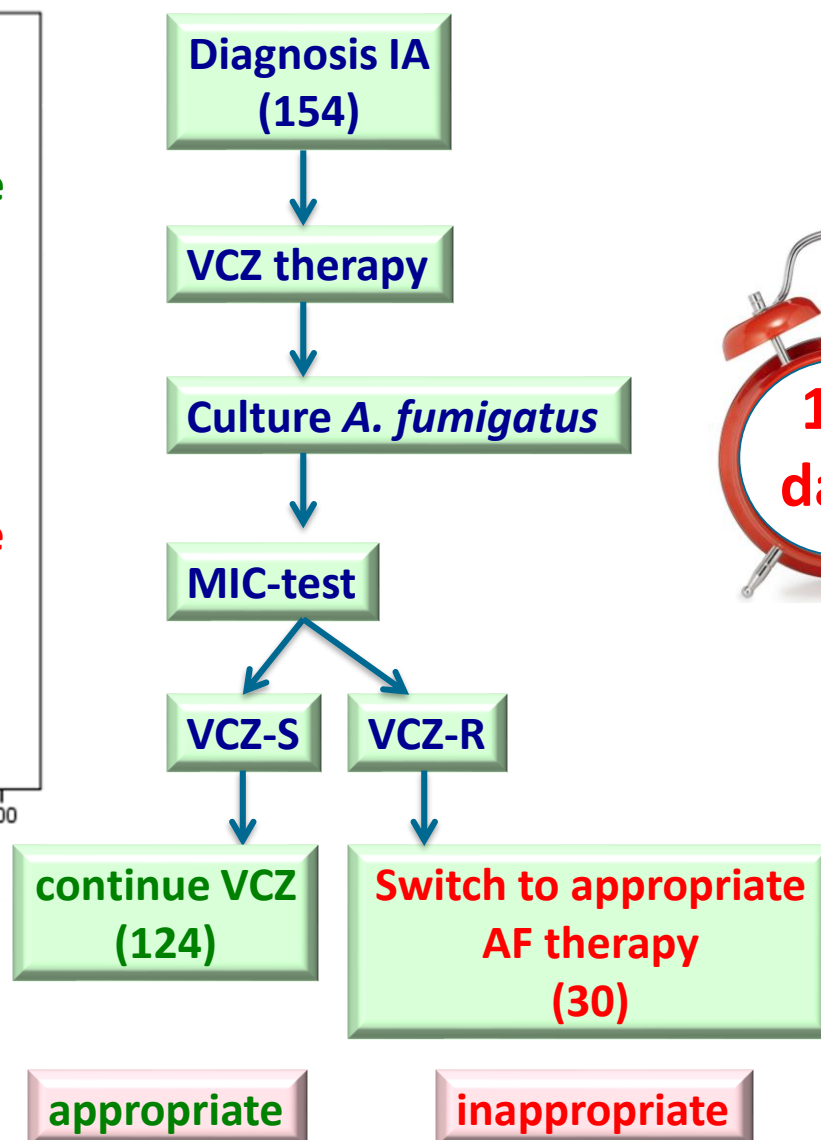
37 patients with VCZ-R invasive aspergillosis



Resistance diagnosis and appropriateness of antifungal therapy



Intensive R-screening of cultures



The promise of commercial resistance PCRs

MycoGenie® (AdemTech, Pessac, France)

- quadriplex real-time PCR assay
- *A. fumigatus* (28SrRNA gene)
- Cyp51A-gene: TR₃₄, L98H
- internal control

AsperGenius® assay (PathoNostics, Maastricht, The Netherlands)

- 2 different real-time quadriplex amplification mixtures
- Aspergillus species multiplex assay targets the 28S rRNA multicopy gene
- *A. fumigatus* complex (Af), *Aspergillus terreus* and *Aspergillus* spp. (Asp sp)
- Cyp51A gene: TR₃₄, L98H, Y121F and T289A
- The distinction between wild-type and mutant *A. fumigatus* strains is performed by melting curve analysis

Resistance PCR and appropriateness of antifungal therapy



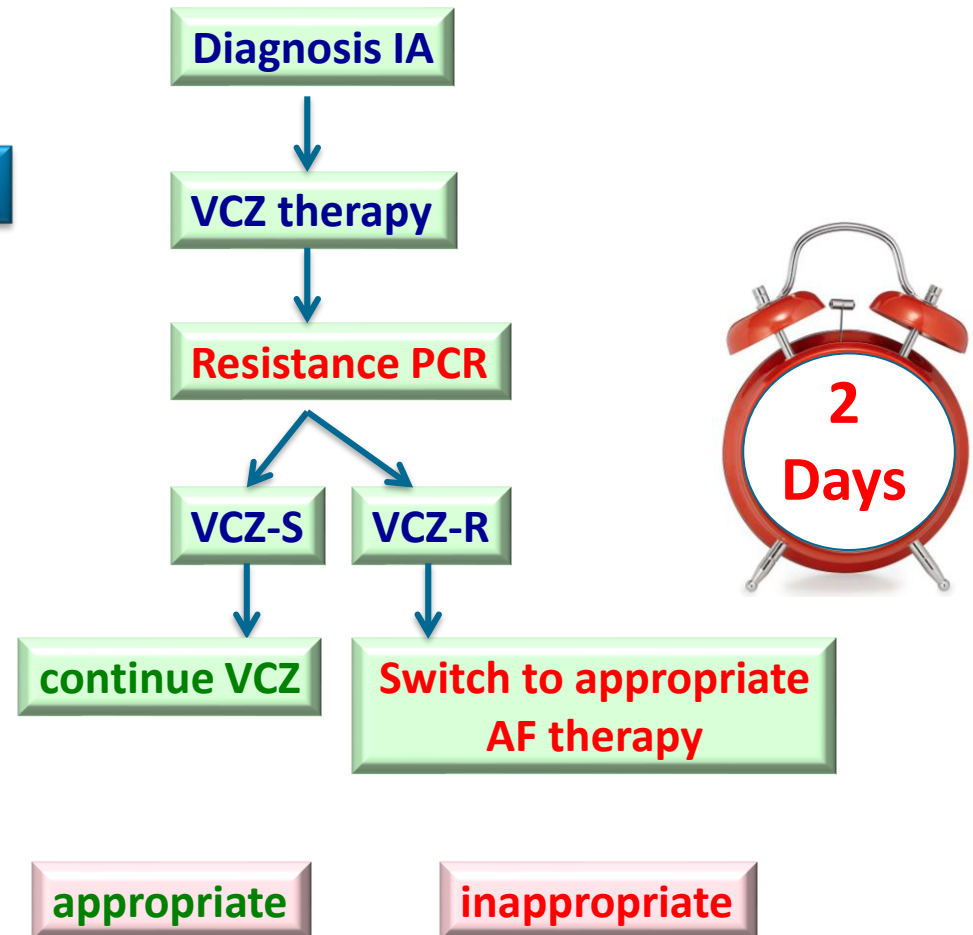
AzorMan study – B. Rijnders



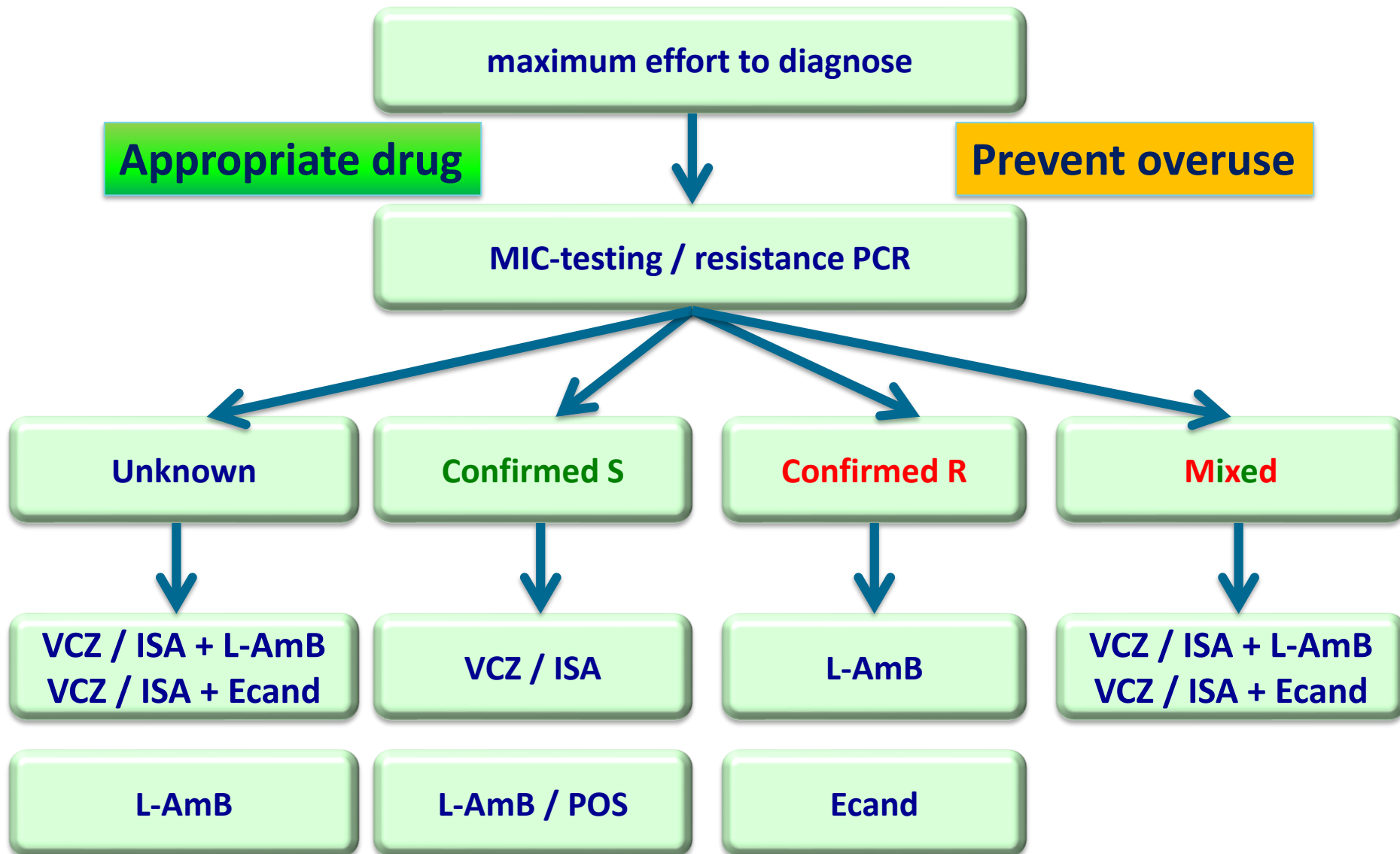
Sensitivity of resistance PCR

Number of mutations detected

Strategy when R-frequency is low



Management strategy.....New SWAB guideline



Multidisciplinary mycology team

ID physician

Pharmacist

Microbiologist

Pulmonologist

Hematologist



Threat?

yes

Global?

Acquired resistance frequency in *A. fumigatus* 2013 - 2017



5 university medical centers

Screening for resistance of unselected clinical isolates using VIPcheck™

Includes clinically not-relevant isolates

Number of patients screened 600 to 814 per annum

Resistance frequency =

$$\frac{\text{number of patients with azoleR isolate}}{\text{number of screened patients}}$$

Anticipated risk: Aspergillus resistance surveillance

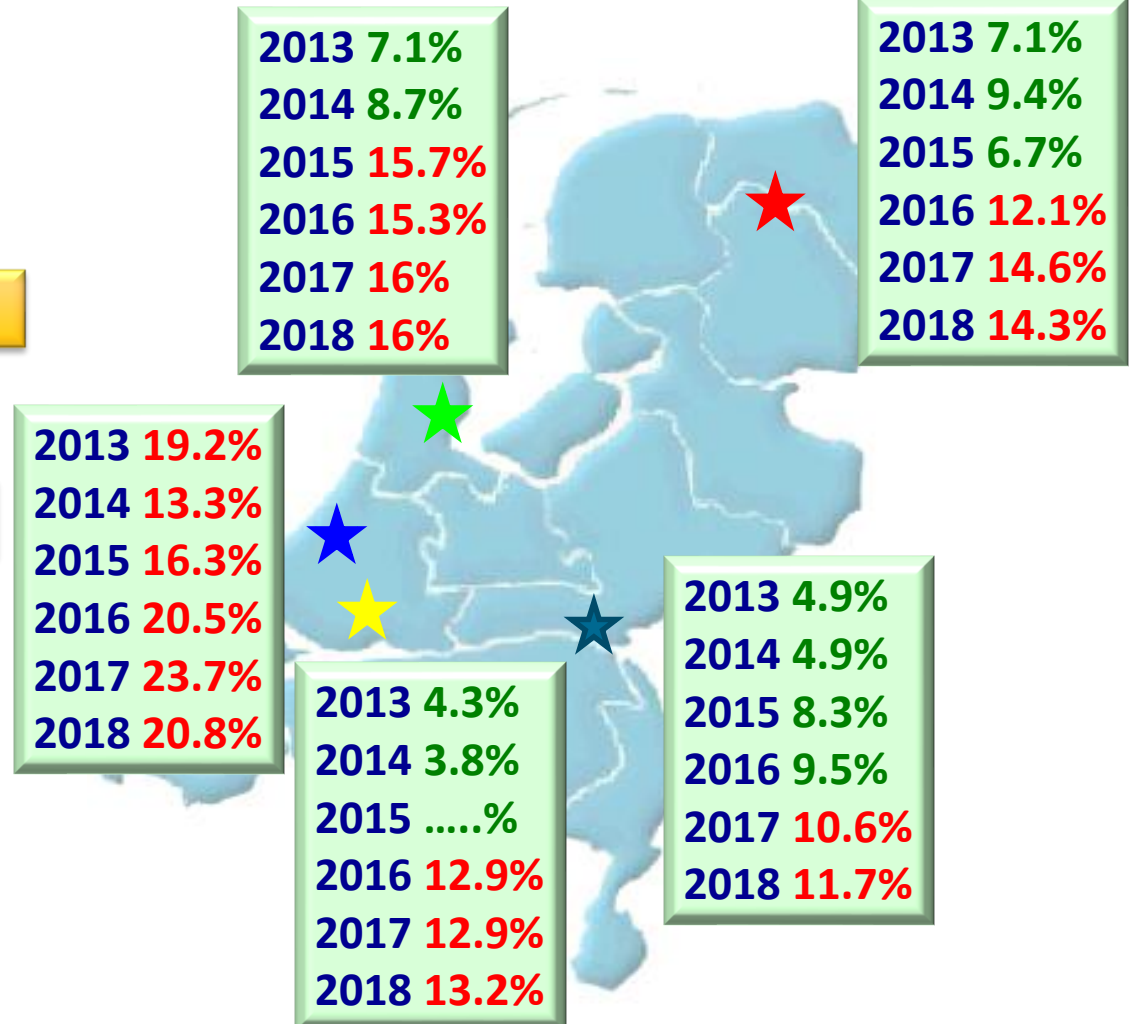


Unselected isolates

Clinical data are not collected

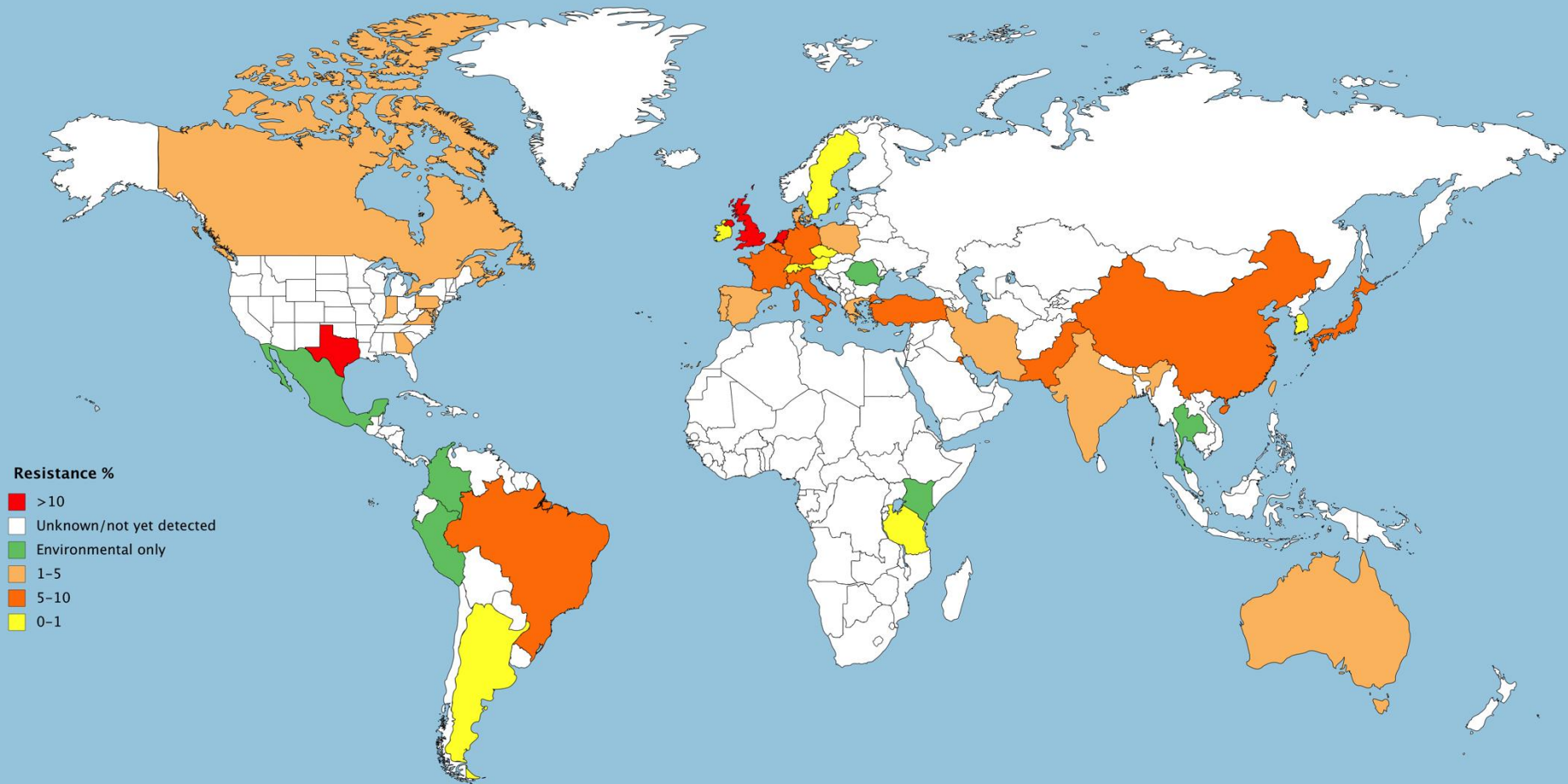
Only culture positives

Variation between hospitals



80% to 90% environmental

Acquired resistance: global?





Does your country
have a resistance
surveillance program
in *Aspergillus*?

**No international
surveillance programs**

**Most clinical
microbiology laboratories
do not perform MIC
testing of molds**

Threat?

yes

Global?

yes

Forgotten?

Do you worry about AMR ?

An antibiotic is an antimicrobial drug used to treat **bacterial** infections in humans and animals.

Like humans, animals (including farm animals) carry **bacteria**. when antibiotics are given to animals, the drugs kill most of the **bacteria**, but resistant **bacteria** can survive and multiply.

WHAT YOU NEED TO KNOW ABOUT AMR (ANTIMICROBIAL RESISTANCE)



food.gov.uk

1 THE FACTS ABOUT AMR

An antibiotic is an antimicrobial drug used to treat bacterial infections in humans and animals. However, bacteria can change and ways to survive the use of an antibiotic. The more we use and misuse antibiotics could increase the resistance of bacteria to them. This is known as antimicrobial resistance (AMR).

AMR is a serious threat to public health. It could mean that antibiotic treatment won't be able to help, the next time you are ill.

The economic cost of AMR, in terms of lost global production, between now and 2050 is estimated at US\$100 trillion.

The O'Neill report estimates that almost 700,000 people worldwide die annually from AMR.

The global impact of AMR could be 10 million deaths annually by 2050.



2 HOW AMR SPREADS

Like humans, animals (including farm animals) carry bacteria. When antibiotics are given to the animals, the drugs kill most of the bacteria, but resistant bacteria can survive and multiply. These resistant bacteria can be passed on, in the following ways.

Faecal contamination, when the animal is slaughtered or when used as manure, could transfer AMR microbes to the environment and meat products.

Vegetables, fruit and shellfish can become contaminated if the water used to grow them, or which they live in, has AMR microbes.

Food may be contaminated by AMR microbes in the environment.

If food is handled on contaminated surfaces or without the right hygiene practices, AMR bacteria can spread from one type of food to another (cross-contamination).



3 HOW YOU CAN AVOID IT

Remember the 4Cs when handling food – clean well, cook thoroughly, chill correctly and avoid cross-contamination.

Handle and cook your meat – such as poultry, pork, minced beef and lamb – so that the meat is steaming hot throughout, there is no pinkness and any juices run clear.

Thoroughly wash and/or peel fruits and vegetables if you are eating them raw.

Make sure you use antibiotics only when necessary, and complete your prescribed course, so they can work effectively.



4 HOW THE FSA IS TACKLING AMR

We are funding research to find out about AMR microbes in the food chain and help us fill in the gaps in our knowledge.

We are working with other government departments and industry as they develop action plans to reduce the levels of AMR microbes in food.

Our Advisory Committee on the Microbiological Safety of Food has established an AMR sub-group to consider issues in the food chain.

We are also working with consumers to raise awareness of AMR and food, and provide practical advice.



For more information, visit food.gov.uk/amr and nhs.uk/nhsengland/arc/pages/aboutarc.aspx

Join the conversation on [Facebook](https://www.facebook.com/foodgovuk) and [Twitter](https://twitter.com/foodgovuk) using #AMR

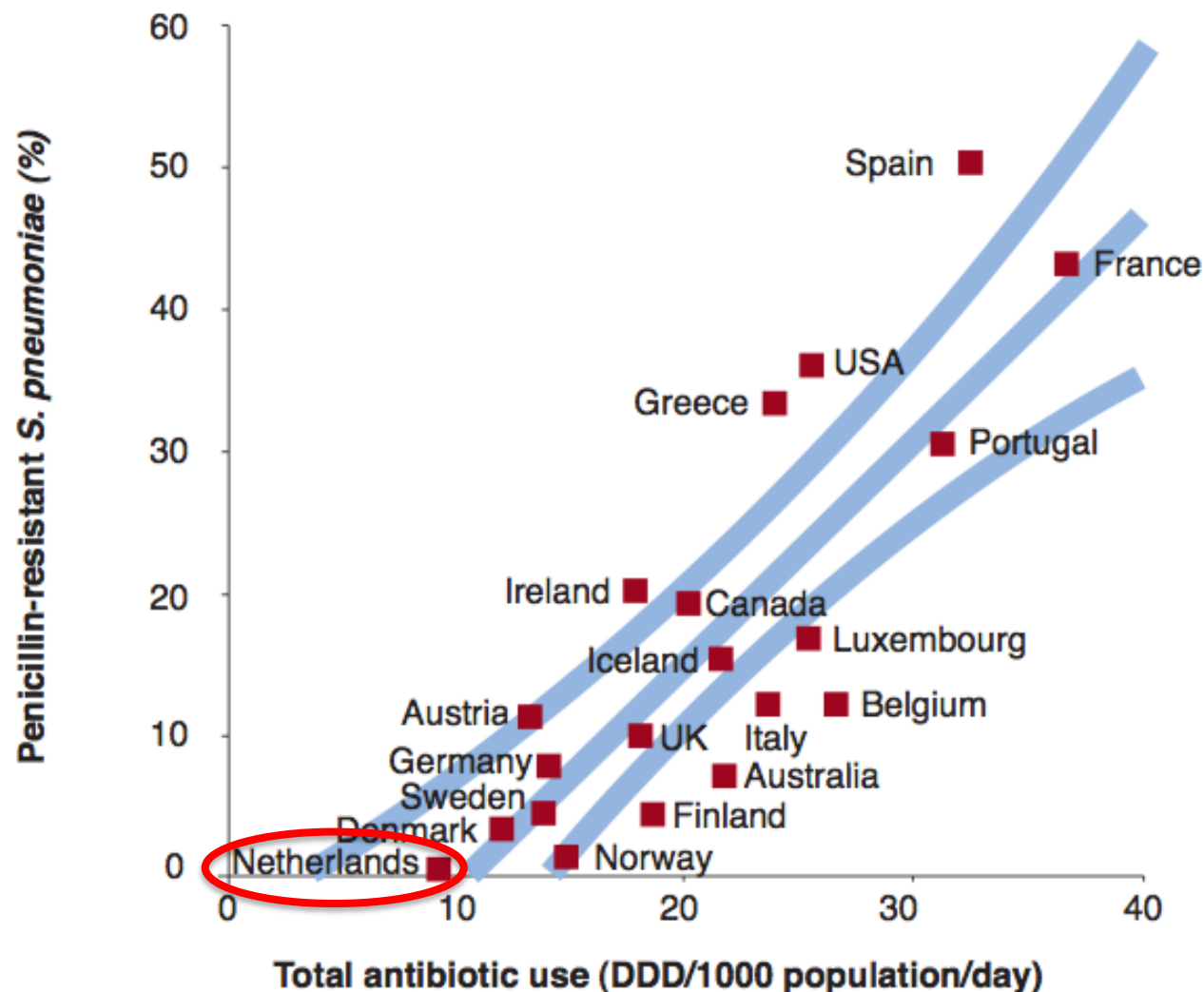
Sign up to our alerts on [food.gov.uk/email](mailto:food.gov.uk@email) Watch us on [YouTube](https://www.youtube.com/foodgovuk) View our pins on [Pinterest](https://www.pinterest.com/foodgovuk)

Sources

- O'Neill Review on Antimicrobial Resistance, 2014 (see www.amr-review.org)
- CMA Report #4
- www.nhs.uk/nhsengland/arc/pages/aboutarc.aspx

For more information, visit food.gov.uk

Figure 2.6 Antibiotic use and AMR from 1990–2000 in selected countries



DDD: Defined Daily Doses

Total antibiotic use in outpatients versus prevalence of penicillin-nonsusceptible *Streptococcus pneumoniae* in 20 industrialized countries.

AMR $\neq$ ABR

Funding of infectious disease projects by ZonMw

Program	Period	Budget (M€)	Molds included?	No projects awarded	No fungal projects
Infectieziektebestrijding	2006 - 2011	12.6	Tolerated	33	1
Q-koorts	2010-2014	3	No	14	0
Infectieziektebestrijding	2014 - 2017	17	Fungal resistance excluded		
Ronde 1 (Non-alimentaire zoönosen)				6	0
Ronde 2				14	0
Ronde 3 (Non-alimentaire zoönosen)				9	0
Ronde 4				9	0
Priority Medicines Antimicrobiële Resistentie	2009 - 2018	14.76	Fungi excluded	28	0
Antibioticaresistentie	2016 - 2023	16	Fungi excluded		
Ronde 1				10	0
Ronde 2				6	0
Totaal		63.36		129	1 (0.8%)

Fungal disease not perceived as a public health threat

Fungal disease unit?

Cib/RIVM



eCDC



WHO



CDC



2013



Azole resistance in *A. fumigatus*: Under the radar.....



Medical

Not a public health threat....

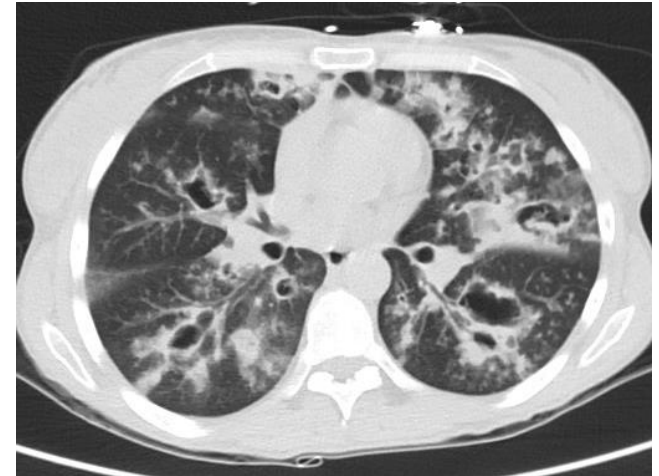


Agricultural

Not a plant pathogen....



Lack of awareness



An very unfavorable combination.....



Influenza

Aspergillosis



Occurs in **1 in 5** patients admitted to the ICU with influenza

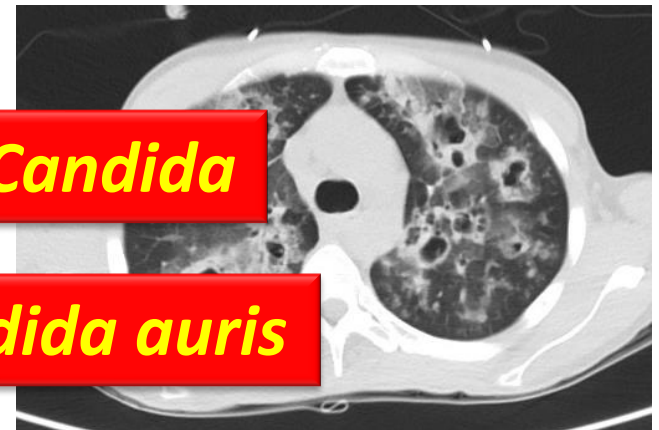
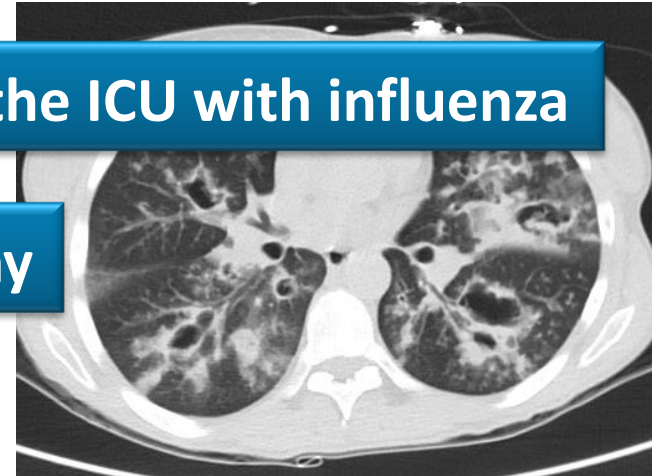
1 in 3 patients were previously healthy

1 in 3 patients had azole resistance

1 in 2 patients died

Emergence of drug resistance in *Candida*

Emergence of drug resistant *Candida auris*



Fungal disease not perceived as a public health threat

Fungal disease expertise?

Cib/RIVM



Center of Expertise
in Mycology
Radboudumc/CWZ



eCDC



WHO



CDC

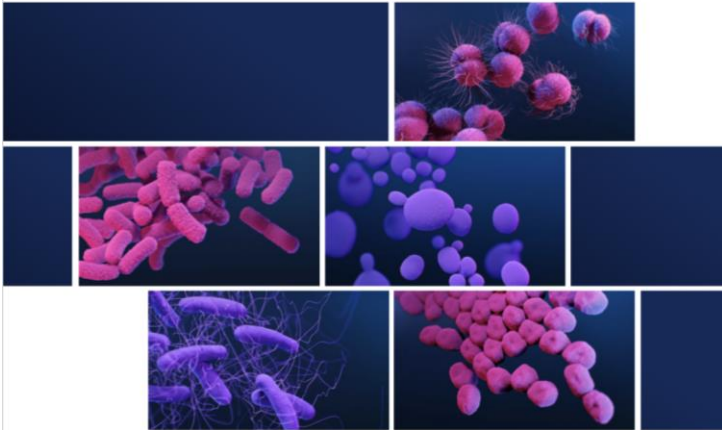


antibiotic resistance threats

CDC – antibiotic resistance threats

ANTIBIOTIC RESISTANCE THREATS IN THE UNITED STATES

2019



Urgent Threats

- Carbapenem-resistant *Acinetobacter*
- *Candida auris* (*C. auris*)
- *Clostridioides difficile* (*C. difficile*)
- Carbapenem-resistant Enterobacteriaceae (CRE)
- Drug-resistant *Neisseria gonorrhoeae* (*N. gonorrhoeae*)



Serious Threats

- Drug-resistant *Campylobacter*
- Drug-resistant *Candida*
- Extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae
- Vancomycin-resistant *Enterococci* (VRE)
- Multidrug-resistant *Pseudomonas aeruginosa* (*P. aeruginosa*)
- Drug-resistant nontyphoidal *Salmonella*
- Drug-resistant *Salmonella* serotype Typhi
- Drug-resistant *Shigella*
- Methicillin-resistant *Staphylococcus aureus* (MRSA)
- Drug-resistant *Streptococcus pneumoniae* (*S. pneumoniae*)
- Drug-resistant Tuberculosis (TB)



Concerning Threats

- Erythromycin-resistant group A *Streptococcus*
- Clindamycin-resistant group B *Streptococcus*

Watch List

- Azole-resistant *Aspergillus fumigatus* (*A. fumigatus*)
- Drug-resistant *Mycoplasma genitalium* (*M. genitalium*)
- Drug-resistant *Bordetella pertussis* (*B. pertussis*)



Threat?

yes

Global?

yes

Forgotten?

yes

The environment: Deadly flower power?

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Deadly flower power? Imported tulip bulbs spread anti-fungal resistance

Date: May 18, 2017

Source: Trinity College Dublin

Summary: Tulip and narcissus bulbs imported into Ireland from the Netherlands may be acting as vehicles for the international spread of a drug-resistant fungus -- with potentially fatal consequences. Experts advise people not to plant bulbs near hospitals or to gift them to at-risk patients.

Share:      

RELATED TOPICS

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- > Pharmacology
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Plants & Animals

FULL STORY

Tulip bulbs imported into Ireland from the Netherlands may be helping to spread resistance to vital antifungal medicines called Triazoles that treat potentially fatal fungal infections. That is according to research showing that the inter-country transfer of bulbs of Holland's iconic flowers may inadvertently have opened up a new transport route for a particularly nasty fungal pathogen called *Aspergillus fumigatus*.

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this week

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Starving Prostate Cancer With What You Eat: Apple Peels, Red Grapes, Turmeric



Does Consuming Low-Fat Dairy Increase the Risk of Parkinson's Disease?



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Promiscuous Salamander Found to Use Genes from Three Partners Equally

EARTH & CLIMATE

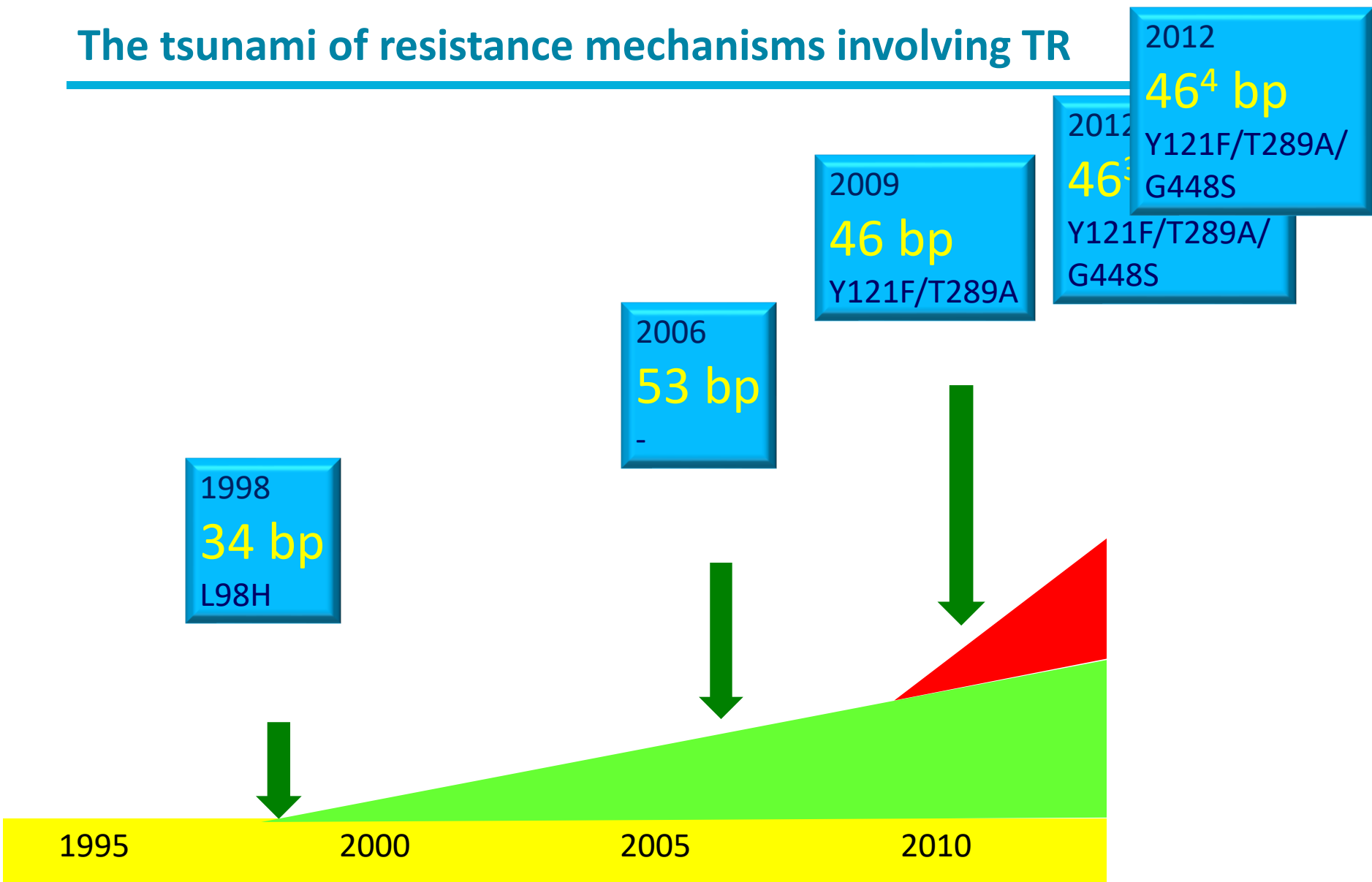


Large Canadian Arctic Climate Change Study Cancelled Due to Climate Change



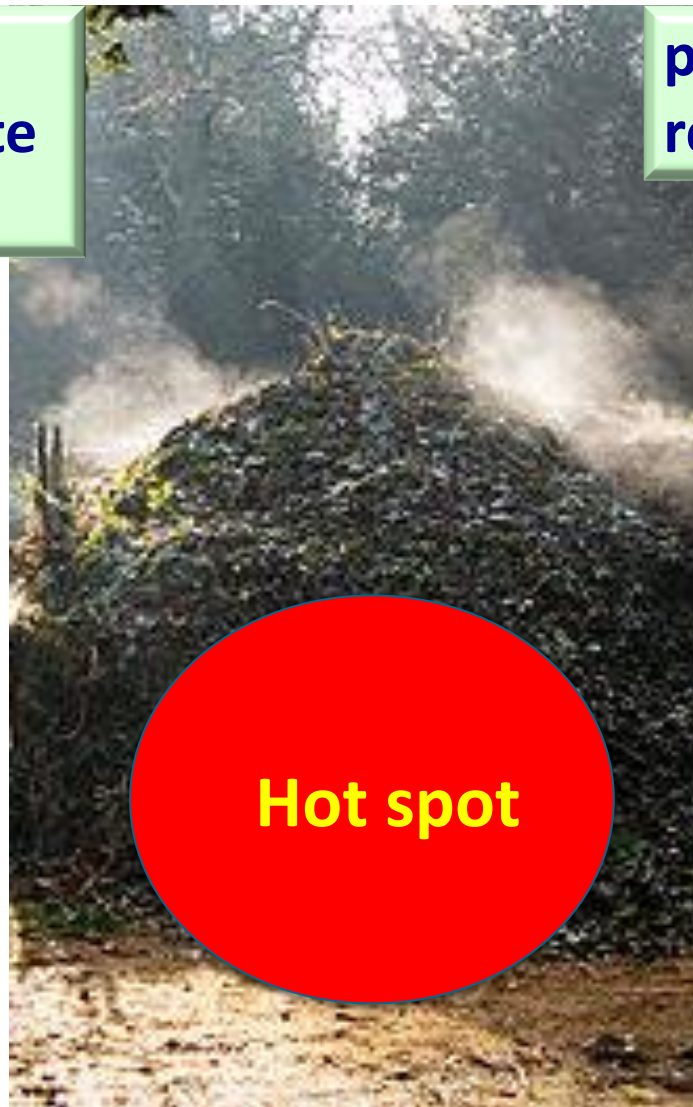
Solar Paint Offers Endless Energy from Water Vapor

The tsunami of resistance mechanisms involving TR



***A. fumigatus* is
able to complete
its life cycle**

**presence of azole
residues**



Flower bulb waste

Green waste

Wood chippings waste

Strawberry waste

Where?



application of fungicides



collection of organic waste



composting



mature compost



Commercial composting



Where?



application of fungicides



collection of organic waste



composting



mature compost



Ministerie van Landbouw,
Natuur en Voedselkwaliteit

Bezoekadres Postbus 20401 2500 EK Den Haag

Discuss at EU-level

De Voorzitter van de Tweede Kamer
der Staten-Generaal

Directoraat-generaal Agro
Directie Plantaardige Agroketens
en Voedselkwaliteit

Bezoekadres

Intensify surveillance and develop better diagnostic tests

Postbus 20401
2500 EK Den Haag

Source control – re-evaluate authorized azole fungicides

F 070 378 6100 (algemeen)
www.rijksoverheid.nl/lnv

Prevent storage and facilitate early composting

ns kenmerk
GA / 19209421

de schimmel *Aspergillus fumigatus* tegen azolen

Geachte Voorzitter,

Op 17 juni jl. heb ik uw Kamer geïnformeerd over het onderzoeksrapport van het RIVM over de ontwikkeling van resistentie bij de schimmel *Aspergillus fumigatus* tegen azolen (Kamerstuk 27858, nr. 460). Ik heb in deze brief toegezegd uw Kamer te informeren over het advies van het College voor de toelating van

How are patients infected? – potential sources



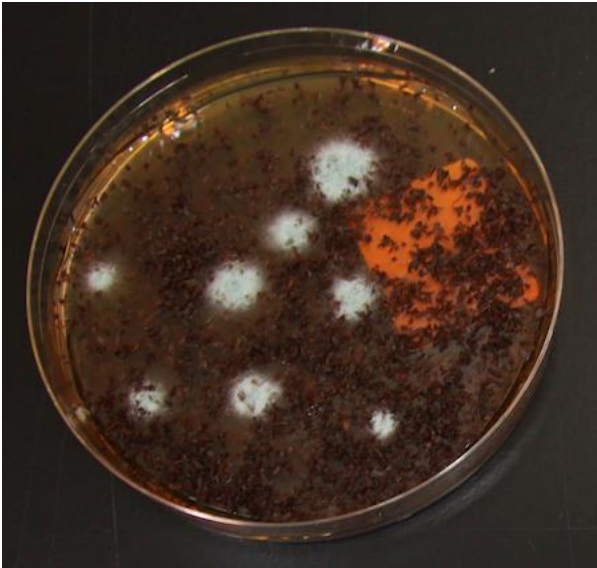
other?



How ?



How are patients infected? – potential sources



tea



pepper



onions

Threat?

yes

Develop better diagnostic tests
New drug targets

Global?

yes

Set up international surveillance
Include fungal resistance in One-health programs

Forgotten?

yes

Prioritize fungal resistance research
Include fungal resistance in AMR initiatives