Clinical relevance of resistance in Aspergillus

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Steps to establishing clinical validity of resistance/susceptibility for pathogens

1. Identify some resistant isolates
2. Develop susceptibility testing methodology that separates susceptible from resistant isolates
3. Road test best method to establish robustness in clinical laboratories (intra and inter-laboratory reproducibility etc)
4. Establish breakpoints for clinical interpretation based on:
   - wild type distribution
   - observational clinical studies
   - pharmacodynamic studies in well established models

Aspergillus fumigatus

Resistant isolate selection

Strain no.	NCPF no.	Patient details; source of strain	Therapeutic response data
1, AF65	7097	Acute leukaemia; lung biopsy	Intermediate response to high dose amphotericin B despite neutrophil recovery; subsequent relapse
2, AF71			Good response to itraconazole[33]
3, AF210	7101	Laparostomy for Crohn's disease; liver surface	Excellent response (cure) to amphotericin B 1 mg/kg[34]
4, AF294	7102	Multiple myeloma and profound neutropenia; bronchoalveolar	Failed amphotericin B 1 mg/kg over 12 days despite neutrophil recovery
5, AF72	7099	Cured Hodgkin's disease;	Isolated during itraconazole therapy with good serum concentrations[23]
6, AF91		sputum	Isolated during itraconazole therapy with good serum concentrations[23]

Likely AmB resistant

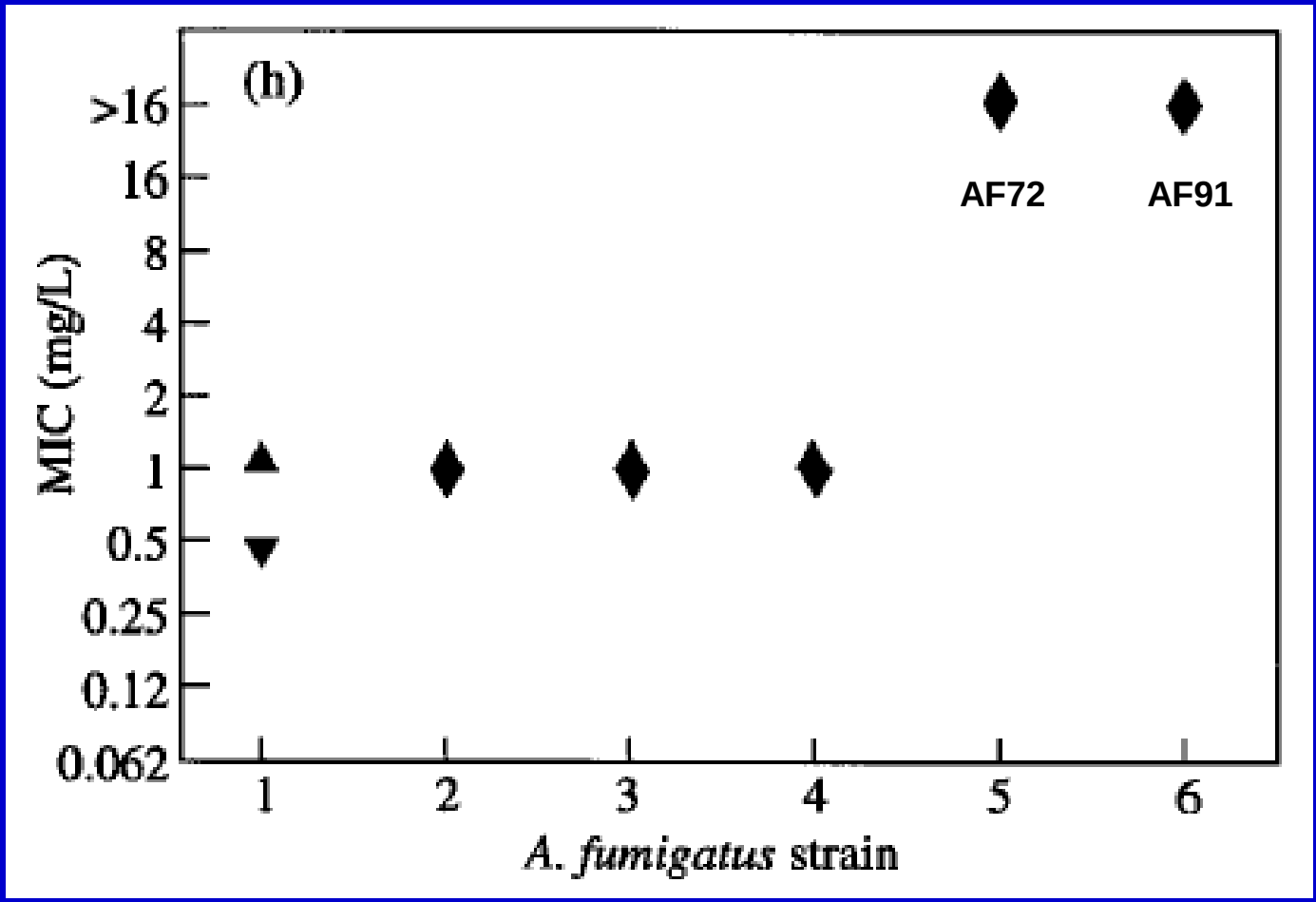
Likely Itra resistant

NCPF, National Collection of Pathogenic Fungi.

Denning et al, JAC 1997;40:401

MIC test development (itraconazole)

microtitre, RPMI 2% glucose 35°C 48 hrs



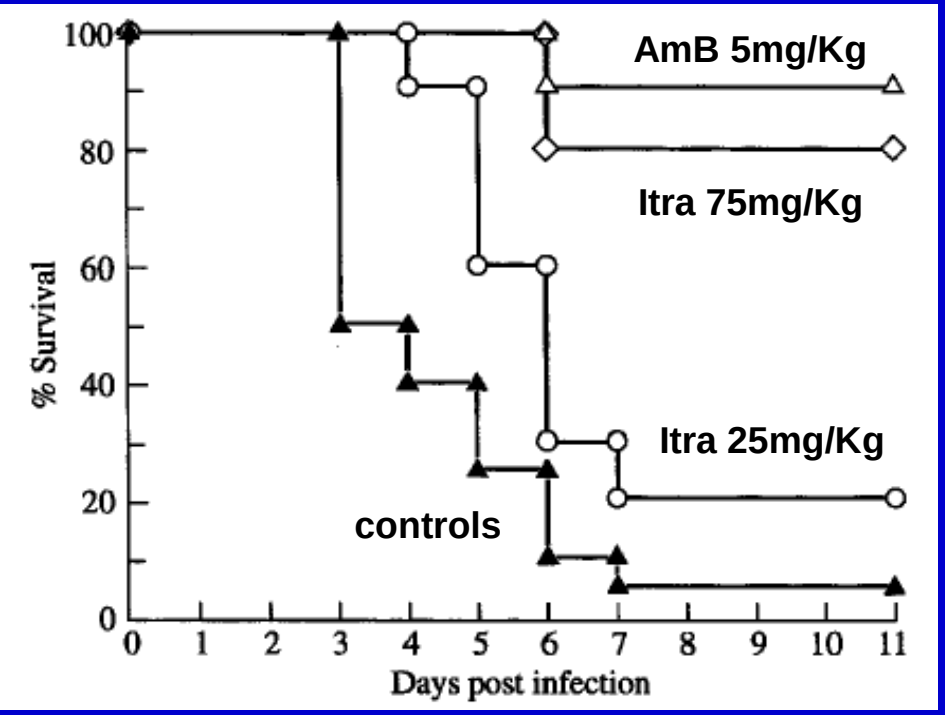
Test inoculum

2×10^6/mL

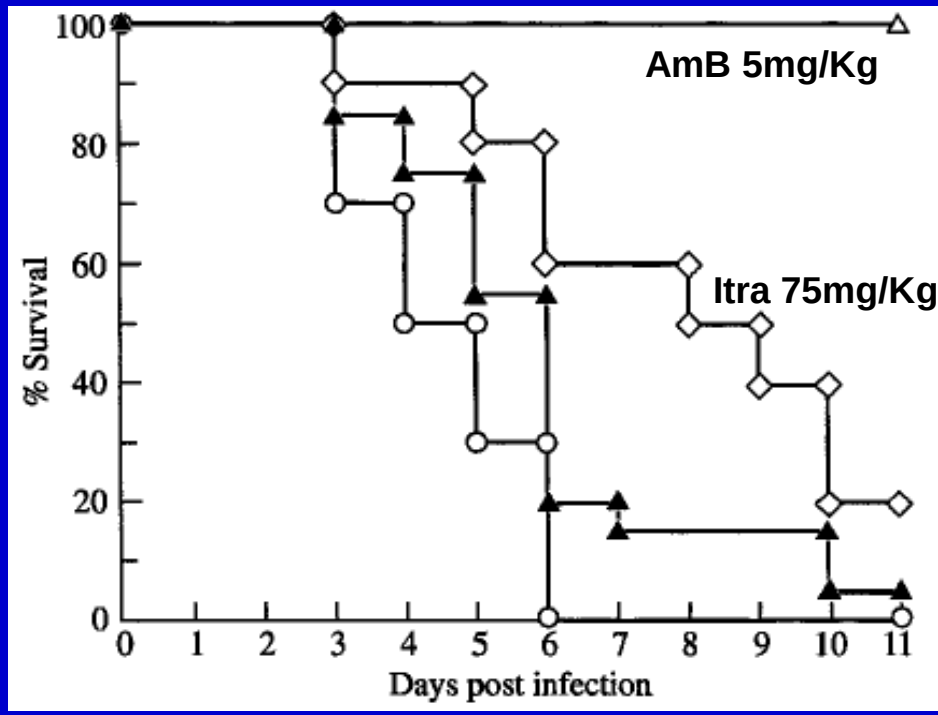
Denning et al, JAC 1997;40:401

MIC test development (itraconazole)

confirmation in vivo



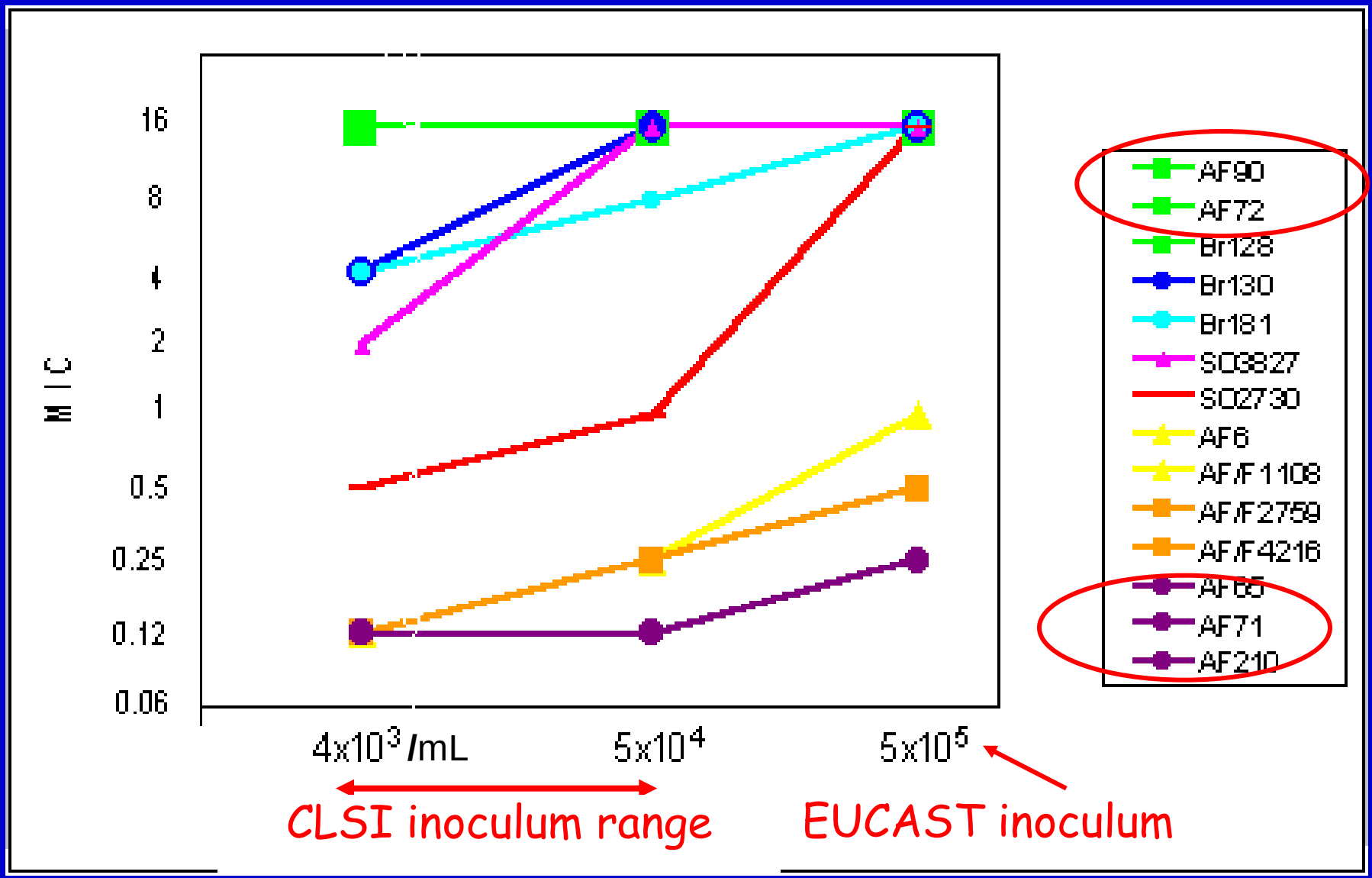
Strain 5 (AF 72)
G54 CYP51A mutation



Strain 6 (AF 91)
M220 CYP51A mutation

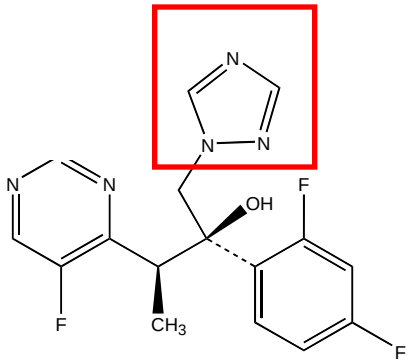
Denning et al, JAC 1997;40:401

Inoculum and MIC interdependence

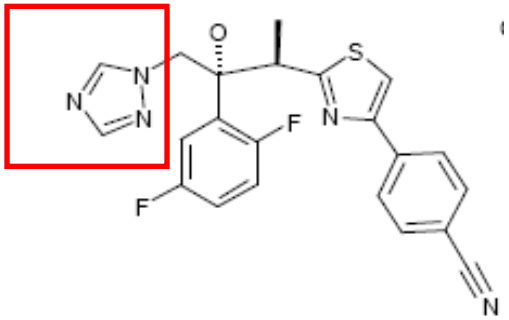


Mosquera ICAAC 2000 and Mosquera & Denning AAC 2002;46:556

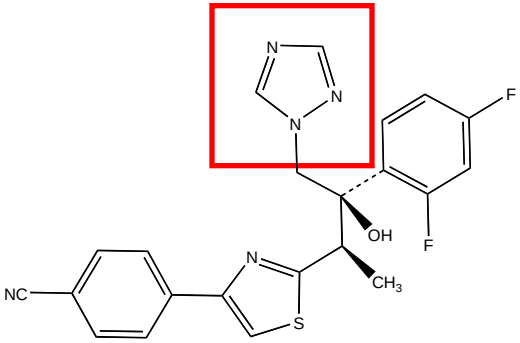
Cross resistance in *Aspergillus fumigatus* to other azoles?



Voriconazole

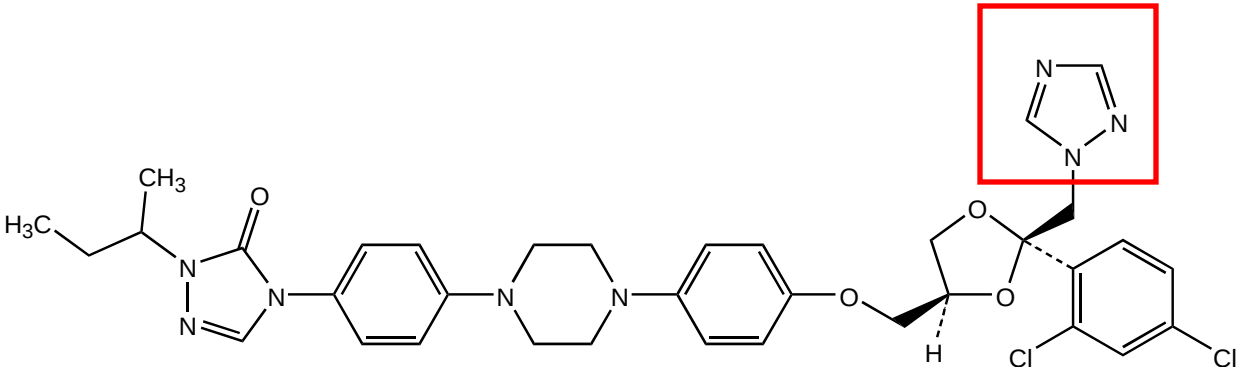


Isavuconazole

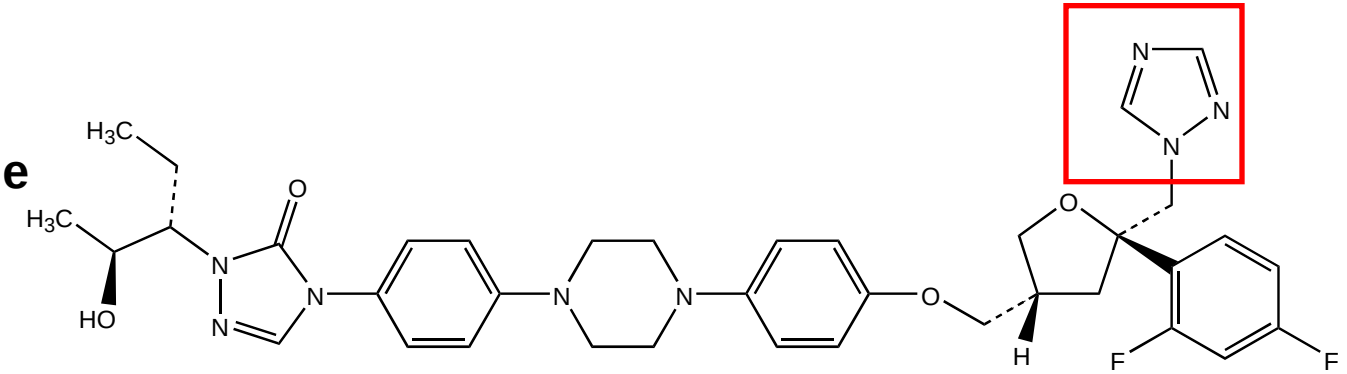


Ravuconazole

Itraconazole



Posaconazole



Itraconazole resistance reports

• Chryssanthou	4/107 (3.7%)	>32 mg/L
• Dannaoui	4/156 (2.6%)	>16 mg/L
• Gomez-Lopez	3/185 (1.6%)	>4 mg/L

Limited clinical correlation

Chryssanthou Scand J ID 1997;29:509 Dannaoui JAC 1999;44:553 Gomez-Lopez AAC 2003;47:3085

Voriconazole resistance

- Verweij 1/150 (0.7%) >4 mg/L
- Gomez-Lopez 3/185 (1.6%) >4 mg/L

as drug used more, expect more resistance

see Verweij et al M-2018 ICAAC 2007

Verweij, JAC 1998;42:389-92; Gomez-Lopez, AAC 2003;47:3085

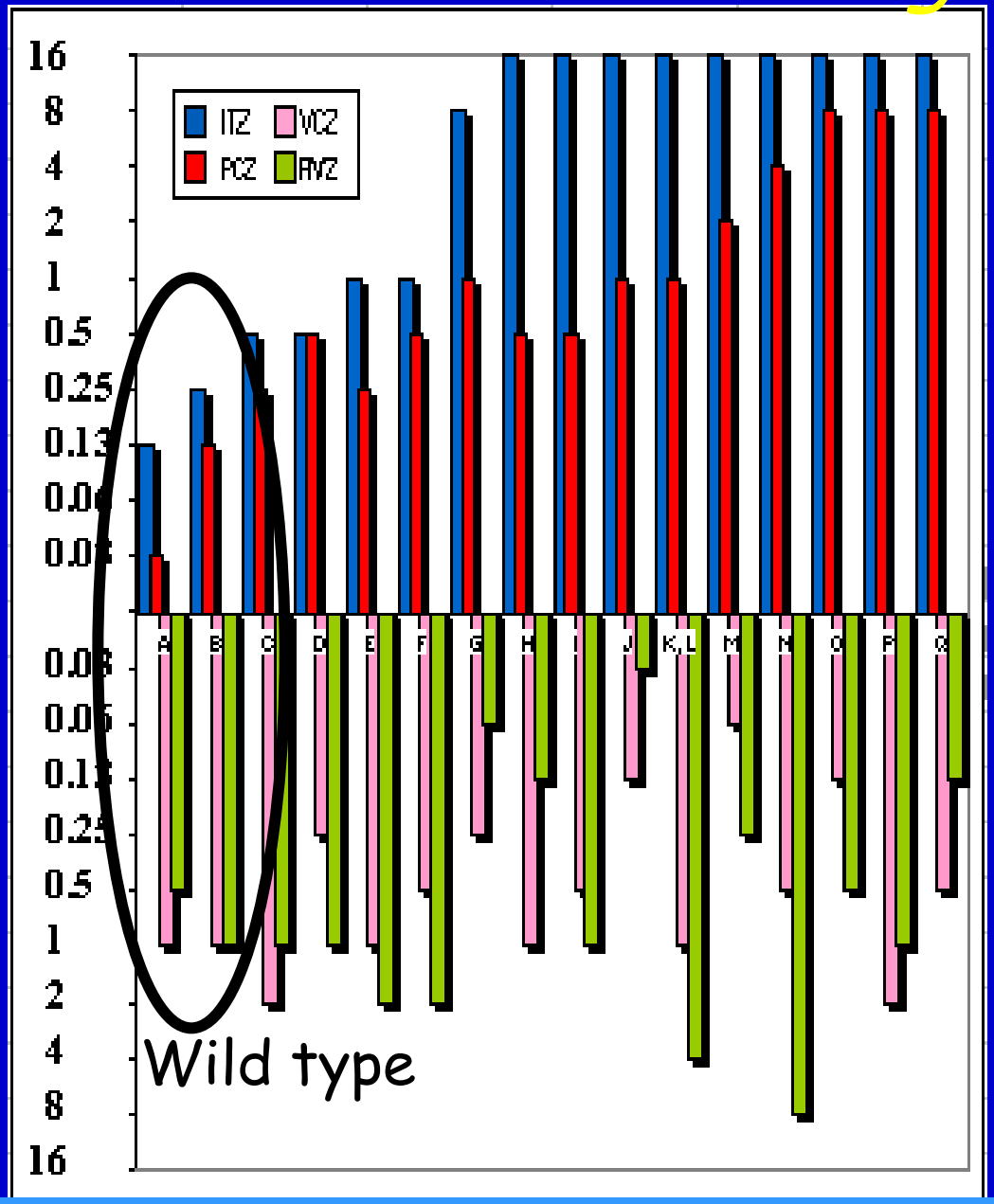
Azole resistance – Manchester
A. fumigatus

			Breakpoint
Itraconazole	33/315	(10.5%)	≥4 mg/L
Voriconazole	27/285	(9.5%)	≥4 mg/L
Voriconazole	9/314	(2.9%)	≥8 mg/L
Posaconazole	Not enough data		
Ravuconazole	No data		
Isavuconazole	No data		

Could be slightly overstated because inoculum used 1997-2002 was higher than now

Howard et al, unpublished

Cross resistance in *A. fumigatus*



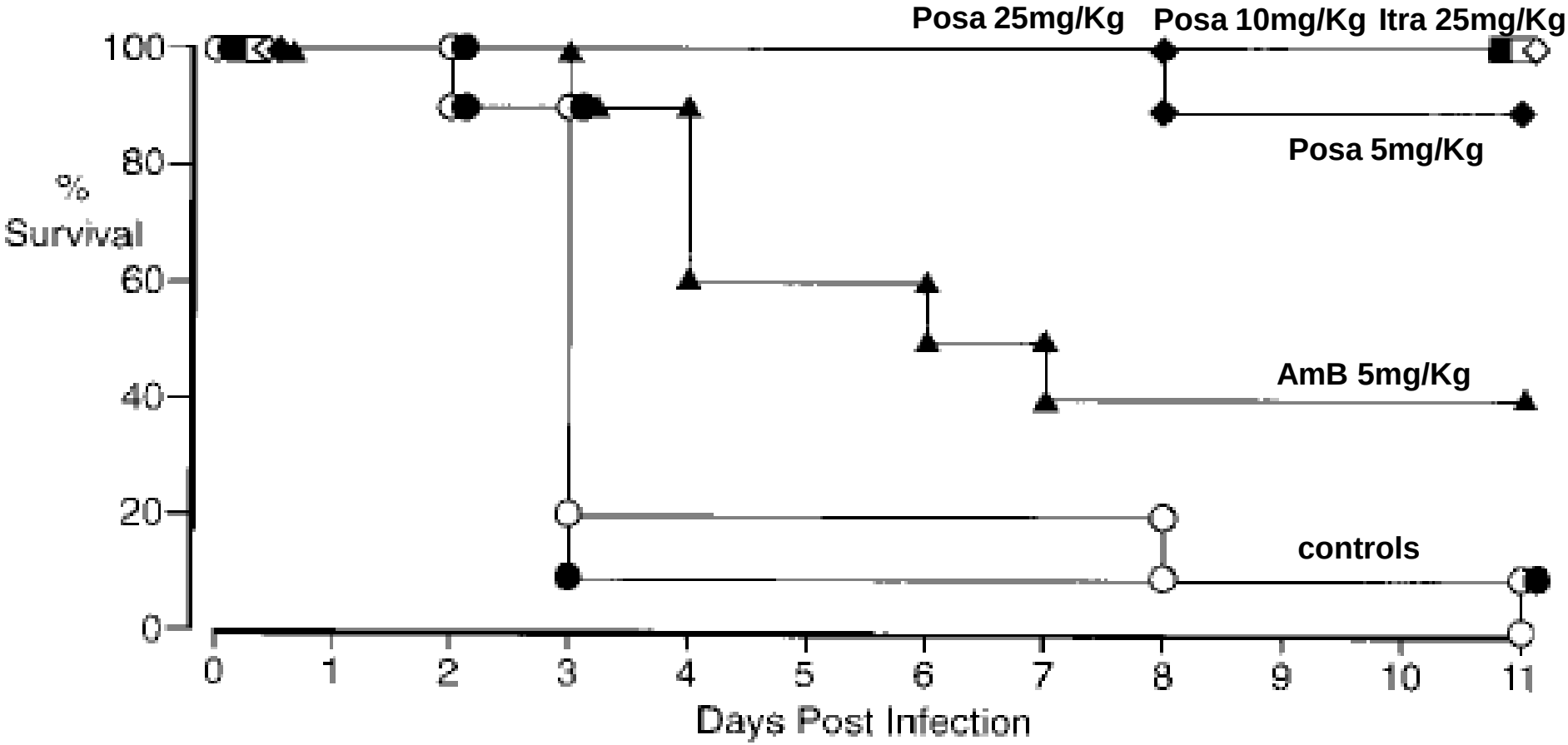
Test inoculum

$5x10^4$/mL

Mosquera & Denning AAC 2002;46:556

MIC test confirmation (posaconazole)

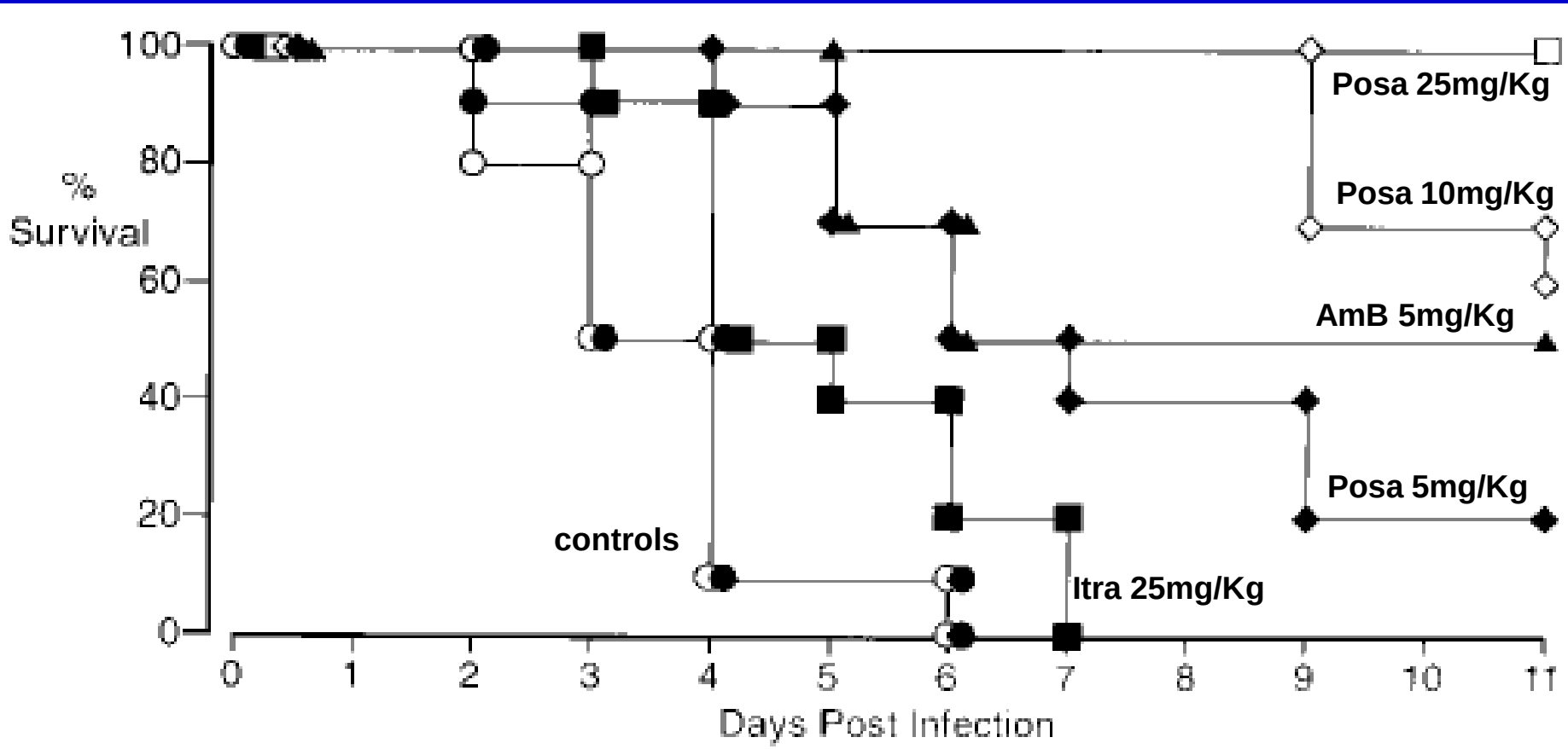
AF 71 (not 72) Itra MIC = 0.25mg/L & posa MIC = 0.03



Oakley et al, *AAC* 1997;41:1504

MIC test confirmation (posaconazole)

AF 90 Itra MIC = >8mg/L & posa MIC = 1.0



Oakley et al, *AAC* 1997;41:1504

MIC test confirmation (posaconazole)

Outcome showing MIC/drug exposure relationship

TABLE 3. Concentrations of SCH and ITZ in serum 6 h after dosing on day 7

Isolate	Concn (μg/ml) in serum for the following group[a]:			
	SCH (25)	SCH (10)	SCH (5)	ITZ (25)
AF71	8.0	4.0	1.2	1.8
AF90	7.6	4.2	2.1	5.1

Oakley et al, *AAC* 1997;41:1504

Resistance in other *Aspergilli*?

Itraconazole resistance - Manchester

A. fumigatus complex	33/315	(10.5%)	≥4 mg/L
A. flavus complex	5/36	(13.9%)	≥4 mg/L
A. niger complex	8/34	(23.5%)	≥4 mg/L
A. terreus complex	3/17	(17.6%)	≥4 mg/L

Could be slightly overstated because inoculum used 1997-2002 was higher than now

Howard et al, unpublished

Aspergillus fumigatus complex and resistance

Table 1 Minimal inhibitory concentrations (MICs) of different antifungal against clinical isolates of *Aspergillus* section *Fumigati*

Isolate no.	Molecular identification			MICs (μg/ml)*					
	Origen	ITSs	CytB	AmB	ITC	VCZ	RVC	POS	TRB
CM-237	Lung Biopsy	*A. fumigatus*	*A. fumigatus*	0.25–0.5	0.12–0.25	0.25–0.5	0.5–1.0	0.03–0.06	4.0–8.0

Balajee AAC 2004;48:1197; Mellado, Med Mycol 2006;44 Suppl:367

Resistance in context of invasive aspergillosis

Multi-azole resistance in *A. fumigatus*

Itraconazole = >16 mg/mL

Voriconazole = 2.0 - >16 mg/mL

Posaconazole = 0.5 – 1.0 mg/mL

Prevalence of elevated MICs	1945-1998	2002-2007
Multi-azole resistance	0%	10/81 (12%)

Verweij, NEJM 2007;356:1481

Resistance in context of invasive aspergillosis

Table 1. Characteristics of Nine Patients from Whom *A. fumigatus* Resistant to Multiple Triazoles Was Cultured.

Sex	Yr of Age	Underlying Disease	Date of Isolation	Site of Isolation	Disease Classification*	Previous Azole Exposure	Treatment	Outcome
Male†	15	X-linked chronic granulomatous disease	April 4, 2002	Sputum	Breakthrough invasive pulmonary aspergillosis, proven	Prophylaxis with itraconazole (for 6 yr)	Voriconazole (high-dose)	Survived
Male	73	None	Dec. 3, 2003	Ear swab	Invasive aspergillosis of mastoid cavity, proven	None	Surgery and topical therapy	Survived
Male	16	Hyper-IgE syndrome	Nov. 19, 2004	Bronchoalveolar-lavage fluid	Breakthrough invasive pulmonary aspergillosis, proven	Treatment with voriconazole (for 2 yr)	Surgery and posaconazole	Survived
Female	76	Pulmonary fibrosis	June 26, 2005	Sputum	Invasive pulmonary aspergillosis, possible	None	Voriconazole	Survived
Male	31	Chronic granulomatous disease	Nov. 1, 2005	Lung aspirate	Breakthrough invasive pulmonary aspergillosis, probable	Prophylaxis with itraconazole (for >10 yr)	Caspofungin and posaconazole	Survived
Female	68	Acute myeloid leukemia	Feb. 14, 2006	Bronchoalveolar-lavage fluid	Disseminated invasive aspergillosis, probable	None	Voriconazole	Died
Female	62	Chronic obstructive pulmonary disease	April 5, 2006	Bronchoalveolar-lavage fluid	Invasive pulmonary aspergillosis, possible	None	Voriconazole, amphotericin B, and posaconazole	Survived
Male	19	Chronic granulomatous disease	April 15, 2006	Bone	Breakthrough aspergillus osteomyelitis, proven	Prophylaxis with itraconazole (for >2 yr)	Voriconazole, caspofungin, and posaconazole	Survived
Male	45	Acute myeloid leukemia and allogeneic hematopoietic stem-cell transplantation	May 11, 2006	Nose swab	Breakthrough aspergillus sinusitis, proven	Prophylaxis with itraconazole (for 4 wk)	Posaconazole	Died

Verweij, NEJM 2007;356:1481

Chronic cavitary pulmonary aspergillosis (CCPA) in HIV February 2005

32 yr old from Malawi, on HAART Rx
- haemoptysis
- Aspergillus precipitin titre 1/16

CT scan shows 2 large cavities with aspergillomas, with additional lesions (October 2005)

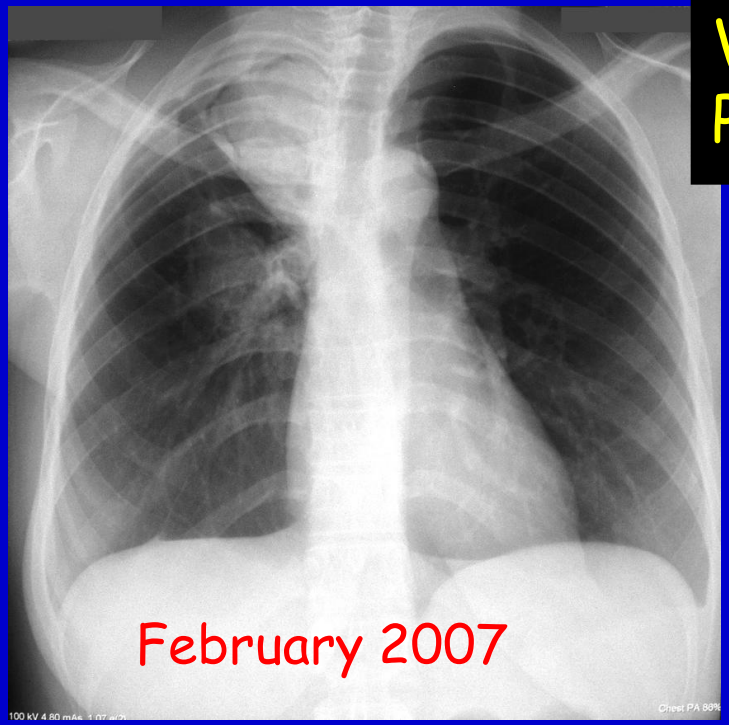
Surgical removal would require a pneumonectomy So treated with itraconazole

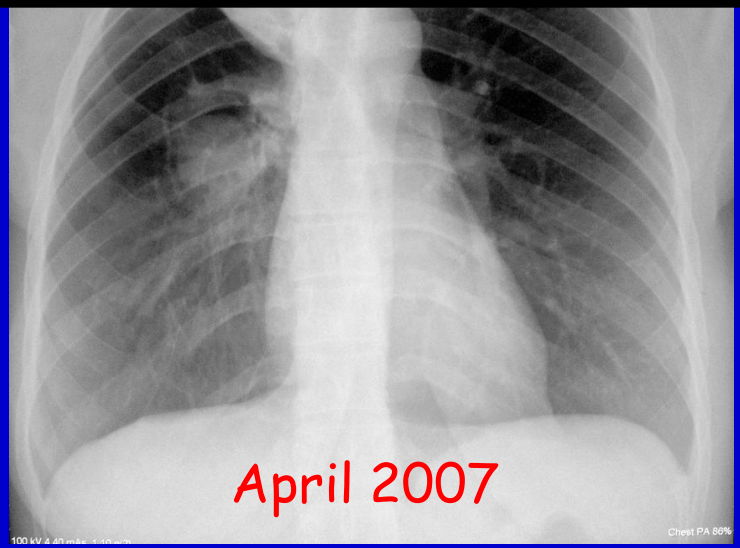
CCPA in HIV February 2007

On HAART Rx, with low viral load, CD4 count >200
- New haemoptysis
- Aspergillus precipitin titre ↑ 1/32

CXR & CT scan showed

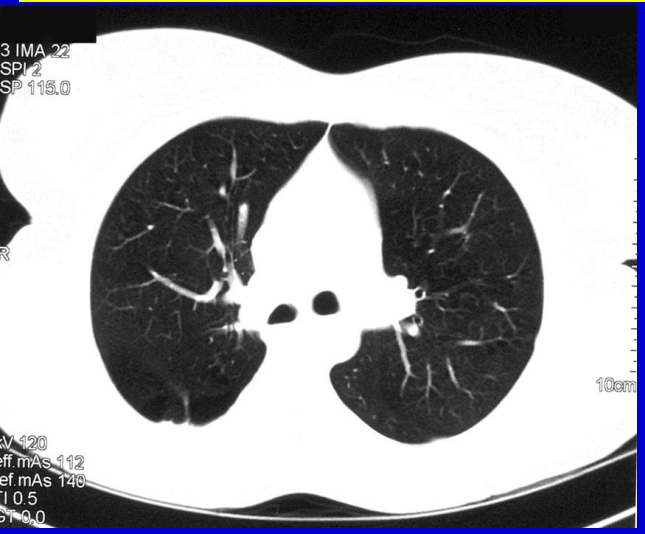
MICs *A. fumigatus* Feb 2007
Itraconazole = >8.0mg/mL
Voriconazole = 0.5 mg/mL
Posaconazole = 1.0 mg/mL

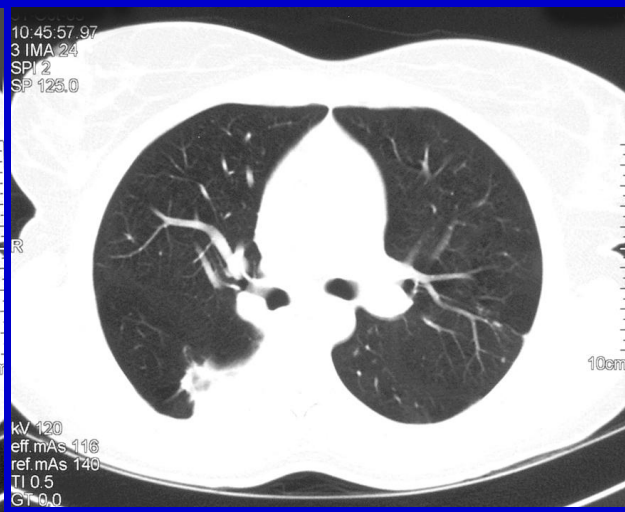




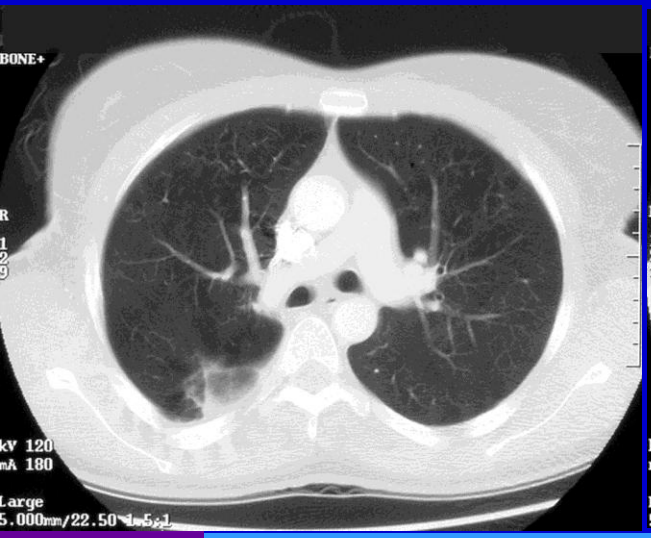
CCPA in HIV - CT scan change

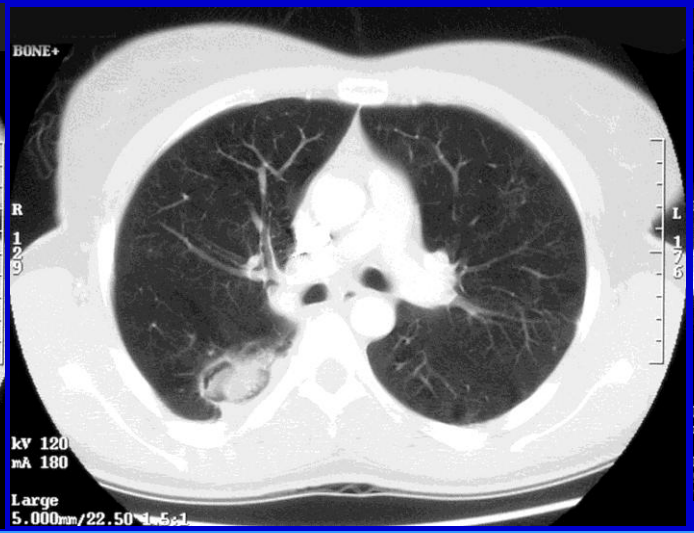
October 2005

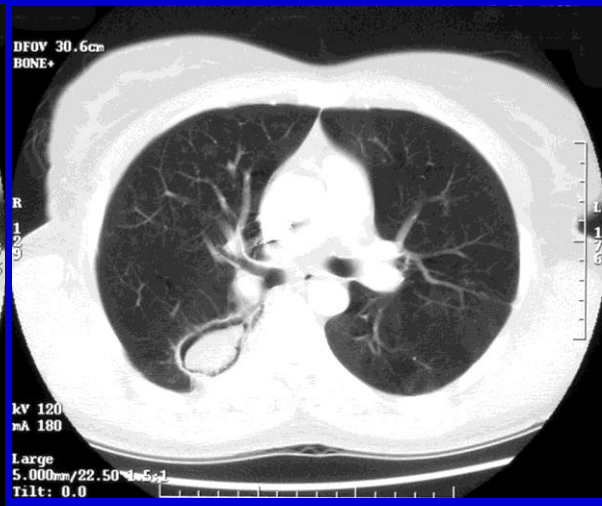




March 2007







CCPA in HIV - low itraconazole concentrations

Itraconazole concentrations	
Nov 05	2.5 mg/L
Dec 05	3.4 mg/L
March 06	4.5 mg/L
July 06	6.7 mg/L
Feb 07	8.4 mg/L

Do low concentrations of antifungal predispose to the development of resistance?

Aspergilloma in context of chronic cavitary pulmonary aspergillosis

<u>MICs A, fumigatus</u>
Itraconazole = >8.0mg/mL
Voriconazole = 2.0 mg/mL
Posaconazole = 0.25 mg/mL

in association with worse cough, marked fatigue and rising Aspergillus precipitins, after treatment for 2 years with itraconazole

Chronic cavitary pulmonary aspergillosis

MICs A. fumigatus

Feb 2004 (x2)
Itraconazole = 0.25 mg/mL
Voriconazole = 0.25 mg/mL
Posaconazole = 0.13 mg/mL

June 2004 (x2)
Itraconazole = >8.0mg/mL
Voriconazole = 0.25 mg/mL
Posaconazole = 1.0 mg/mL

in association with enlargement of his fungal ball and continuing cough
Treated with posaconazole with response

Chronic cavitary pulmonary aspergillosis

<u>*MICs A. fumigatus*</u>

Feb 2004 (x2)
Itraconazole = 0.25 mg/mL
Voriconazole = 0.25 mg/mL
Posaconazole = 0.13 mg/mL

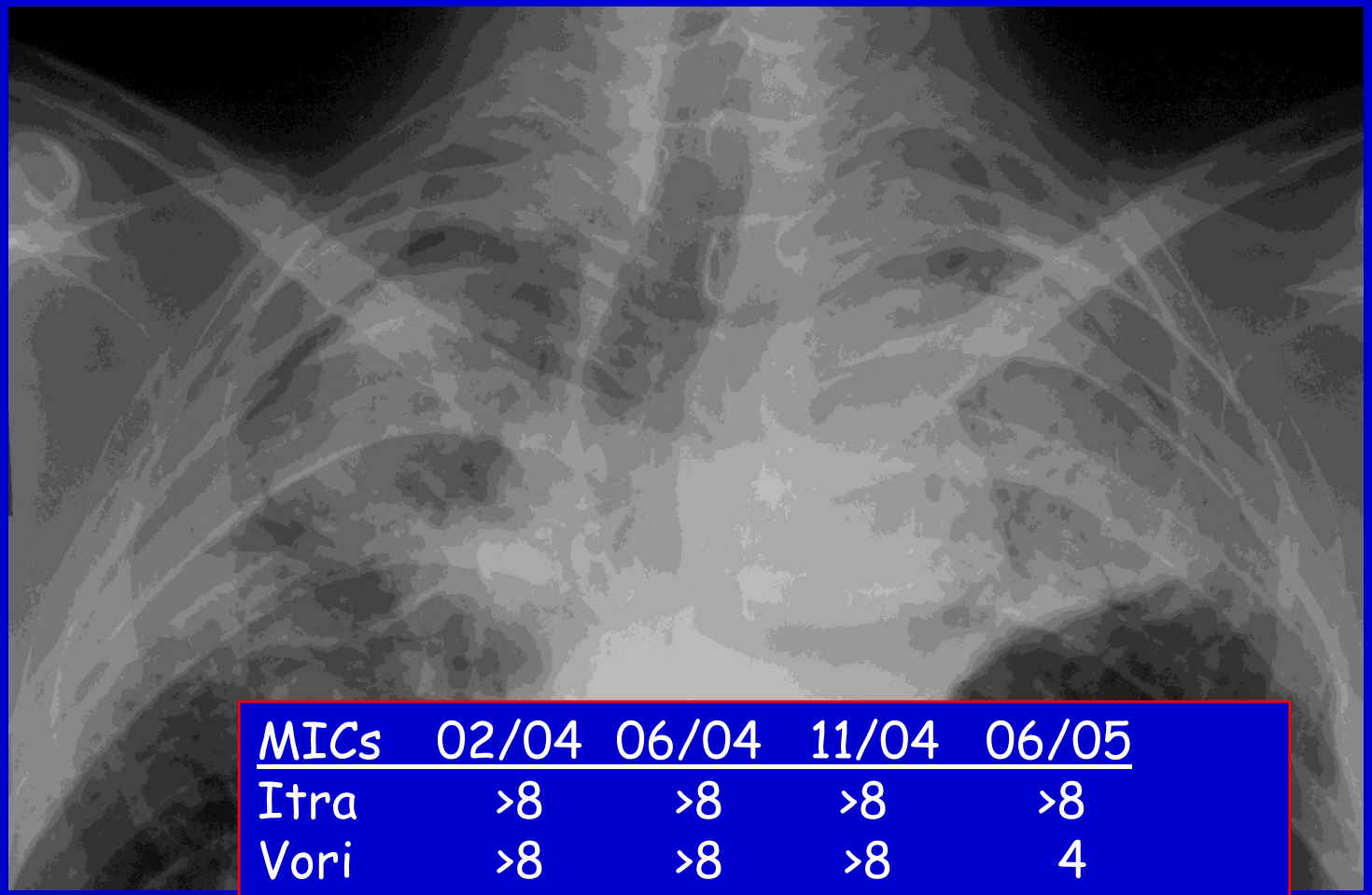
CYP51A G54 wild types
GGG

June 2004 (x2)
Itraconazole = >8.0 mg/mL
Voriconazole = 0.25 mg/mL
Posaconazole = 1.0 mg/mL

CYP51A G54 mutants
AGG
GAG

Consistently low levels of itraconazole (rifabutin interaction)

Chronic fibrosing pulmonary aspergillosis, with bilateral aspergillomas and azole resistance



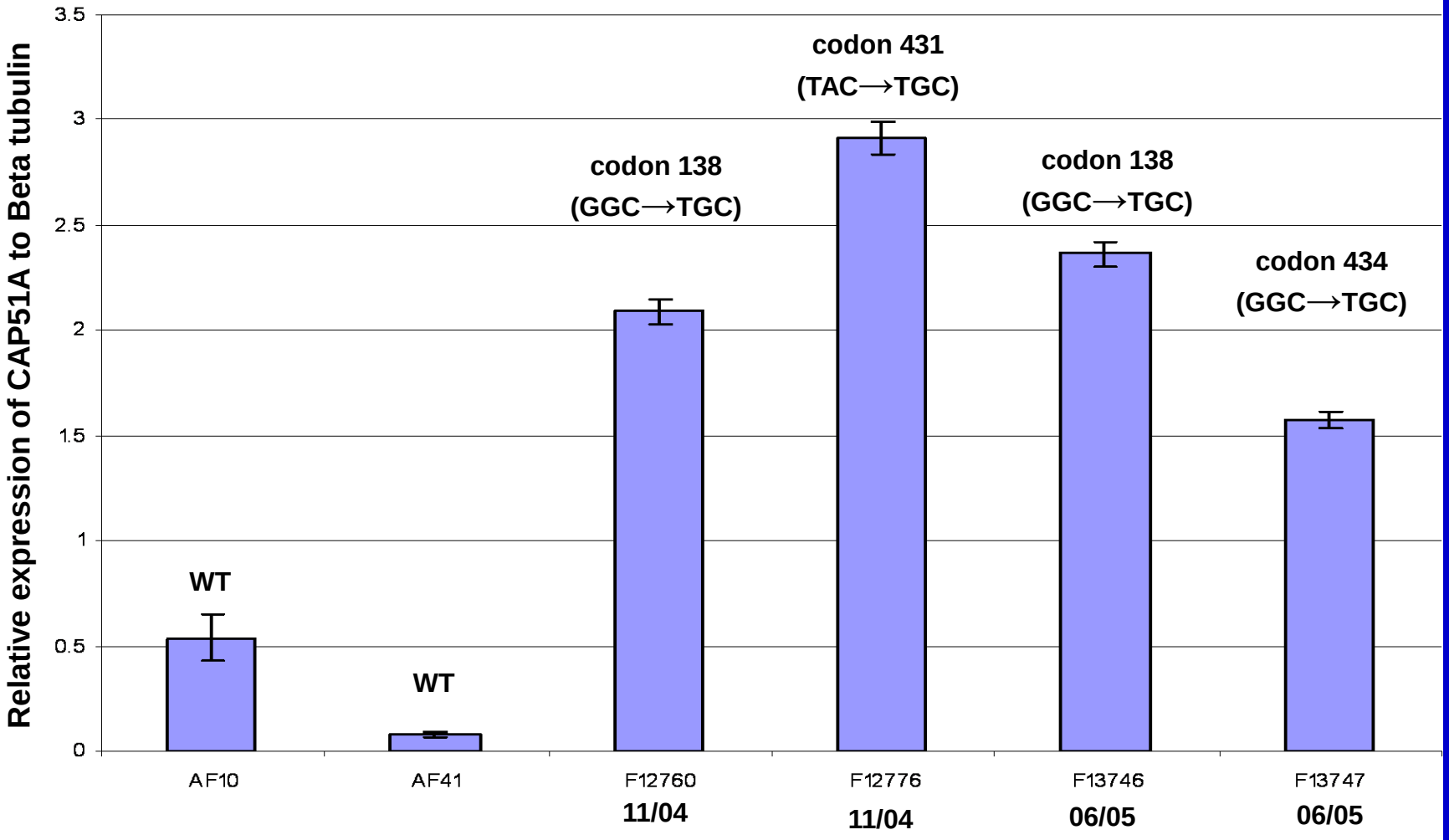
MICs	02/04	06/04	11/04	06/05
Itra	>8	>8	>8	>8
Vori	>8	>8	>8	4
Posa	4	4	2	1

Patient SM
June 2004

After treatment with Itraconazole 200mg daily and later Voriconazole

Howard et al, Int J Antimicrob Ag 2006:28:450

Chronic fibrosing pulmonary aspergillosis and multiple mechanisms of resistance



Al-Barraq unpublished; Howard et al, Int J Antimicrob Ag 2006:28:450

Bronchiectasis + poor pneumococcal antibody response & azole resistance

41 year old female engineer
Haemoptysis with bronchiectasis 1999
Recurrent chest infections (3-4x per year)
Onychomycosis and Rx itraconazole 200mg/d
Poor *S. pneumoniae* antibody response, so immunised

Aspergillus precipitins +ve, titre 1/8 (elevated)
Heavy growth of *A. fumigatus* from sputum

MICs (mg/L)	
Itraconazole	>8 (resistant)
Voriconazole	4 (resistant)
Posaconazole	0.5 (intermediate)

Failed treatment with itraconazole so treated with posaconazole with marked improvement

ABPA + bronchiectasis + mannose binding protein deficiency & azole resistance

44 year old mother with mutiple medical problems
ABPA (IgE 3,000) with mild bronchiectasis 2006
Recurrent exacerbations requiring steroids (2-5x per year)

Commenced itraconazole capsules but levels undetectable
Tried itraconazole solution – nausea, but levels 5.2 mg/L
Some improvement in cough and sputum production, then worsened

A. fumigatus from sputum

MICs (mg/L)	Oct 06	Feb 07
Itraconazole	0.25	>8 (resistant)
Voriconazole	1.0	4 (resistant)
Posaconazole	0.06	0.5 (intermediate)

Breakthrough on itraconazole so treated with posaconazole, marginal improvement

Future perspectives

Mechanisms of resistance

- Target mutations (CYP 51A only)
  ie G54 x5, M220 x3, G138, Y431
- Promoter insertion/repeat
- Cyp51A upregulation/over expression
- Efflux pump upregulation (?)
- Permease downregulation (?)
- Multiple mechanisms simultaneously

Molecular diagnosis of resistance

Wild type susceptible

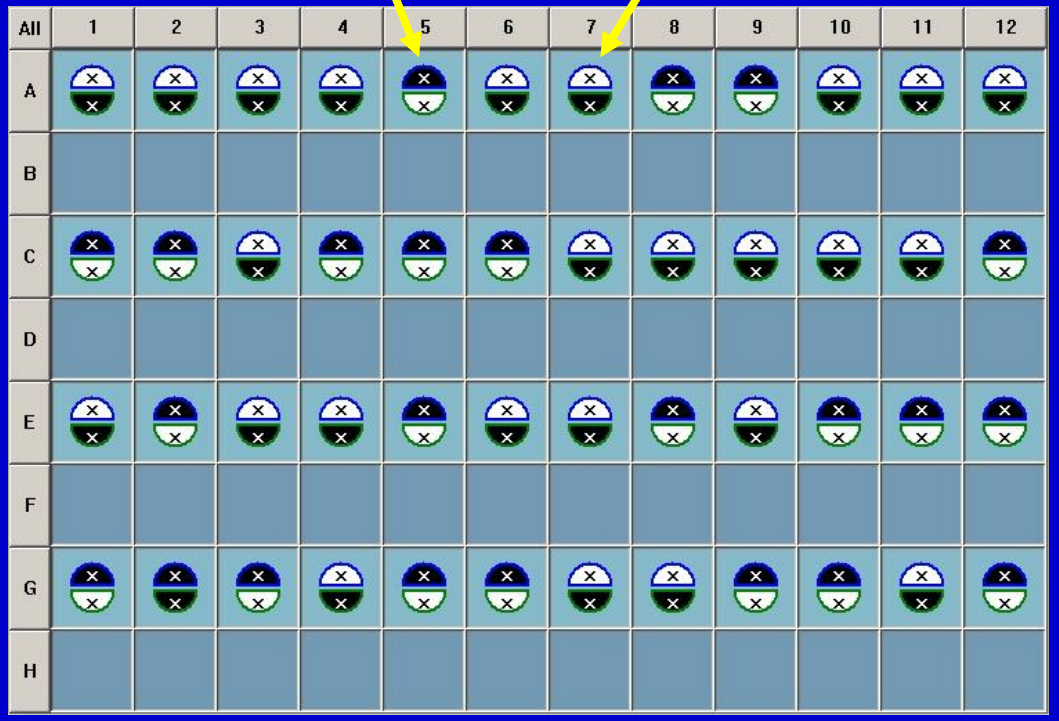
GGG-FAM

GAG-, GAA-, AAG-, AGG-,
CGG-, TGG-, GTG-
HEX

G54 resistant genotype

Susceptible

Resistant

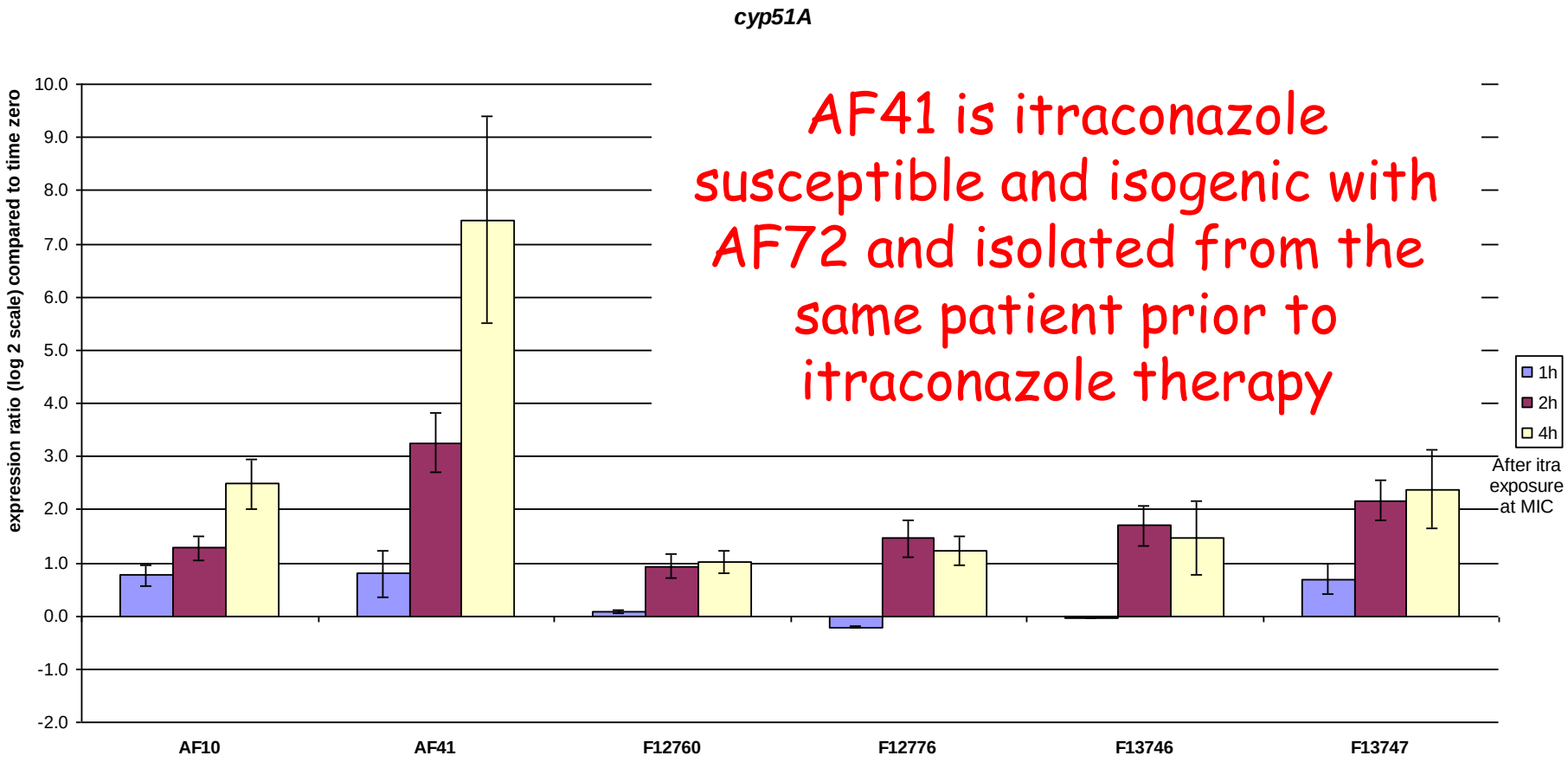


Balashov et al, J Clin Microbiol 1005;43:214

Avoidance of breakthrough resistance

- Multiple cases of breakthrough on or after itraconazole
- Some evidence, mostly unpublished, of sub-optimal drug exposure, suggesting a role for optimising exposure to avoid resistance
- Mechanistic evidence of isolate to isolate variation in adaptation mechanism(s) allowing resistance to emerge

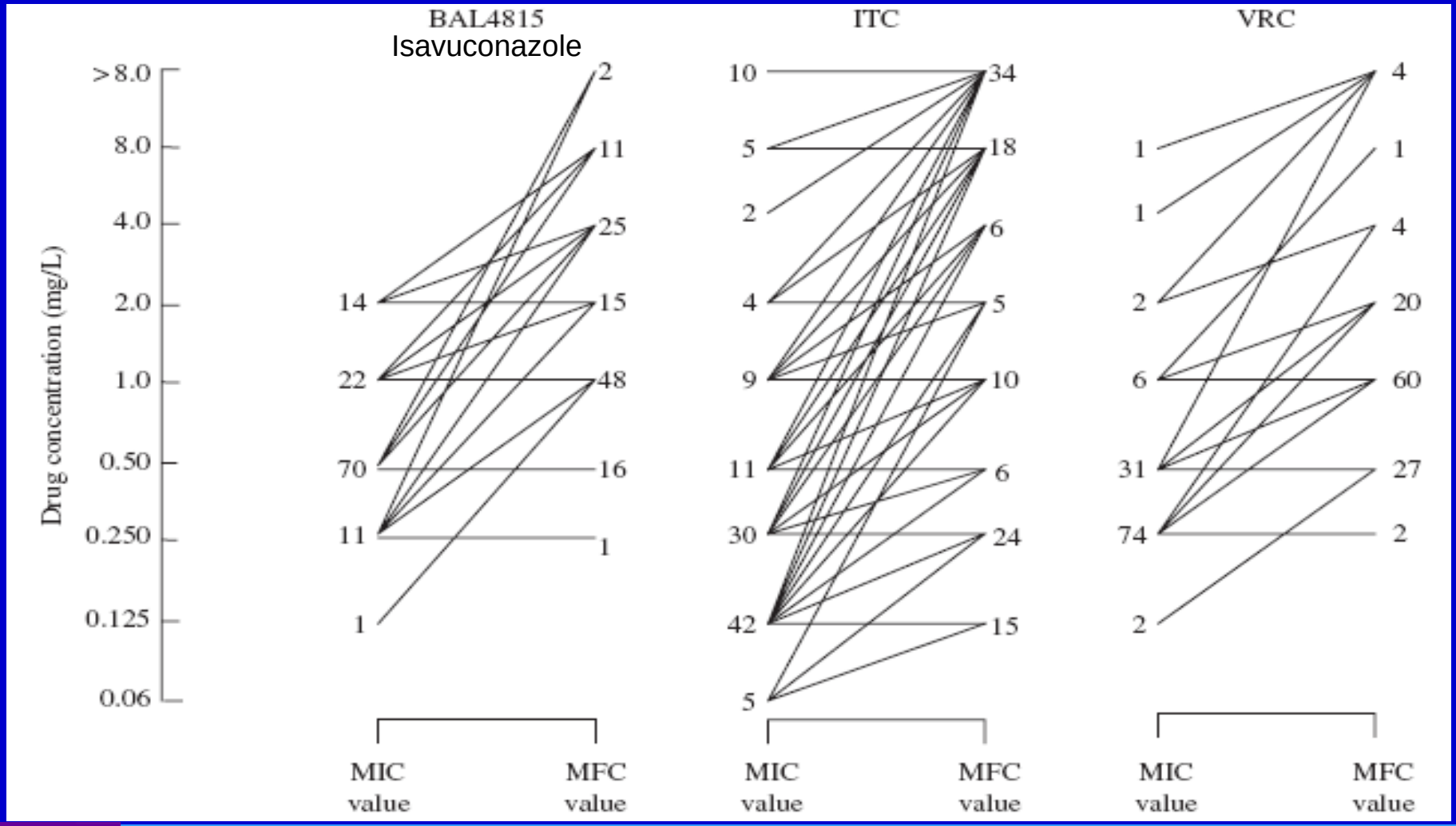
Up-regulation of CYP51A when exposed to itraconazole



AF41 is itraconazole susceptible and isogenic with AF72 and isolated from the same patient prior to itraconazole therapy

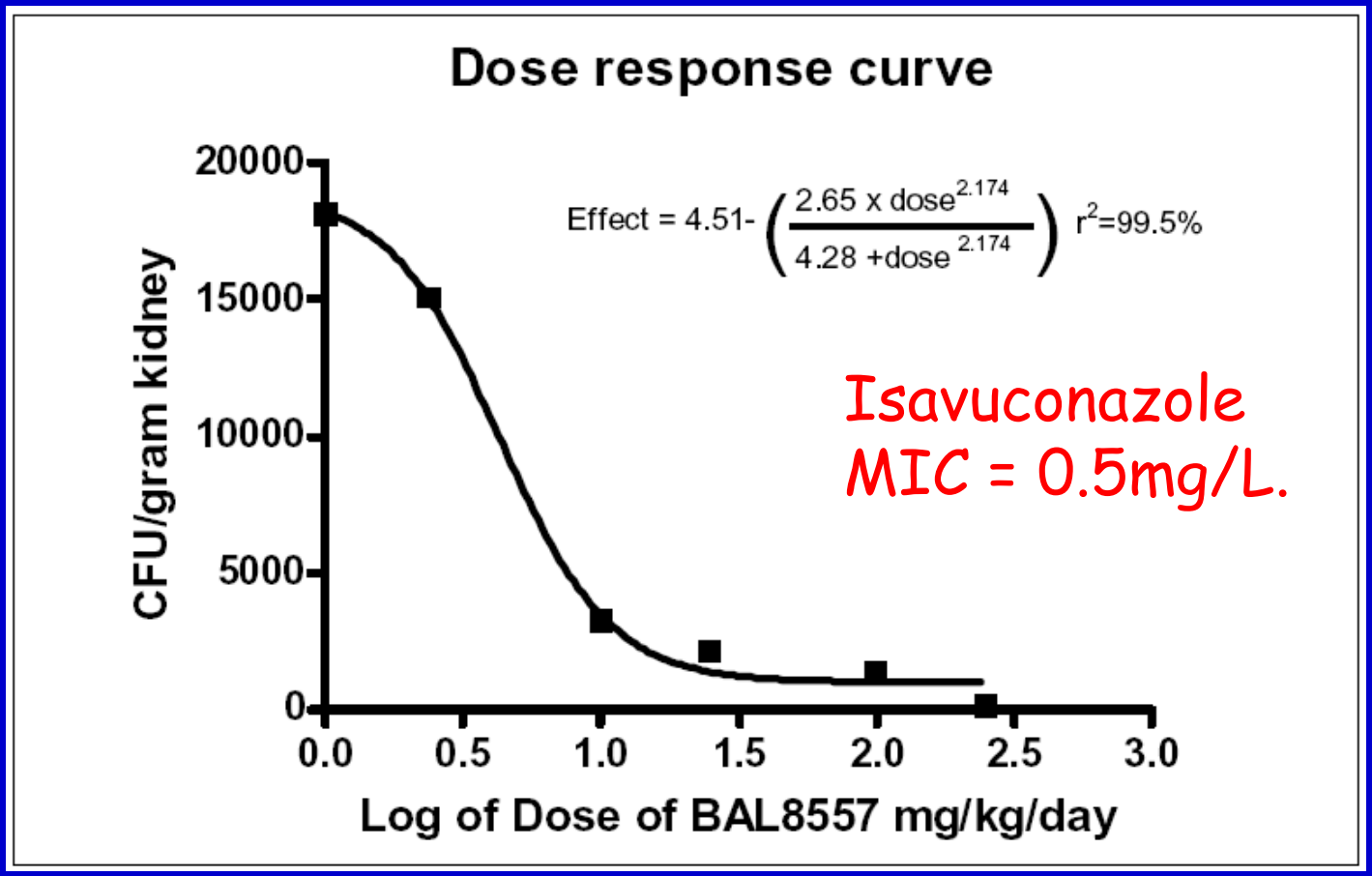
Al-Barraq et al, unpublished; Denning et al, AAC 1997;41:1364

Need to define breakpoints for voriconazole, posaconazole and isavuconazole – using Pk/Pd



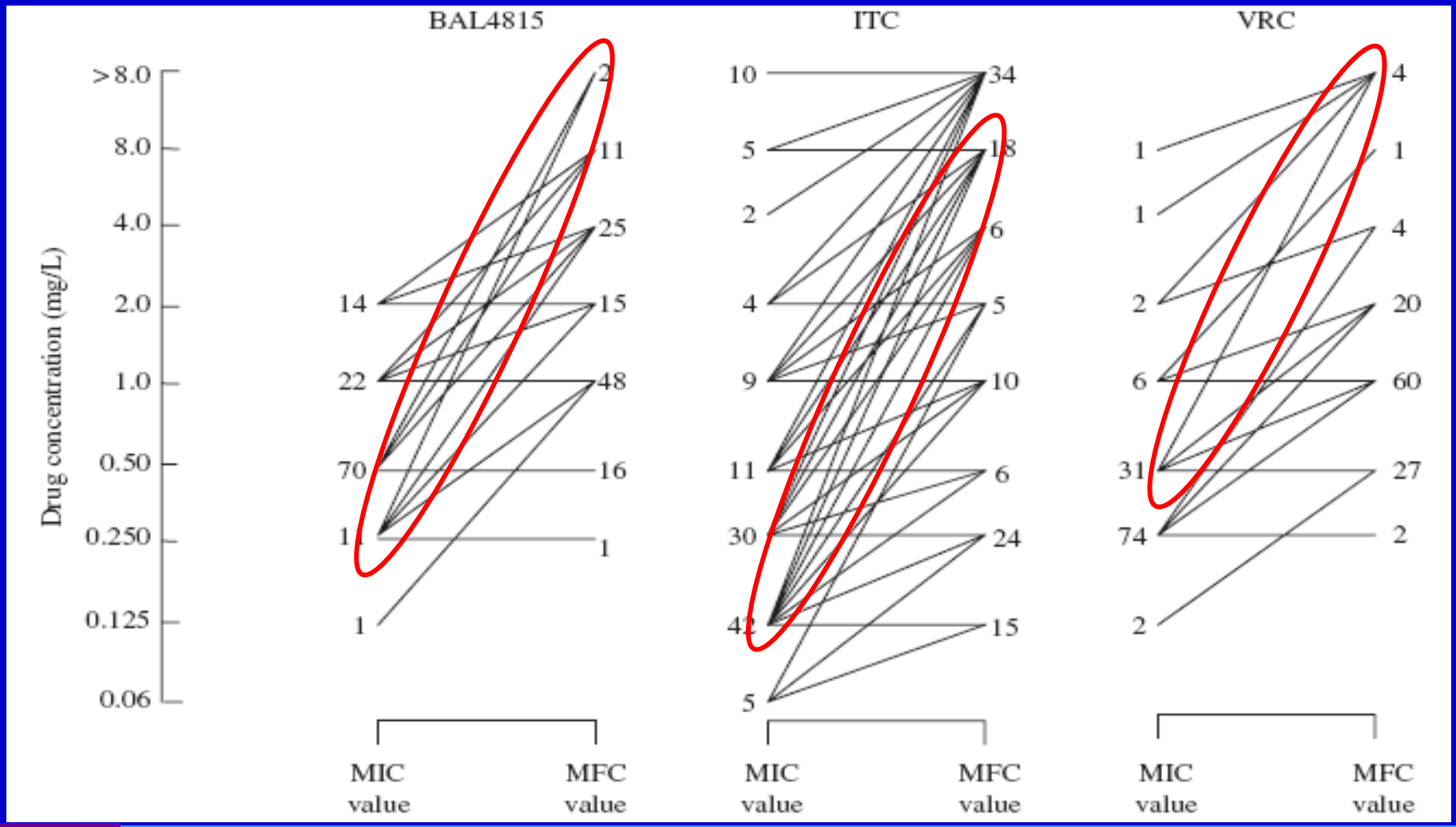
Warn et al, JAC 2006;57:135

Need to define breakpoints for voriconazole, posaconazole and isavuconazole - using Pk/Pd



Warn et al, ICAAC 2006; Abstr A-1120

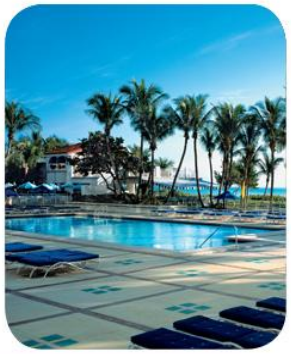
Are minimum fungicidal concentrations relevant?



Warn et al, JAC 2006;57:135

Conclusions

- Azole resistance in Aspergilli exists and is clinically relevant
- Resistance to itraconazole more common with variable patterns of azole cross resistance
- There is a need to define breakpoints for clinical and epidemiological purposes
- Mechanisms of resistance multiple, even in the same patient
- Genotypic markers of resistance may be faster and possibly more precise than MIC testing



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