D.U. Pathologies infectieuses : de la prévention à la thérapeutique

Les antifongiques : cibles, mécanismes d'action, pharmacocinétique et spectre

Résistance des agents fongiques aux antifongiques

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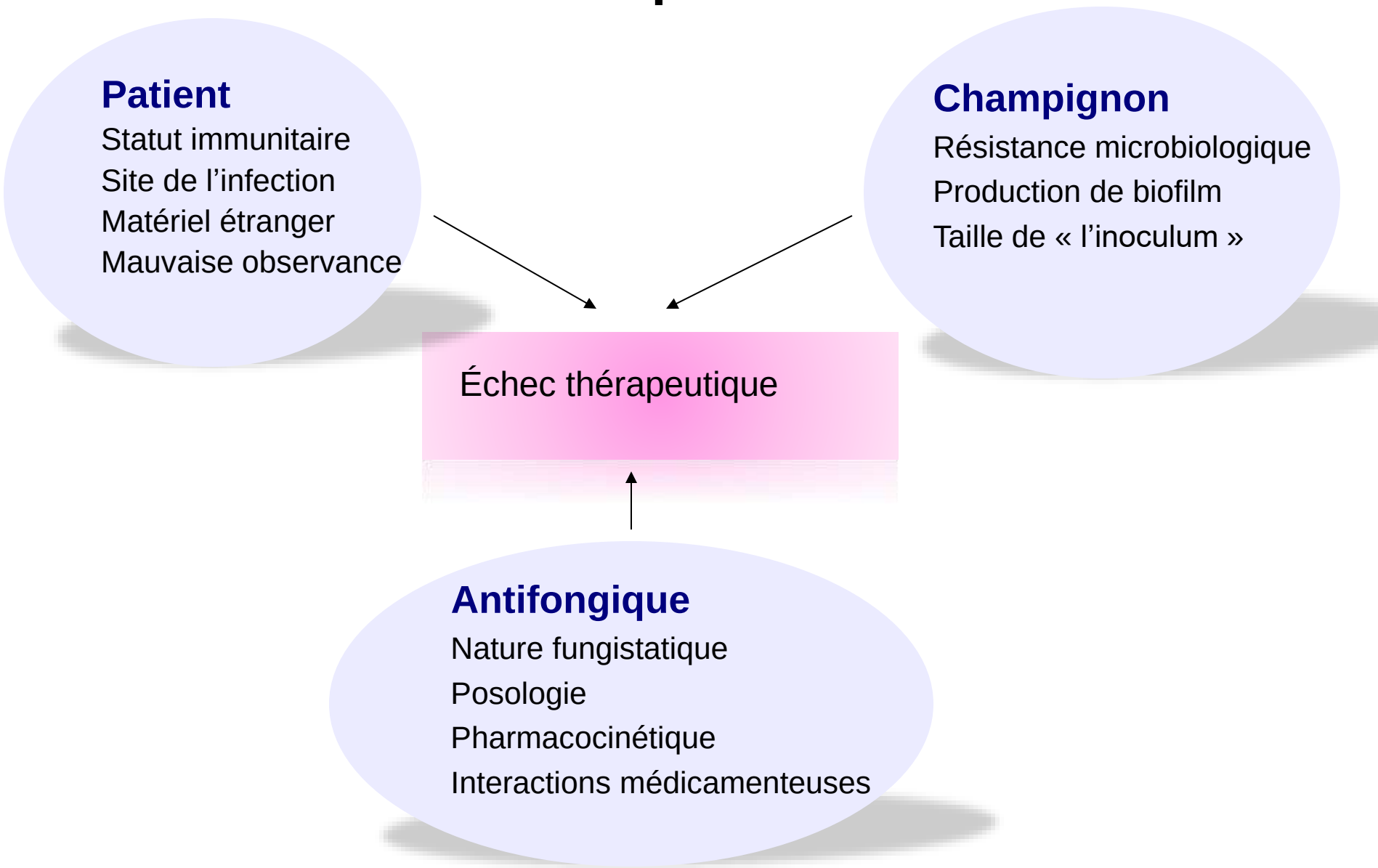
Université Paris Cité

La résistance clinique est multifactorielle



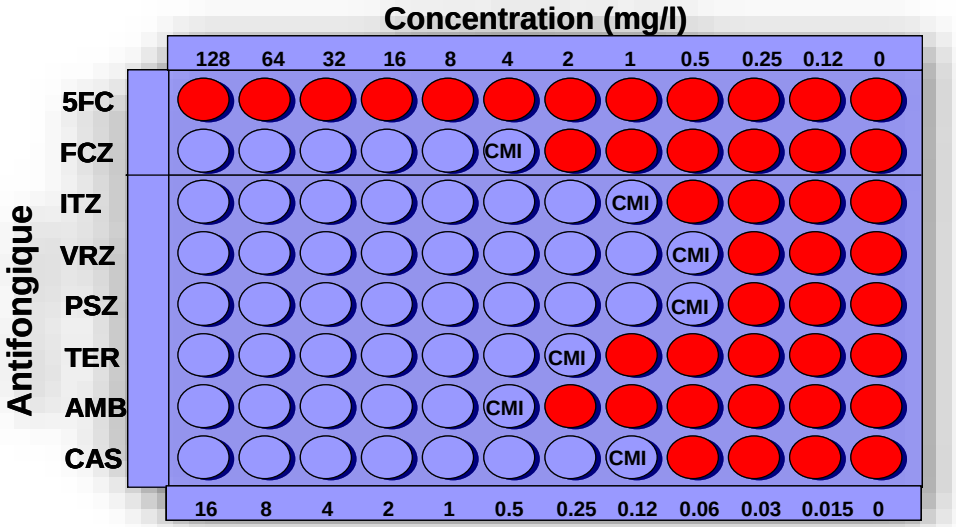
Ne pas confondre résistance clinique et résistance microbiologique

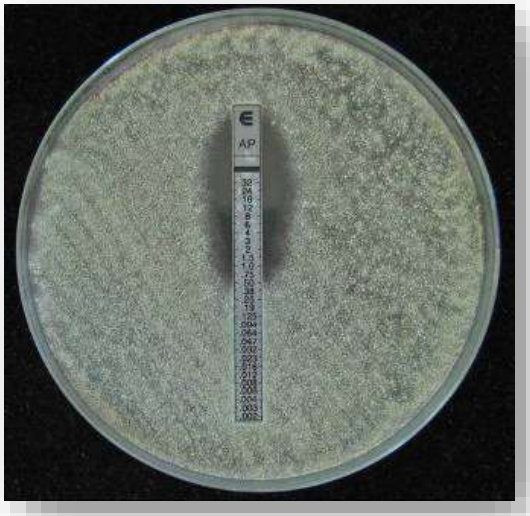
Comment tester la résistance?

Antifongiques systémiques : Comment tester sensibilité / résistance

1. In vitro

✓ Inhibition de pousse : détermination d'une CMI





✓ Courbe de fungicidie

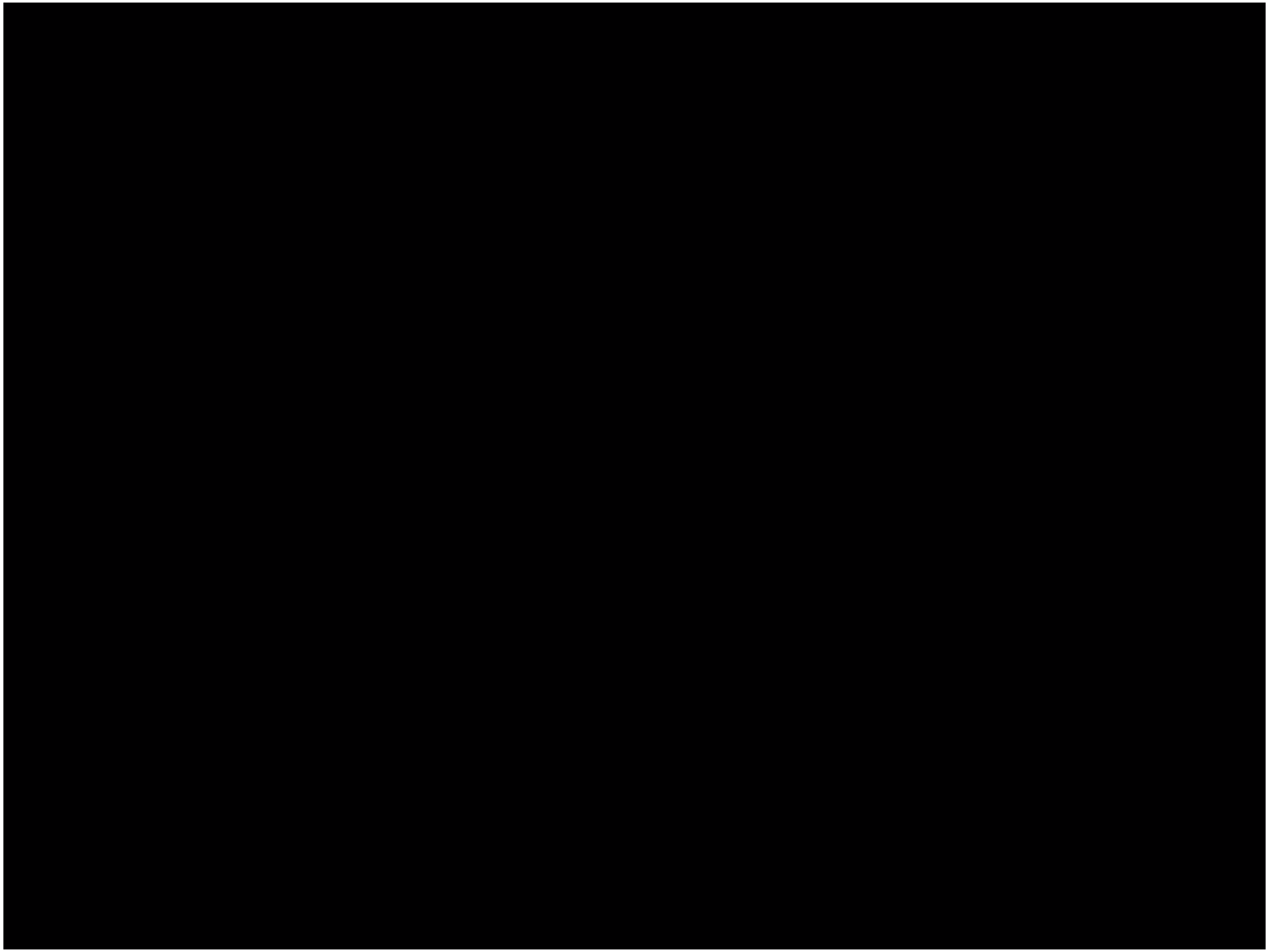
✓ Marqueurs fluorescent de viabilité (CFDA, DIBAC)

2. Animal : modèles expérimentaux

3. Patients: études cliniques

La technique du Etest | Préparation de l'inoculum | Ensemence ment | Incubation | lecture

Gradient exponentiel d'antifongique



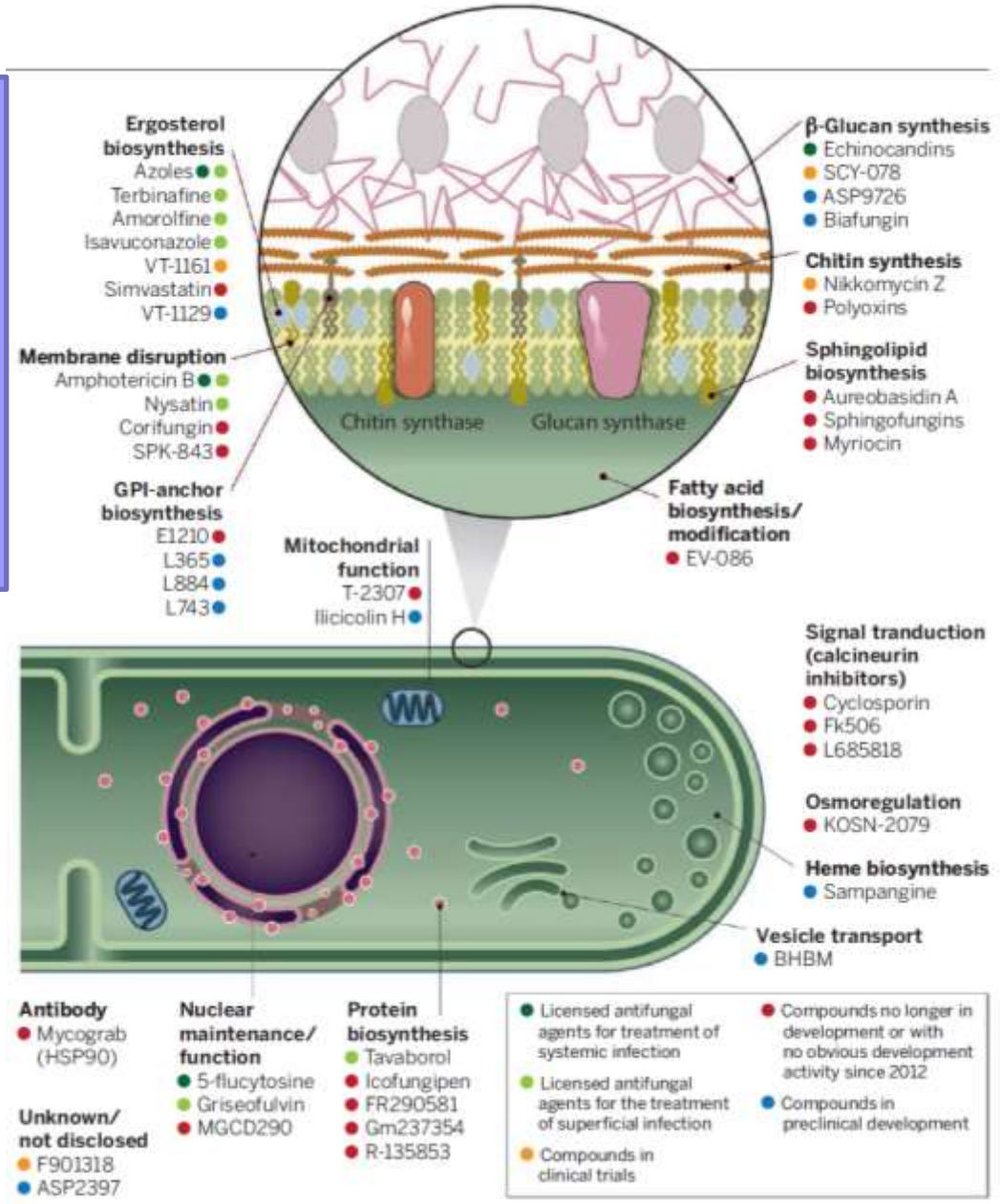
Antifongiques systémiques : les différentes familles

Polyènes	Azolés	Echinocandines	Pyrimidine
Amphotéricine B	Fluconazole	Caspofungine	Flucytosine
	Itraconazole	Micafungine	
	Voriconazole	Anidulafungine	
	Posaconazole		
	Isavuconazole		

Antifongiques infections superficielles: les différentes familles

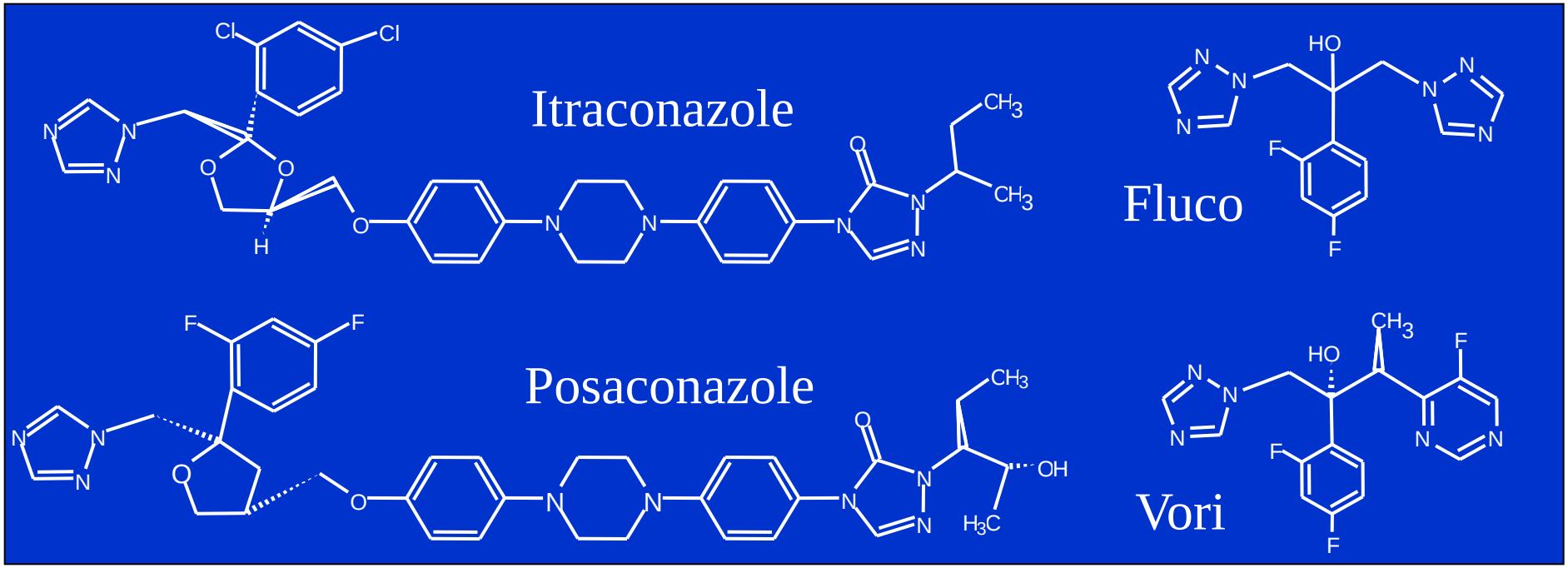
Polyènes	Amphotéricine B	Nystatine		
Azolés	Bifonazole	Clotrimazole	Econazole	Fenticonazole
	Isoconazole	Ketoconazole	Miconazole	Omoconazole
	Oxiconazole	Sertaconazole	Sulconazole	Tioconazole
Allylamines Thiocarbamates	Terbinafine	Tolnaftate		
Morpholines	Amorolfine			
Inhibiteurs mitotiques	Griseofulvine			
Autres	Acide undécylénique	Ciclopiroxolamine	Tavaborole	

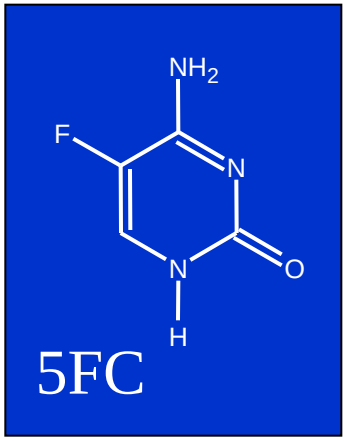
Few drugs are coming to market, but opportunities for drug development exist

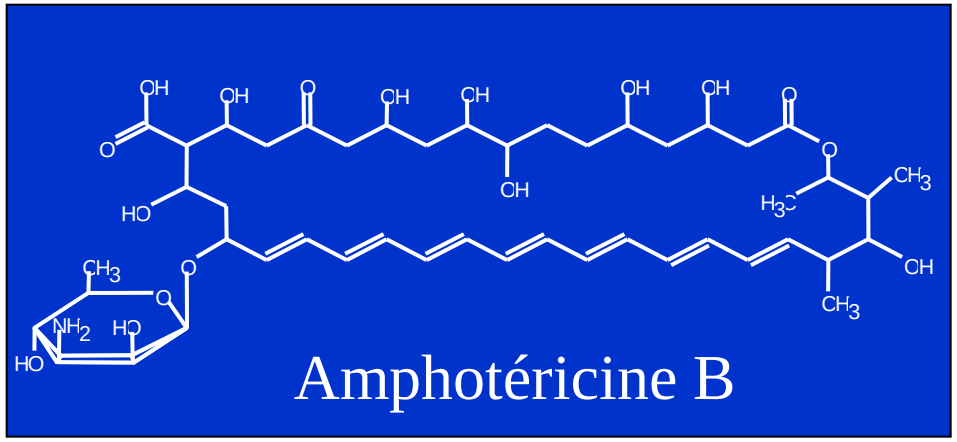


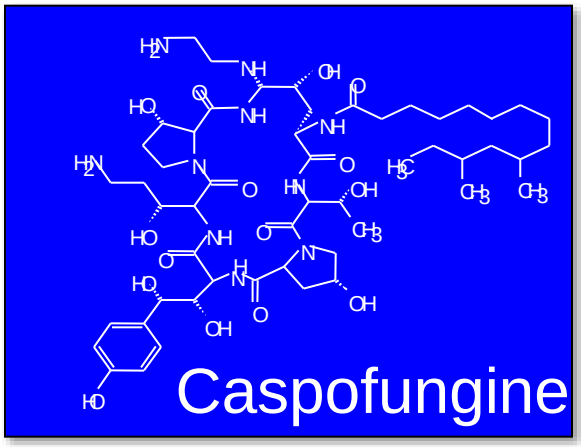
Denning DW, Bromley MJ. 2015. Science 347:1414-1416.

Azolés, Polyènes, 5FC

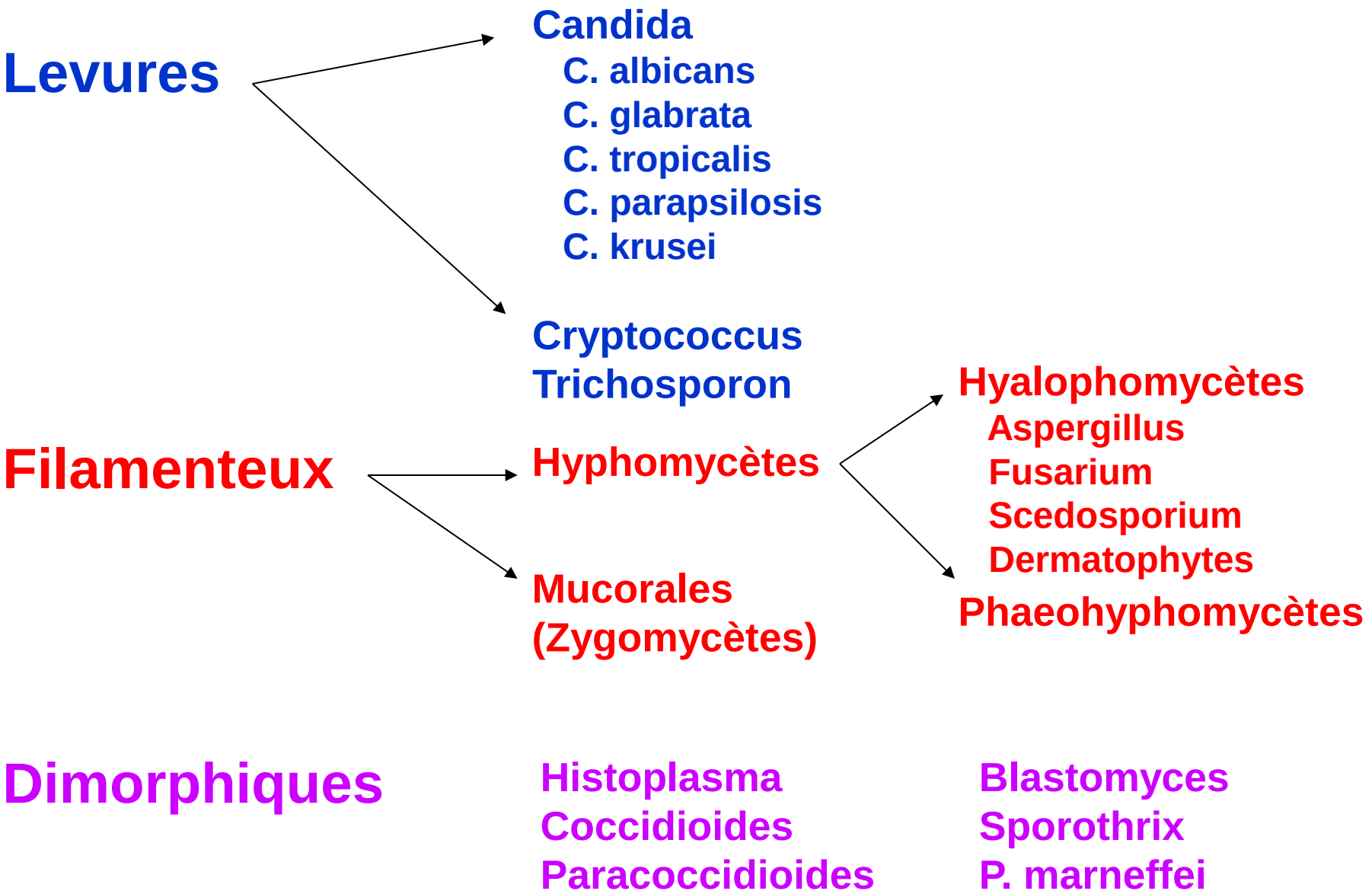








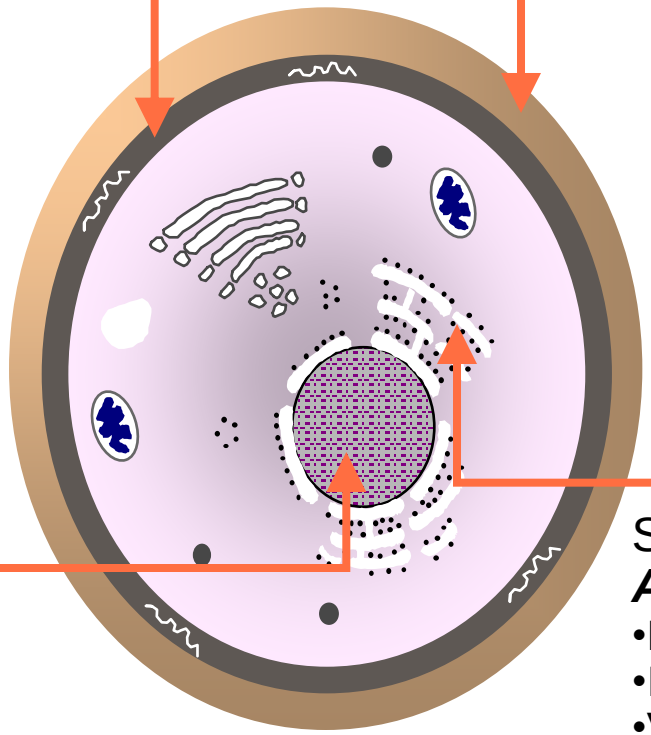
Les différents champignons d'intérêt médical



Mode d'action des antifongiques : overview

Membrane
Polyènes
•Fungizone
•AmBisome
•Abelcet

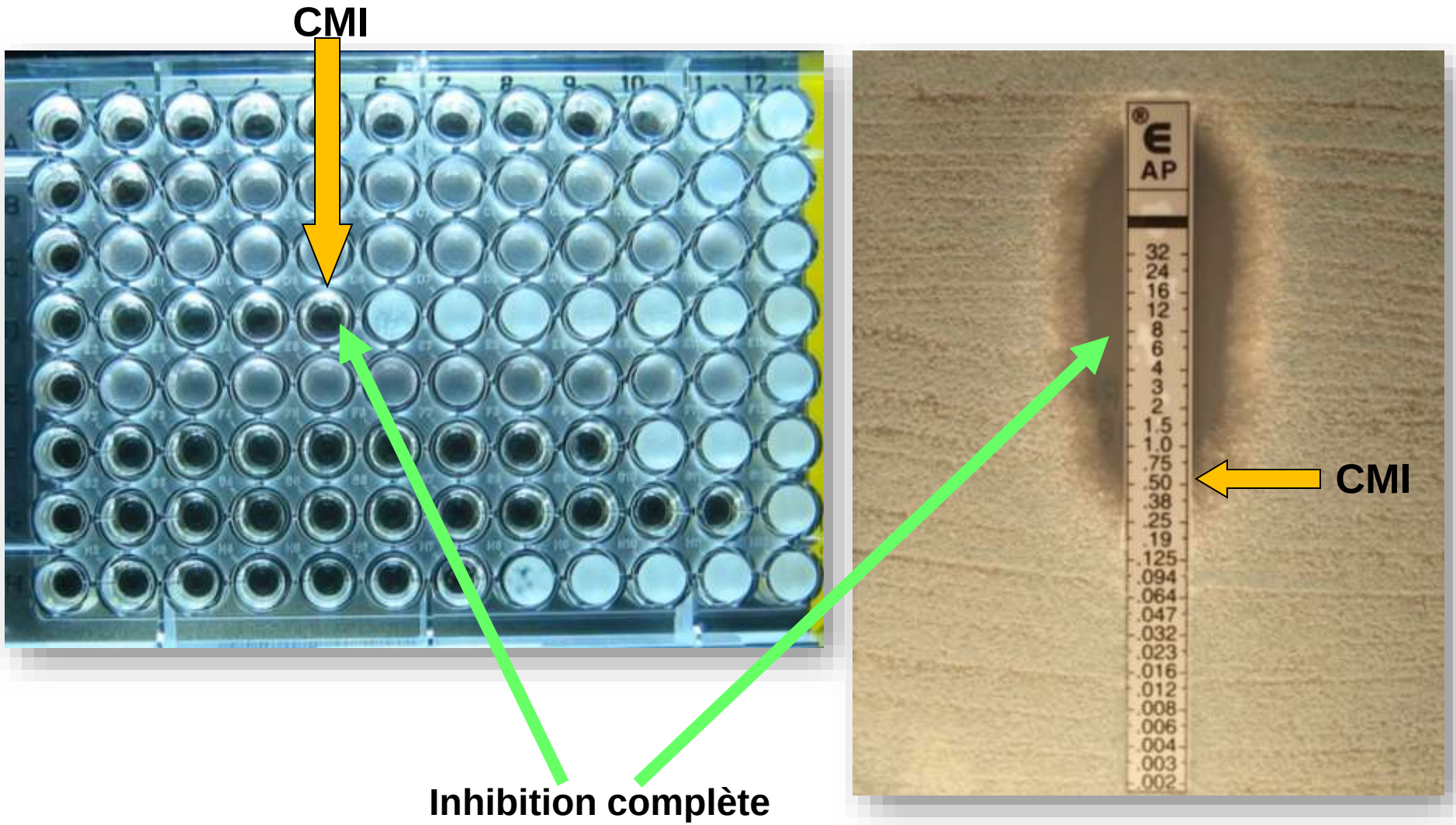
Paroi
Echinocandines
•Caspofungin
•Micafungin
•Anidulafungin



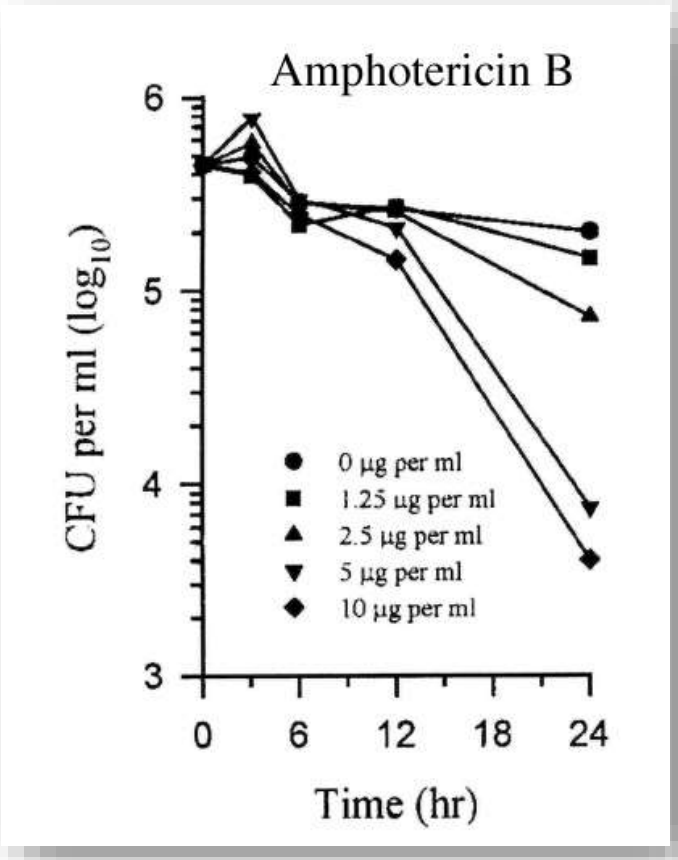
Synthèse d 'Ac. nucléiques
analogues de pyrimidine
•5-Fluorocytosine

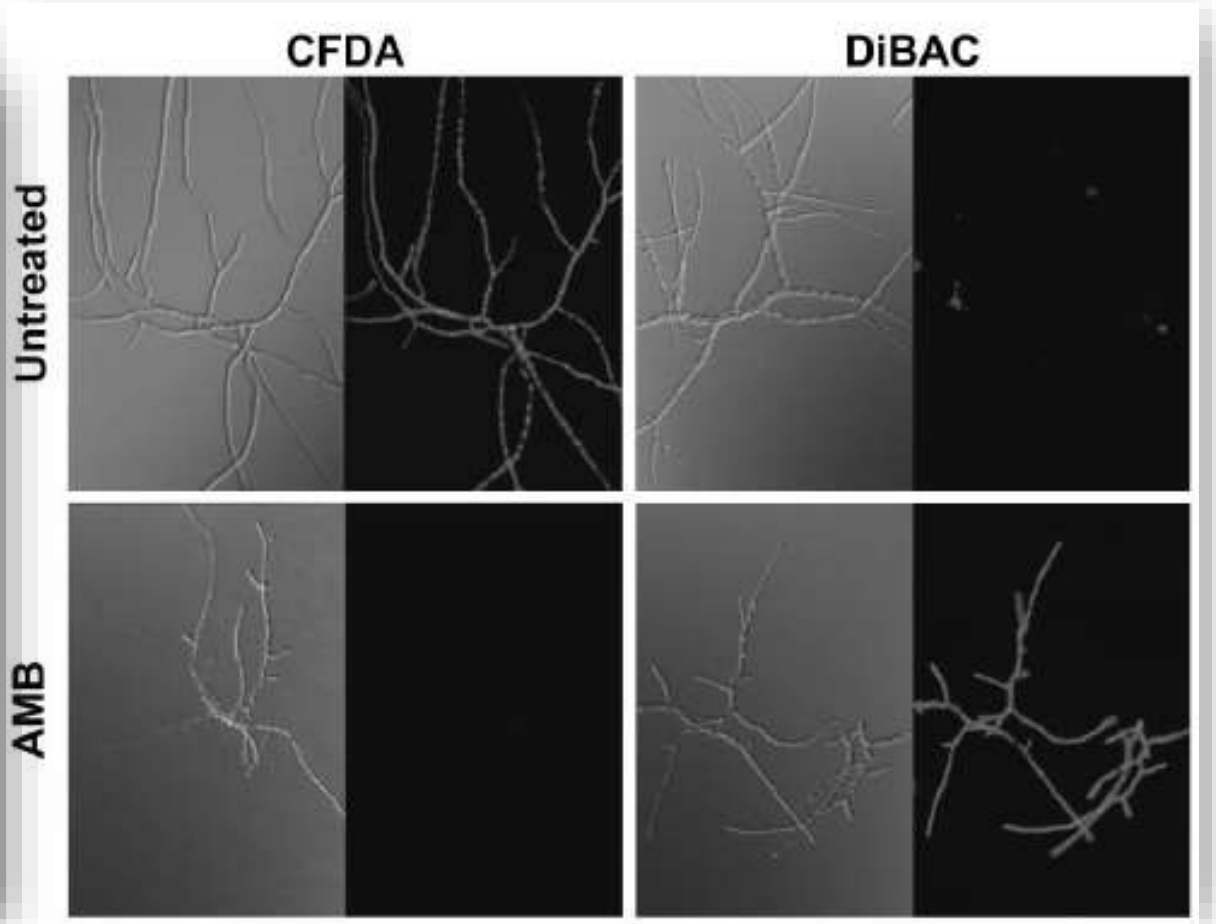
Synthèse des stérols
Azoles
•Fluconazole
•Itraconazole
•Voriconazole
•Posaconazole
•Isavuconazole
Allylamines
Terbinafine

Activité des antifongiques : AMB



Activité des antifongiques : AMB



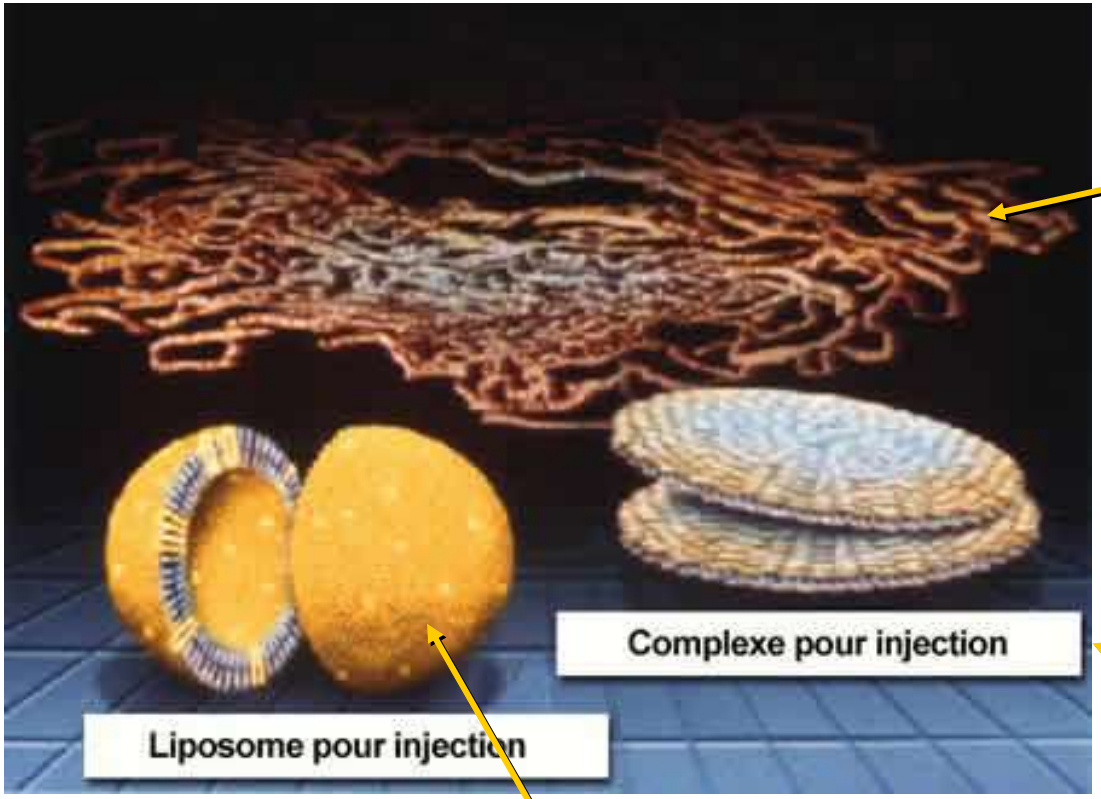


Fungicide sur *Aspergillus fumigatus*

Manavathu et al. Antimicrob Agents Chemother 1998; 42:3018-3021

Bowman, J. C., et al 2002. Antimicrob Agents Chemother 46:3001-3012.

AMB : formulations lipidiques



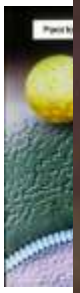
Amphotéricine B
complexe lipidique
Abelcet®
1600 à 6000 nm

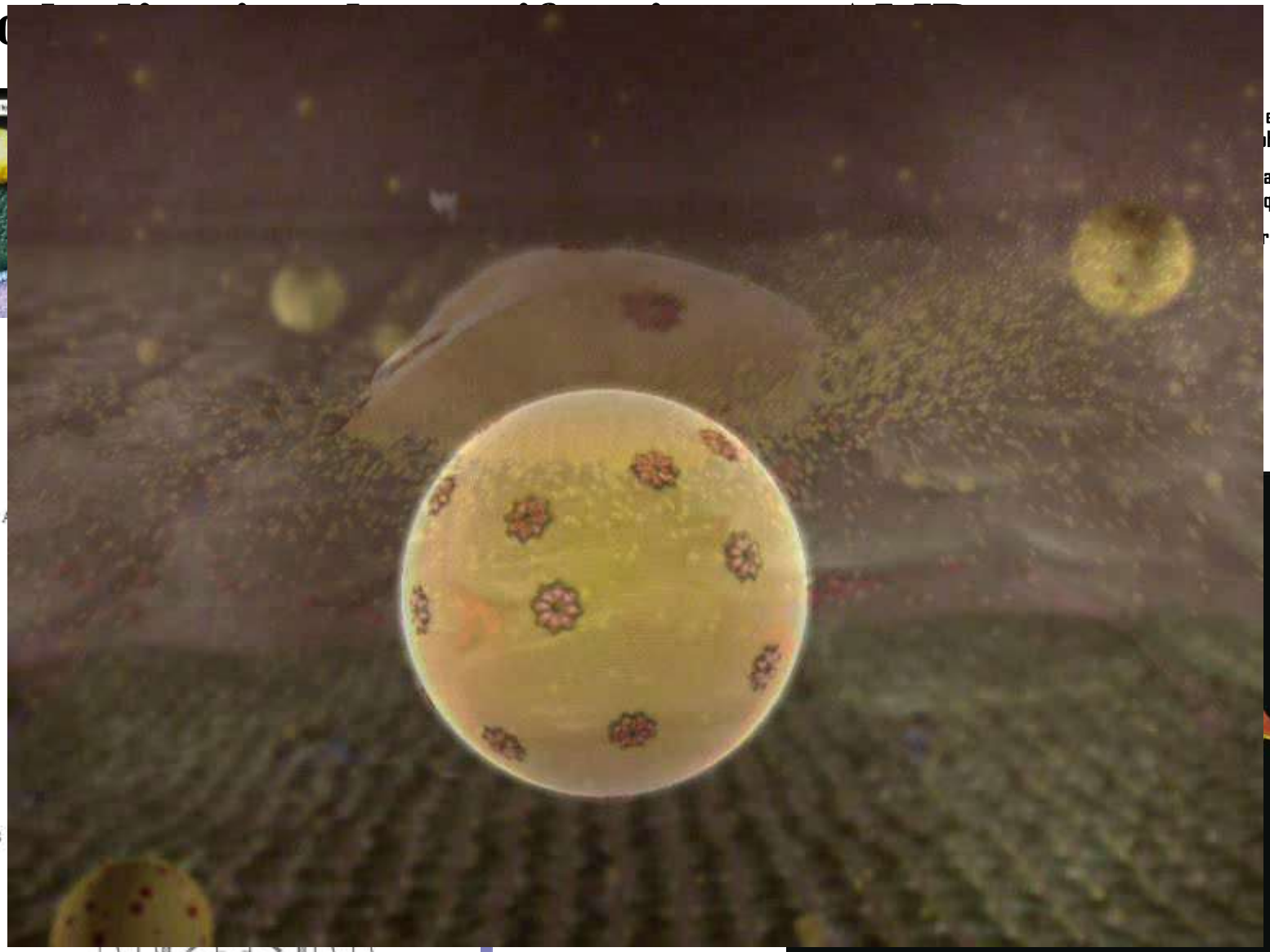
Amphotéricine B
dispersion colloïdale
Amphocil®
122 nm

Amphotéricine B
liposomale
AmBisome®
< 100 nm

Antifongiques : mode d'action

Mo





en contact de
hotéricine B

ation de la membrane
que

ruction de la cellule

B

Ghannoum MA, Rice LB. Clin Microbiol Rev 1999; **12**:501-517.

Gruszecki. FEBS Letters 2002, **524**:92-96

PK/PD

Paramètre PK	AMB (AMB Lipo)
Biodisponibilité orale, %	<5
Voie d'administration	IV
Liaison protéines	>95
½ vie (h)	100-150
TDM	non

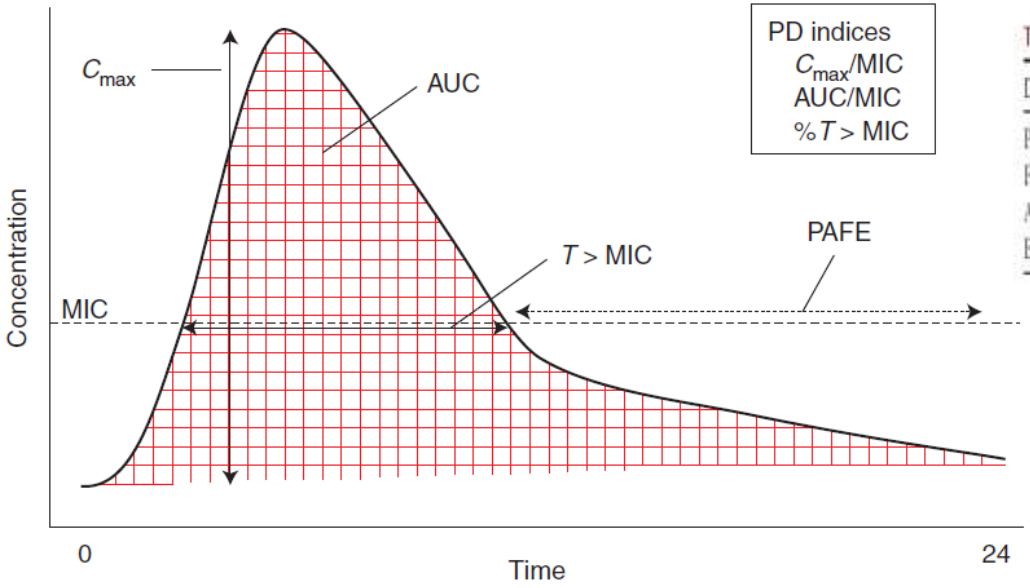
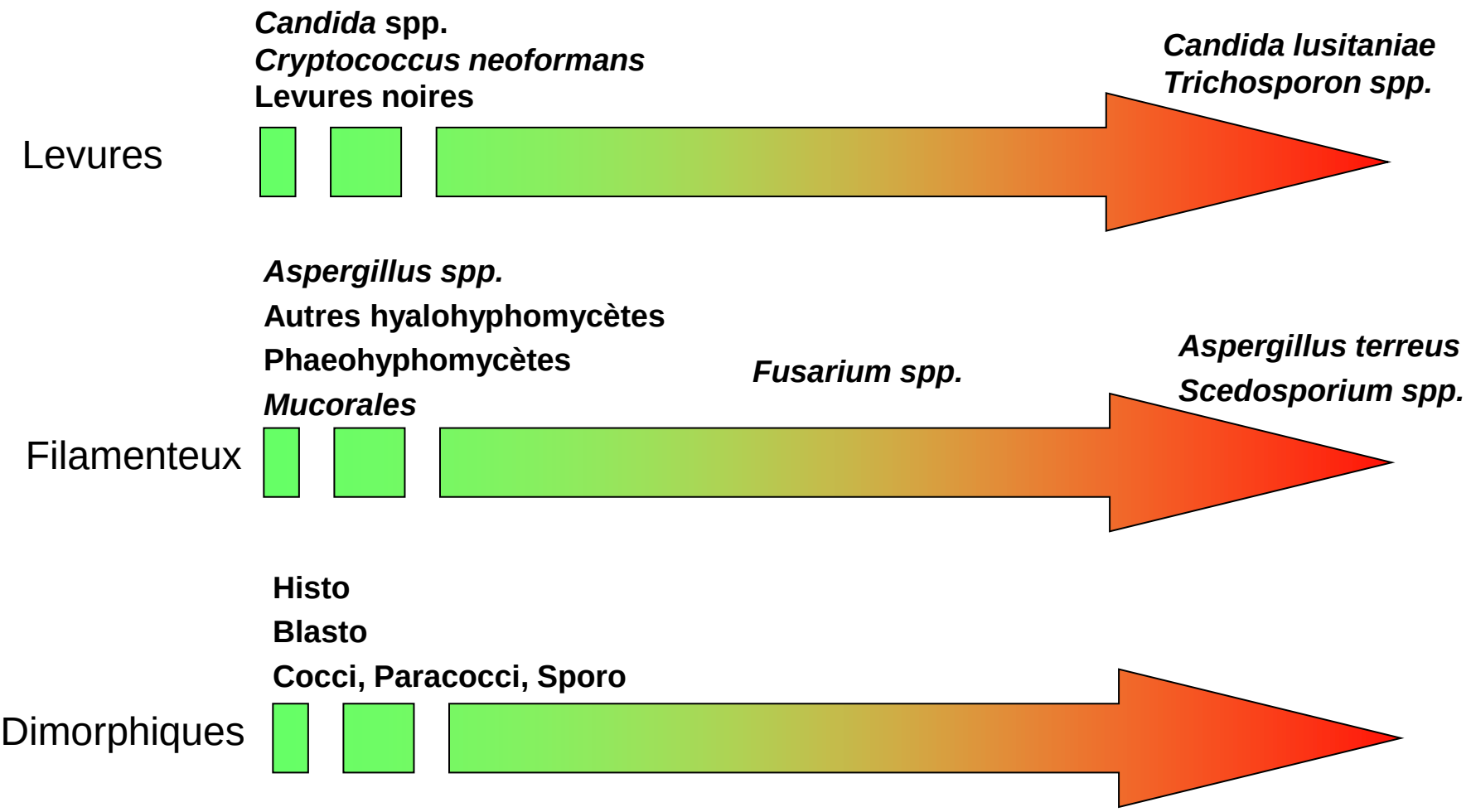


Table 2. The major PD characteristics by fungal drug class

Drug class	Concentration dependent	Prolonged PAFE	PD index predictive of efficacy
Polyene	Yes	Yes	C_{max}/MIC
Flucytosine	No	No	T > MIC
Azoles	No	Yes	AUC/MIC
Echinocandins	Yes	Yes	C_{max}/MIC or AUC/MIC

Cold Spring Harb Perspect Med doi:10.1101/cshperspect.a019653

Amphotéricine B – spectre très large



Amphotéricine B - Indications

Traitement de choix pour:

- Méningite à *C. neoformans*
- Mucormycoses (zygomycoses)
- Infections fongiques invasives ne répondant pas à un autre traitement

Mécanismes de résistance à l'AMB (ex: R intrinsèque *A. terreus*): quelques hypothèses

- Limitation de l'accès à l'ergostérol : modifications de la paroi ?
- Diminution / disparition de l'ergostérol membranaire ?
- Inhibition des mécanismes oxydatifs ?

Mécanismes de résistance à l'AMB

- Limitation de l'accès à l'ergostérol : modifications de la paroi ?

Espèce	CMI amphotéricine B	
	conidies	protoplastes
A. fumigatus	**0.21**	**0. 19**
A. terreus	**1.7**	**1.41**

- Diminution / disparition de l'ergostérol membranaire ?

Stérol	Espèce	
	A.terreus	*A. fumigatus*
Ergosterol	**85.2**	**75.5-78.9**
24-ethyl-cholesta-5,7,22-trienol	10.1	12.7-19.4
Episterol	1.5	0-1.9
Ergosta-5,8,22-trienol	1.4	2.0-2.3
Unknown sterols	1.8	1.8-4.5

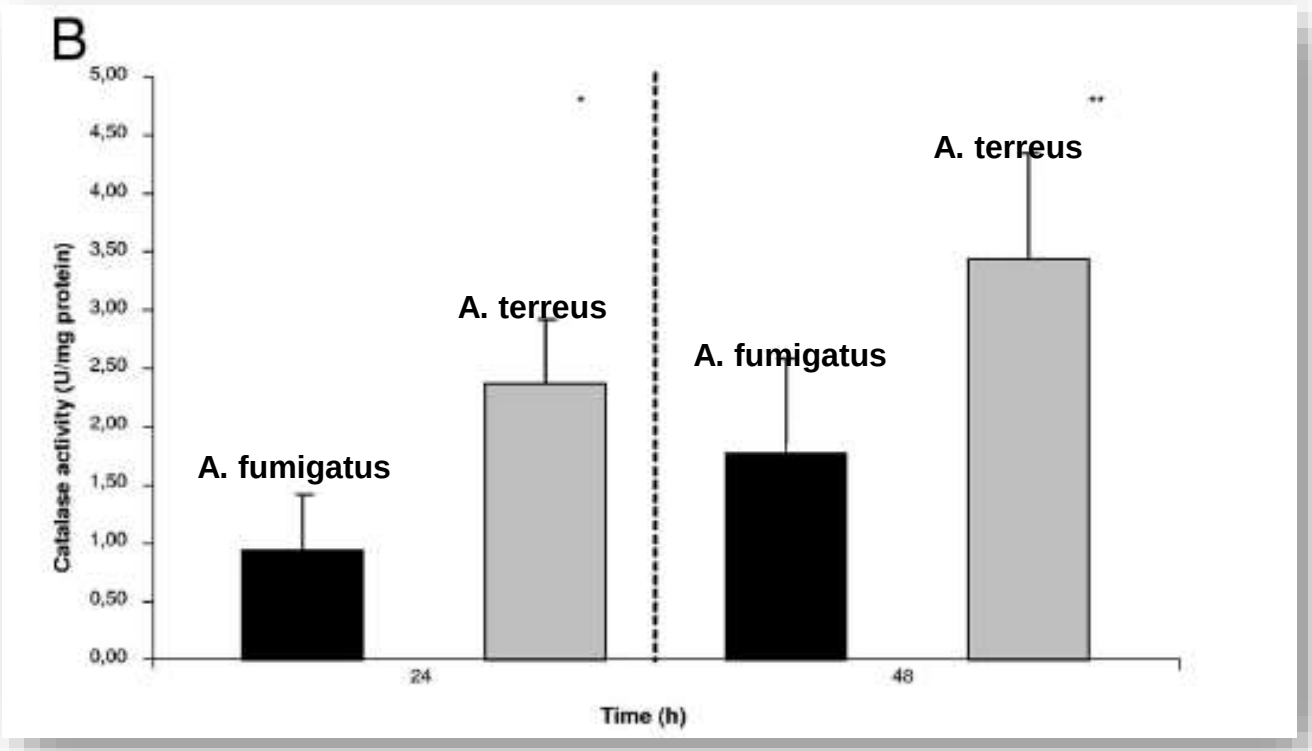
Dannaoui, E., et al. 2000. J. Med. Microbiol. 49:601-6.
Dannaoui, E., et al. 2001 Can J Microbiol. 47:706-10.
Blum, et al. 2008. Antimicrob Agents Chemother 52:1553-5.

Mécanismes de résistance à l'AMB: quelques hypothèses

- Inhibition des mécanismes oxydatifs ?

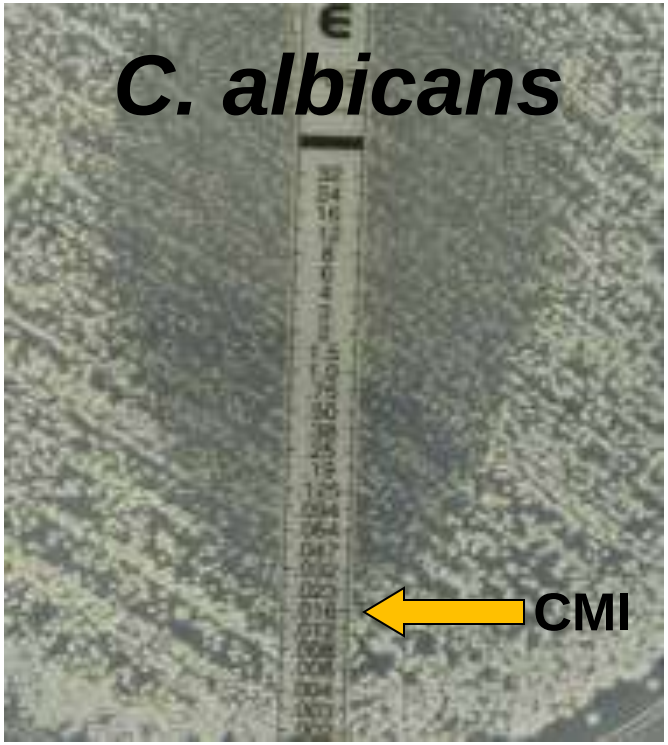
1. Peroxydation des lipides induite par AMB plus importante pour *A. fumigatus* vs *A. terreus*

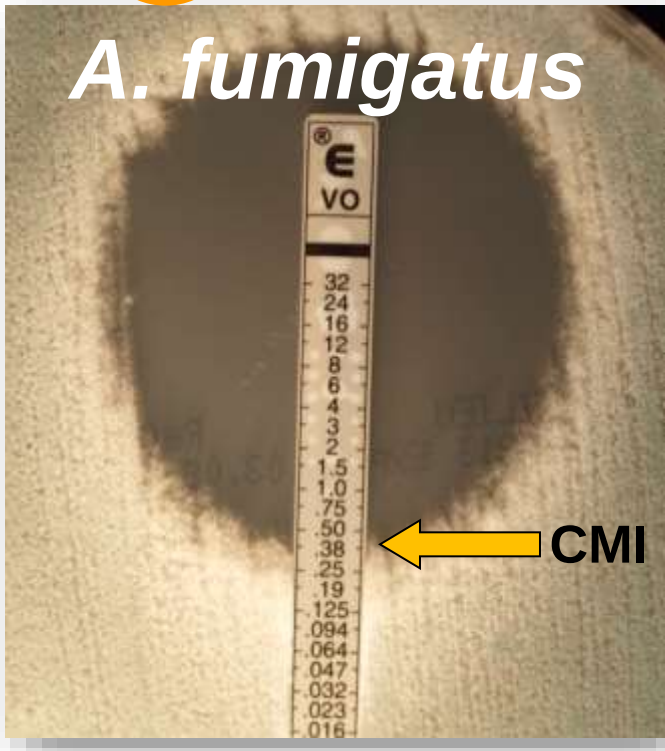
2. Activité catalase



Blum, et al. 2008. Antimicrob Agents Chemother 52:1553-5.

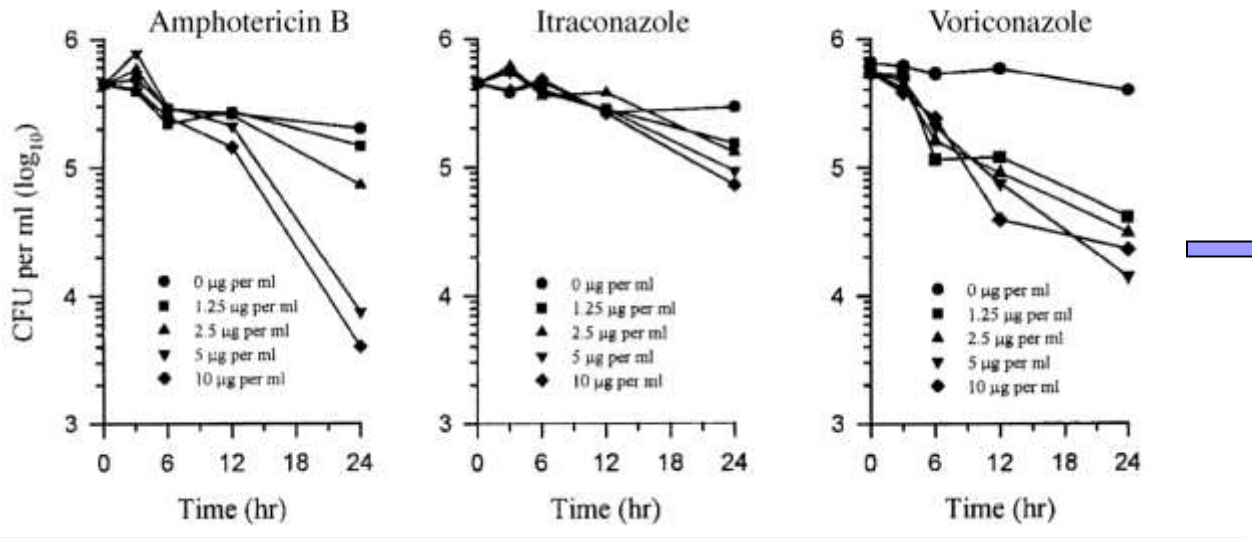
Activité des antifongiques : Azolés





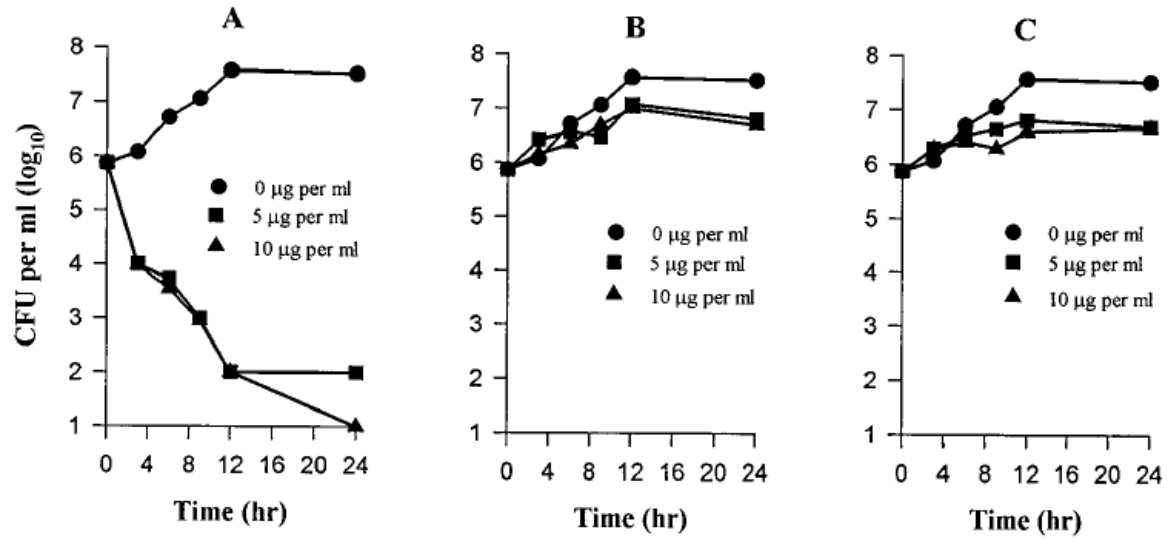
Activité des antifongiques : Azolés

Aspergillus



Fungicide sur *Aspergillus fumigatus*

Candida

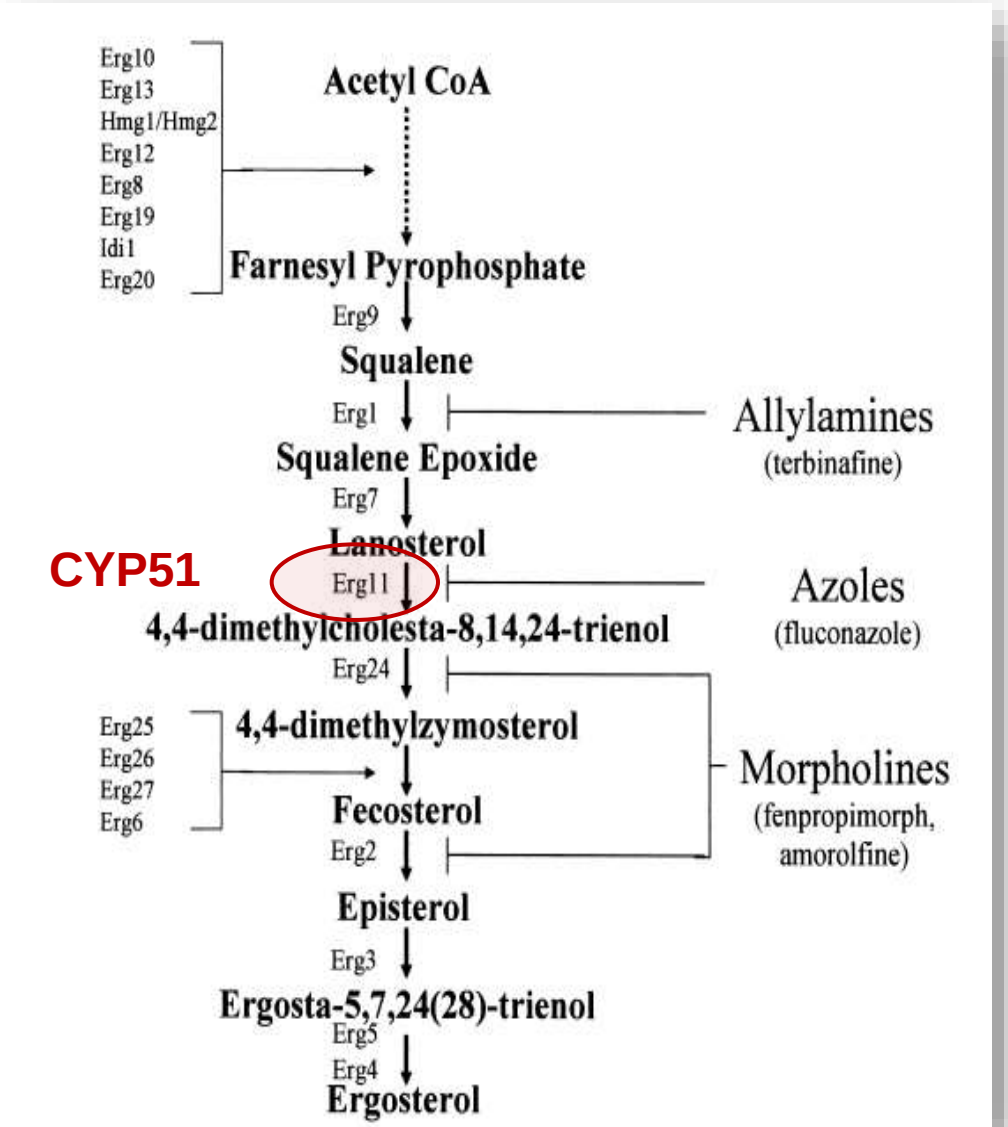


Fungistatique sur *Candida albicans*

Manavathu et al. Antimicrob Agents Chemother 1998; 42:3018-3021

Azoles – Mechanism of action

- **Cytochrome P450-enzyme lanosterol 14-alpha demethylase** responsible for conversion of lanosterol to ergosterol
- Azoles bind to lanosterol 14-alpha demethylase inhibiting the production of ergosterol
- Various mechanisms of azole resistance in fungi



PK/PD

PK parameter	FCZ	ITZ	VRZ	PSZ	ISA
Biodisponibilité orale, %	95	50	96	ND	>95
Voie d'administration	IV/PO	PO	IV/PO	IV/PO	IV/PO
Liaison proteines	10	99.8	58	99	>98
½ vie (h)	31	24	6	25	56-104
TDM	non	oui	oui	oui	?

Table 2. The major PD characteristics by fungal drug class

Drug class	Concentration dependent	Prolonged PAFE	PD index predictive of efficacy
Polyene	Yes	Yes	C_{max}/MIC
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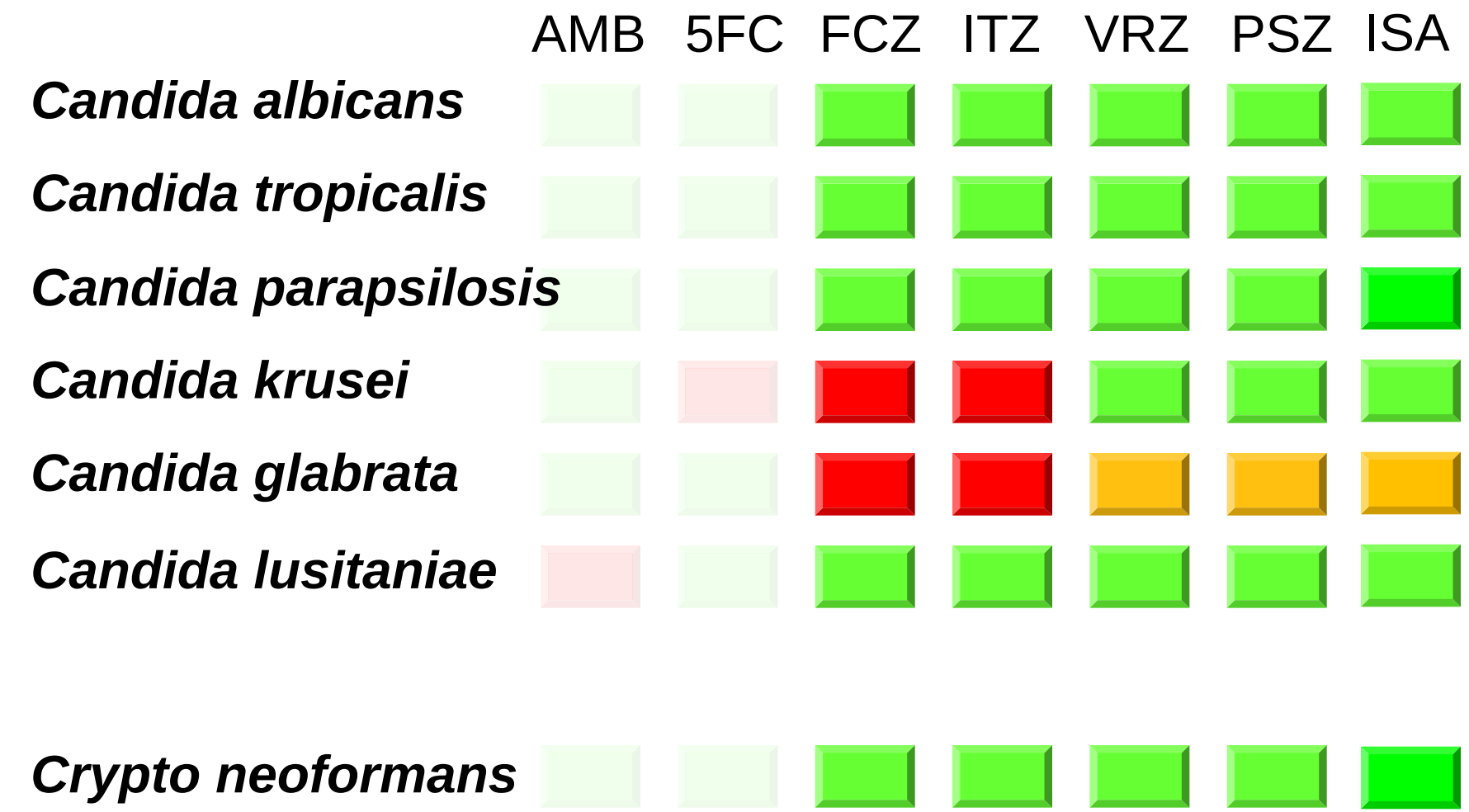
Dodds Ashley et al. 2006. Clin Infect Dis 43:S28-S39.
Seyedmousavi et al. Expert Rev. Anti Infect. Ther. 13(1), 9–27 (2015)

Azoles : same mechanism of action but different spectrum and indications

Azole	Active	Less active	Inactive
Fluconazole	*C. albicans, C. neoformans*	Non-*albicans Candida* (*C. glabrata*)	*C. krusei* Filamentous fungi
Itraconazole	*Aspergillus* spp. Dimorphic - Phaeo *Candida - Cryptococcus*	Non-*albicans Candida*	Mucorales Fusarium spp.
Voriconazole	*C. albicans, C. neoformans* *Aspergillus* *Fusarium*, *Scedosporium*	*C. glabrata*	Mucorales
Posaconazole	*Candida* (*C. neoformans*) *Aspergillus - Fusarium* (R in vitro), *Scedosporium* Dimorphic - Mucorales	*C. glabrata*	
Isavuconazole	*Candida - C. neoformans* *Aspergillus* spp. Mucorales	*Fusarium* *C. glabrata*	

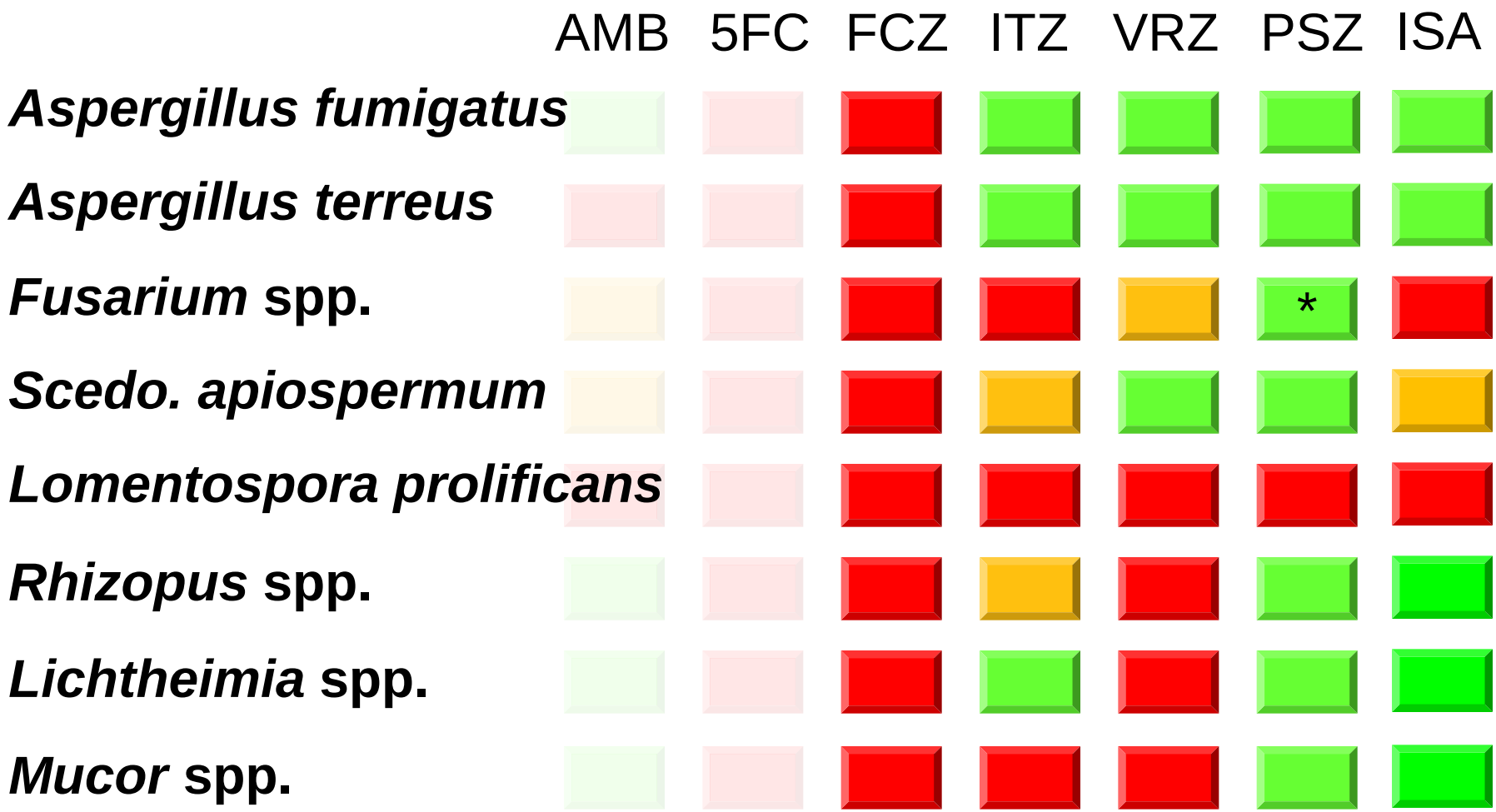
Azolés – spectre variable / molécule

Levures, *Candida, Crypto*



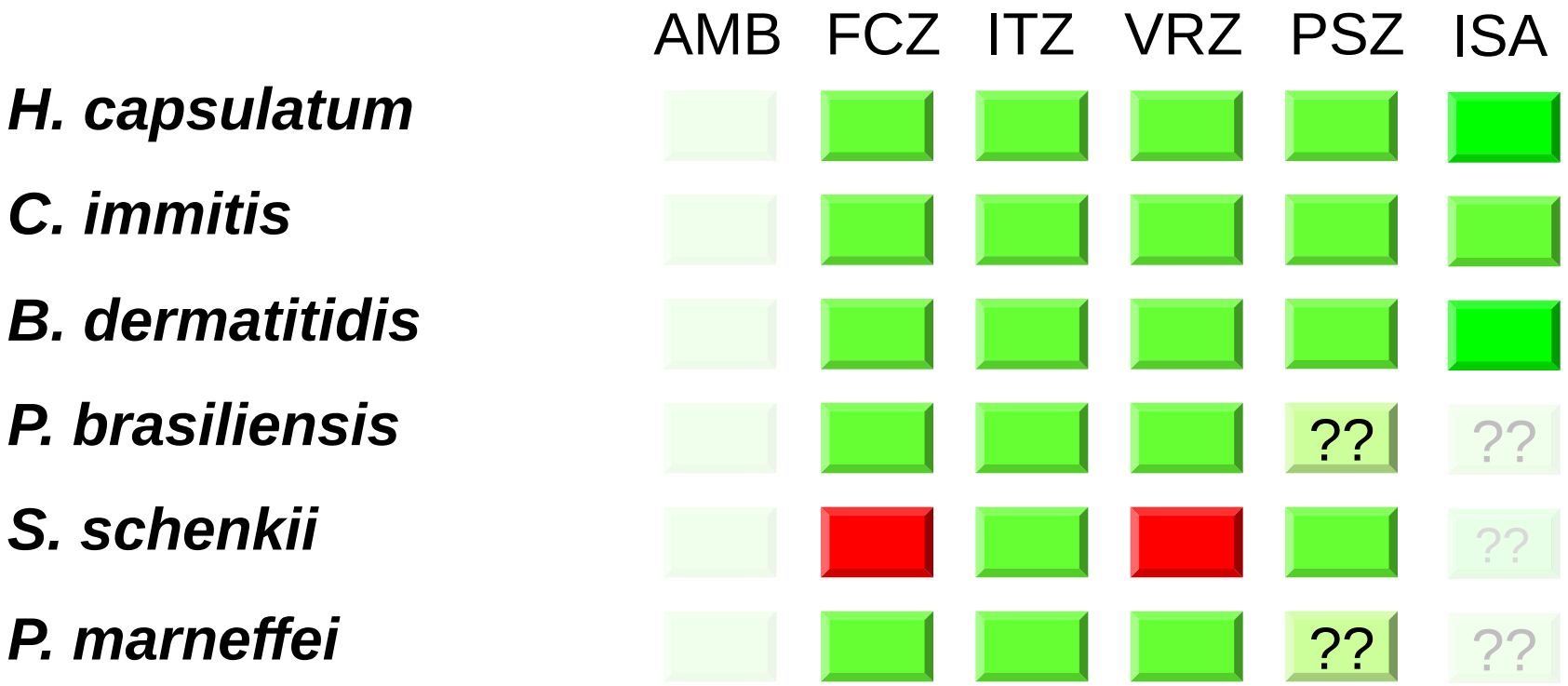
Azolés – spectre variable / molécule

Champignons filamenteux *Aspergillus, Fusarium, Scedo*, Zygo



* Résistant in vitro

Champignons dimorphiques



Azolés – Mécanismes de résistance - Levures

1- Surproduction de la cible CYP51 codée par le gène *ERG11*

*** Augmentation de la transcription de *ERG11* → surexpression**
mRNA augmentés par facteur 3 à 5 (modéré)

*** Amplification génique de *ERG11***
Duplication en plusieurs copies → augmentation mRNA

2 - Modification de la cible : diminution de l ’affinité pour l ’azolé

*** Mutations ponctuelles du gène *ERG11***
Substitution d ’un acide aminé → altération d Azole

Azolés – Mécanismes de résistance - Levures

3- Systèmes d'efflux

*** transporteurs membranaires → pompes**

*** Chez champignons (levures) 2 familles de pompes impliquées dans la résistance aux antifongiques azolés CDR, MDR**

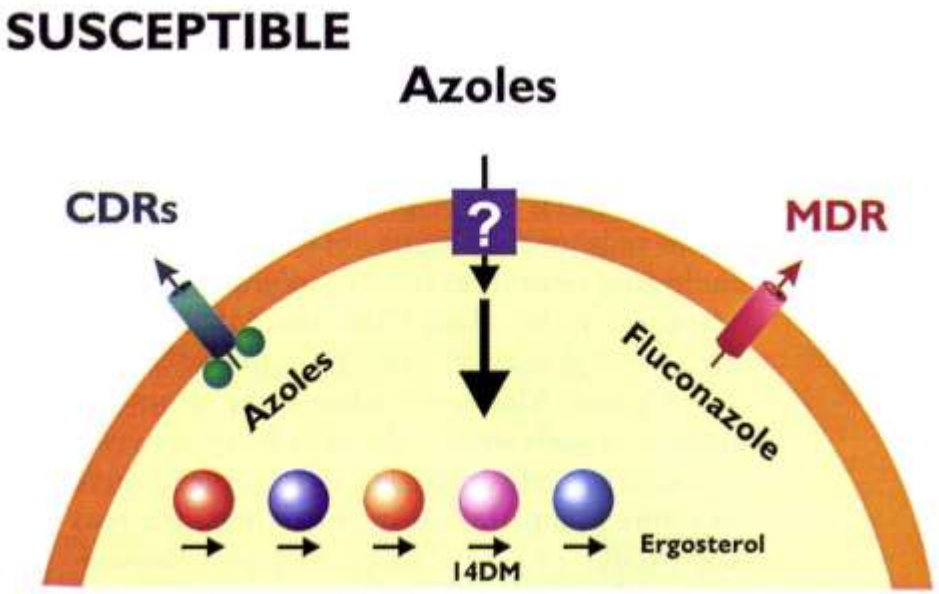
Molecular mechanisms of azole resistance

susceptible cell

azole enter / diffusion.

inhibition Erg11 (pink circle)

efflux pumps expressed at low levels. *CDR* and *MDR*



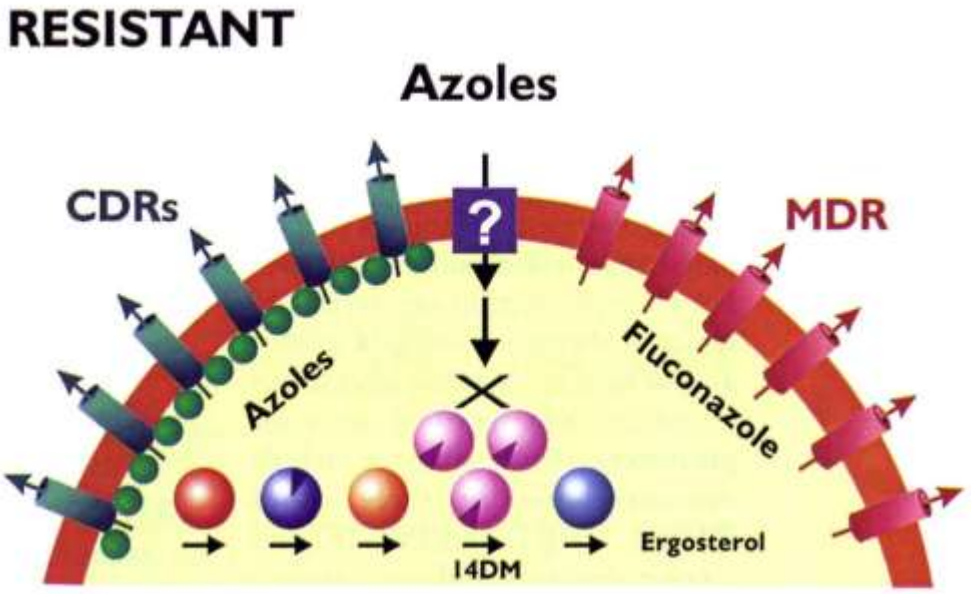
In a "model" resistant

1. azoles less effective against Erg11

   Modification of the enzyme

   Overexpression of the enzyme.

2. azoles removed by overexpression of *CDR* genes (ABCT) and *MDR* (MF).

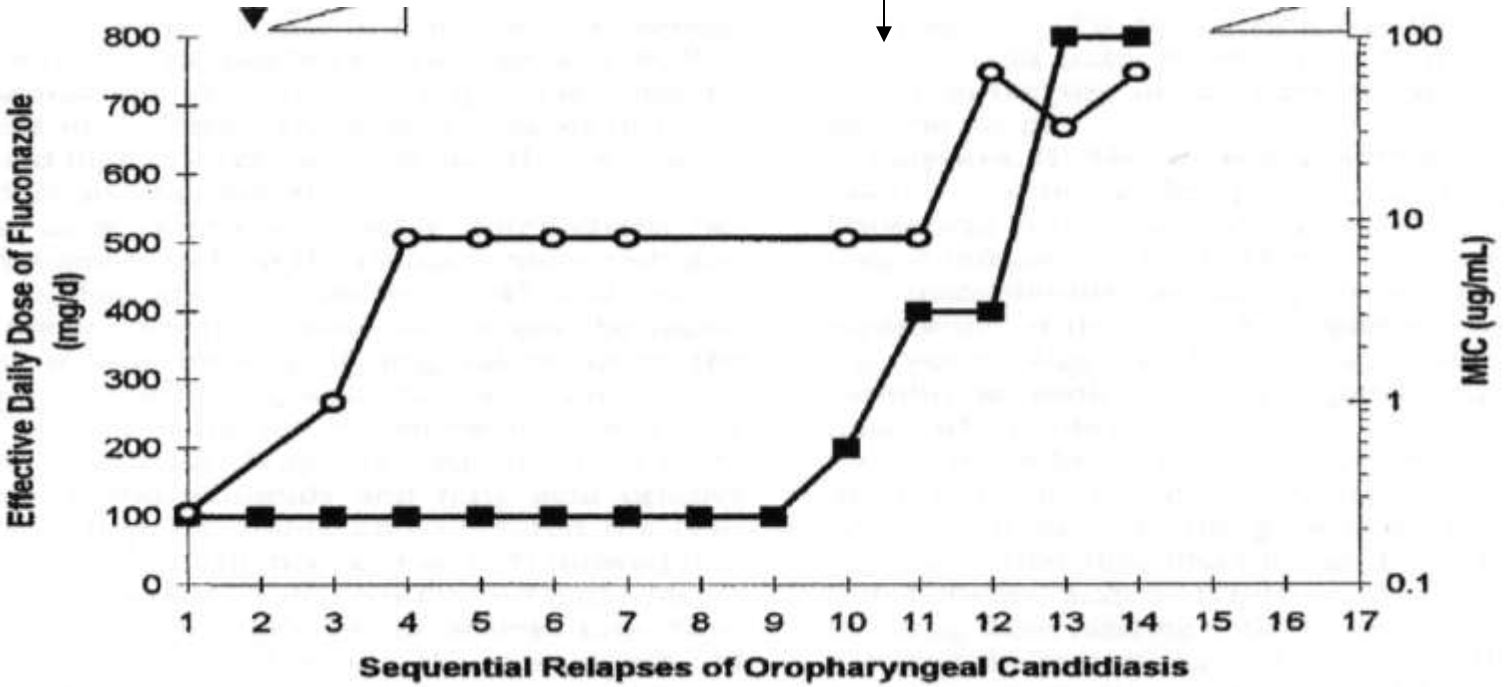


Résistance aux azolés des souches cliniques : succession et accumulation d'événements indépendants chez C. albicans

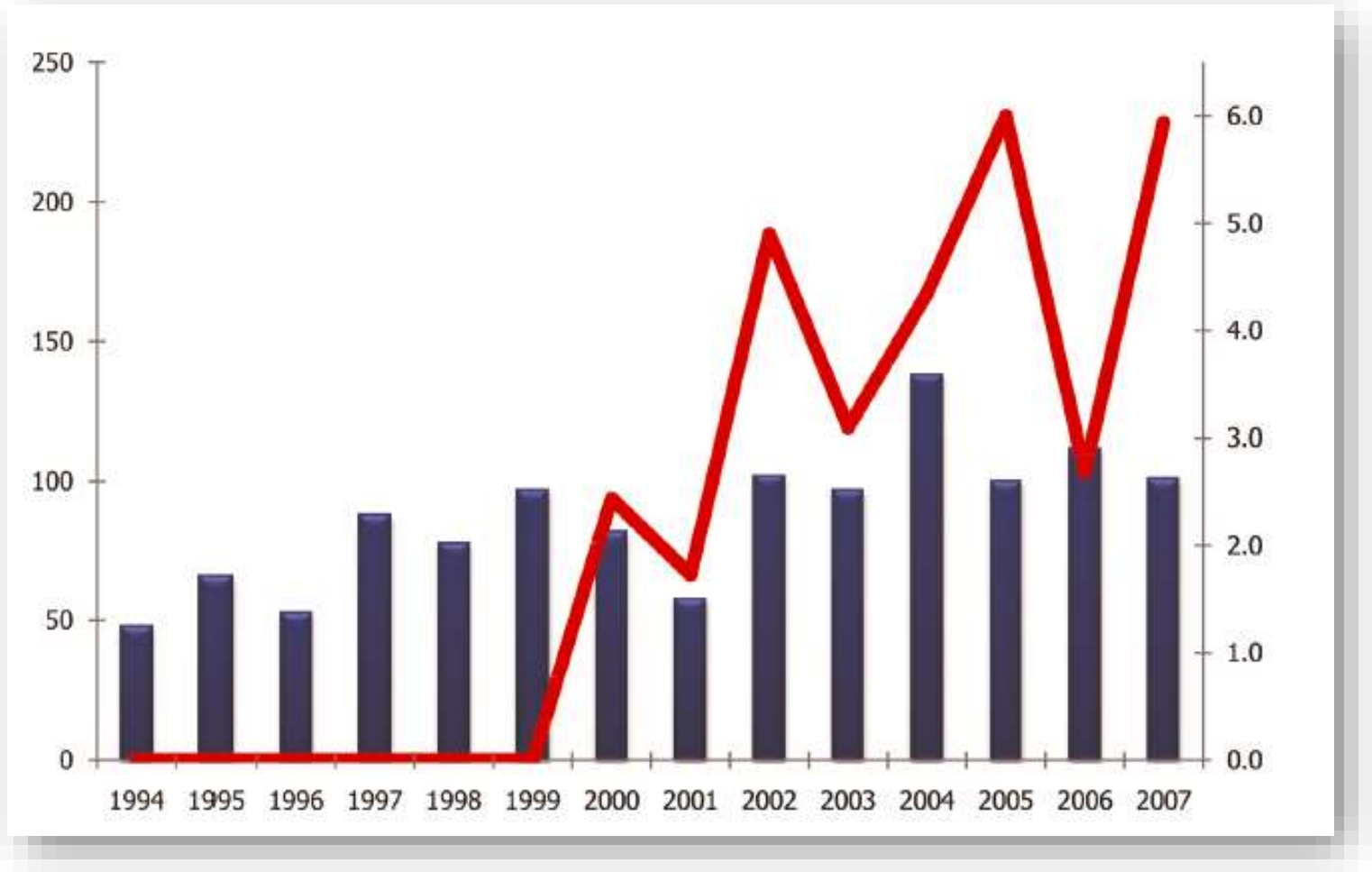
- Mutation de ERG11
- ↘ affinité de CYP51 pour azolés
- ↗ ERG11 mRNA

↗ *MDR* mRNA

↗ *CDR* mRNA



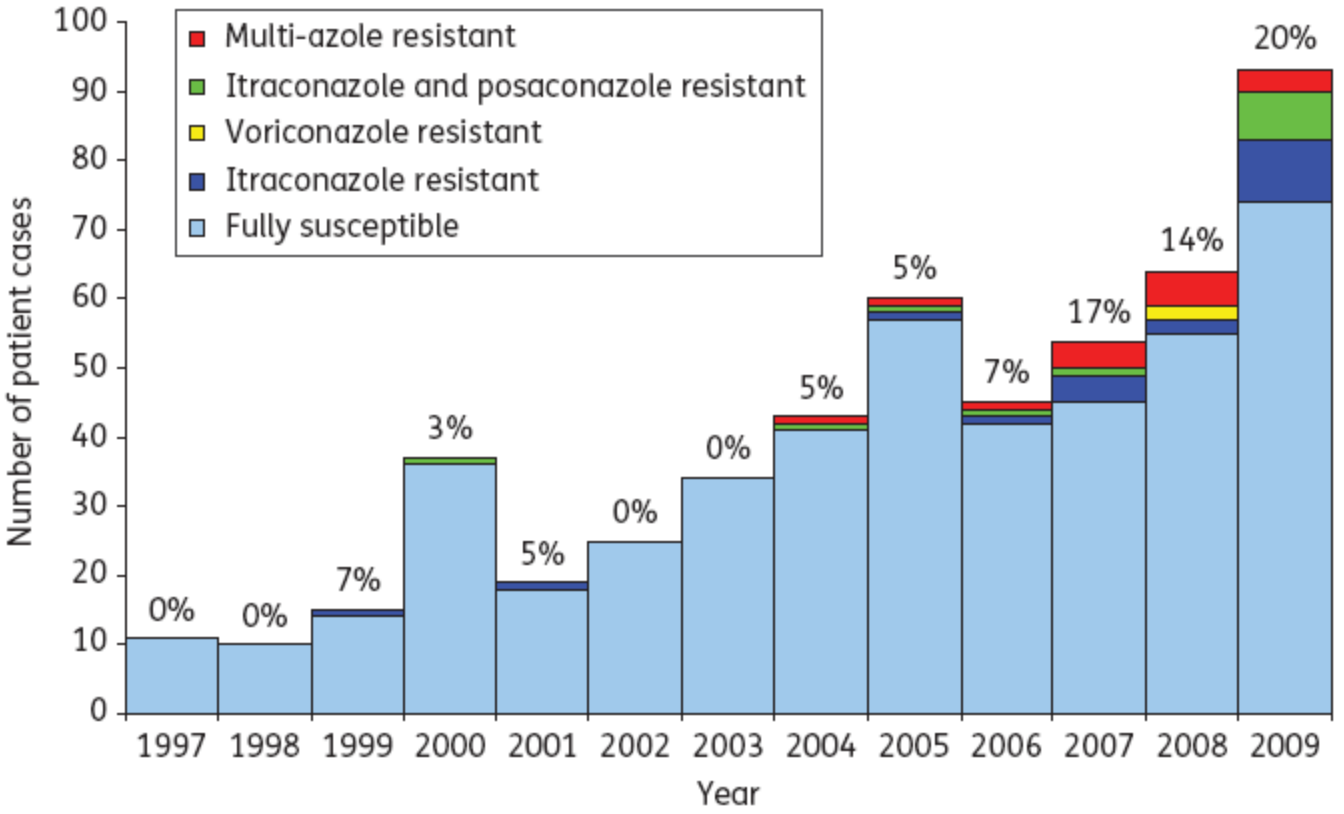
A. fumigatus – Résistance aux azolés



Snelders et al. 2008 PLoS Med. 5:e219

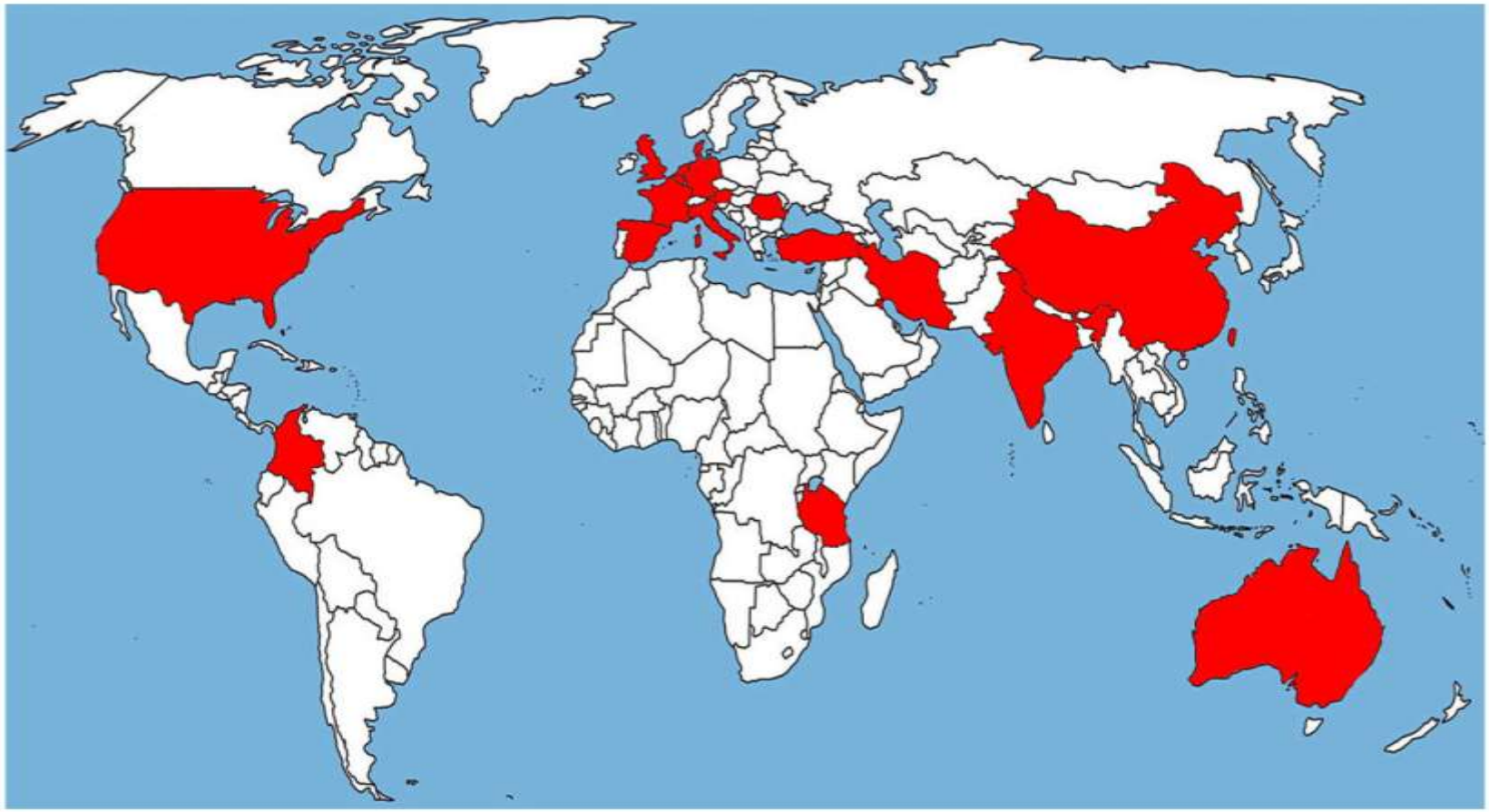
A. fumigatus – Résistance aux azolés

- Resistance a l'itraconazole décrite à partir de 1997: US[1], Suède[2], France[3], Pays Bas[4] , …
- Augmentation de la fréquence: UK[5,6]



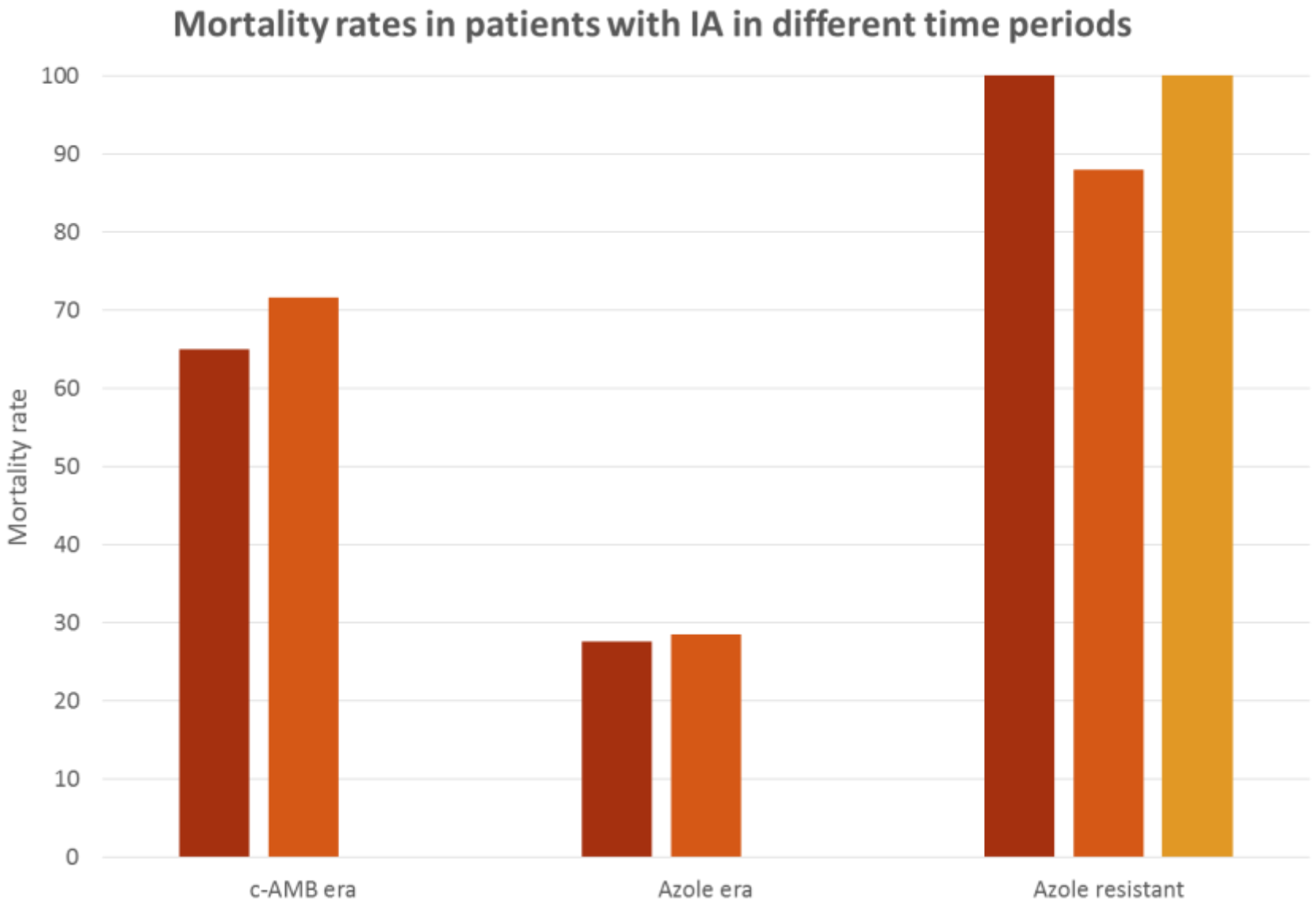
1. Denning, D. W., et al. 1997. *AAC 41:1364-8.*
2. Chryssanthou E. Scand JID. 1997;29:509–12
3. Dannaoui, E., et al. 2001. *JAC 47:333-40.*
4. Warris, A., et al. 2002. NEJM 347:2173-4.
5. Bueid, A. et al. 2010. JAC 65:2116-8.
6. Howard SJ. Et al. EID 2009 15:1068-76

A. fumigatus – Résistance aux azolés



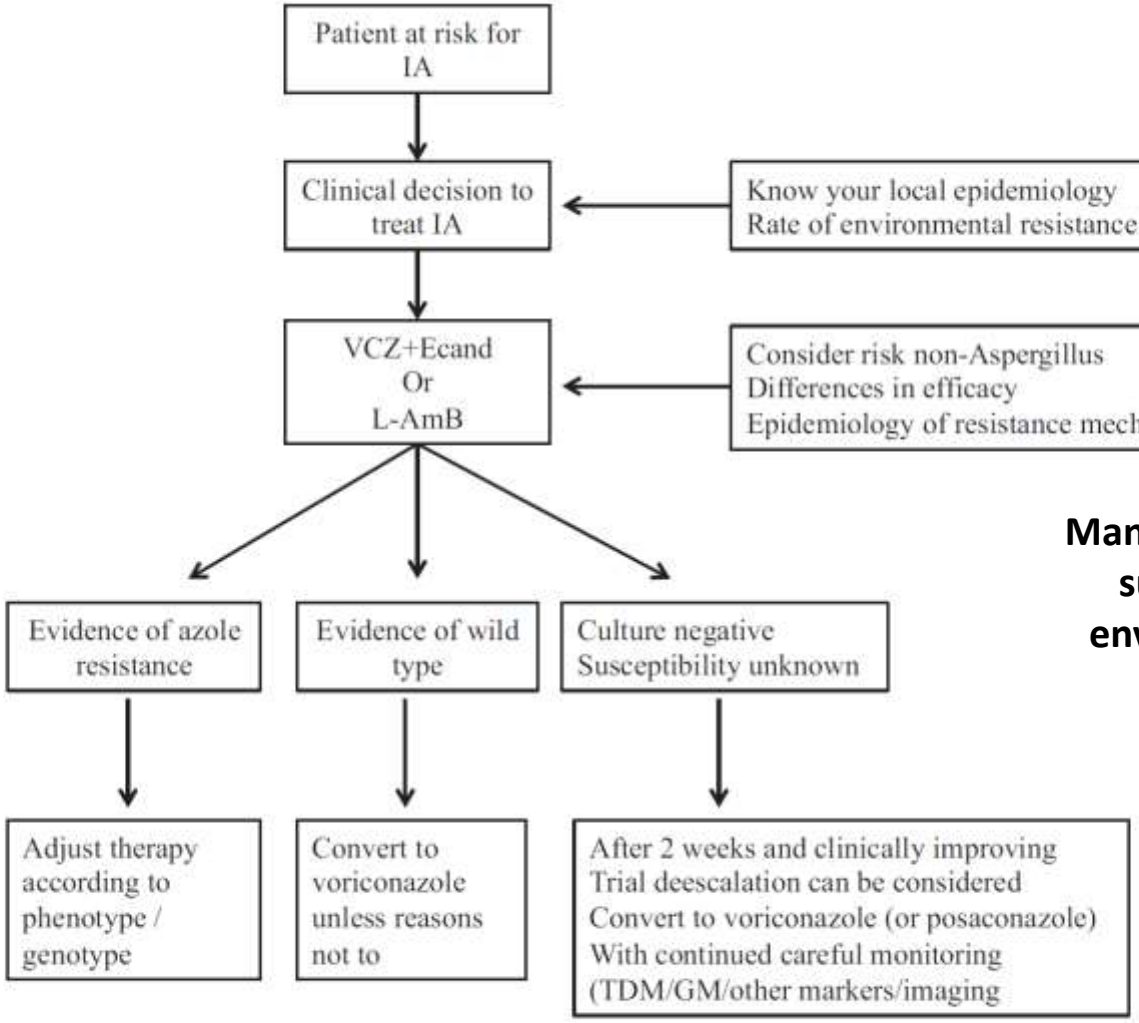
Verweij PE, et al. 2016. Clin Infect Dis **62:**362-368

Antifongiques : résistance



Verweij PE, et al. 2016. CID, 62:362

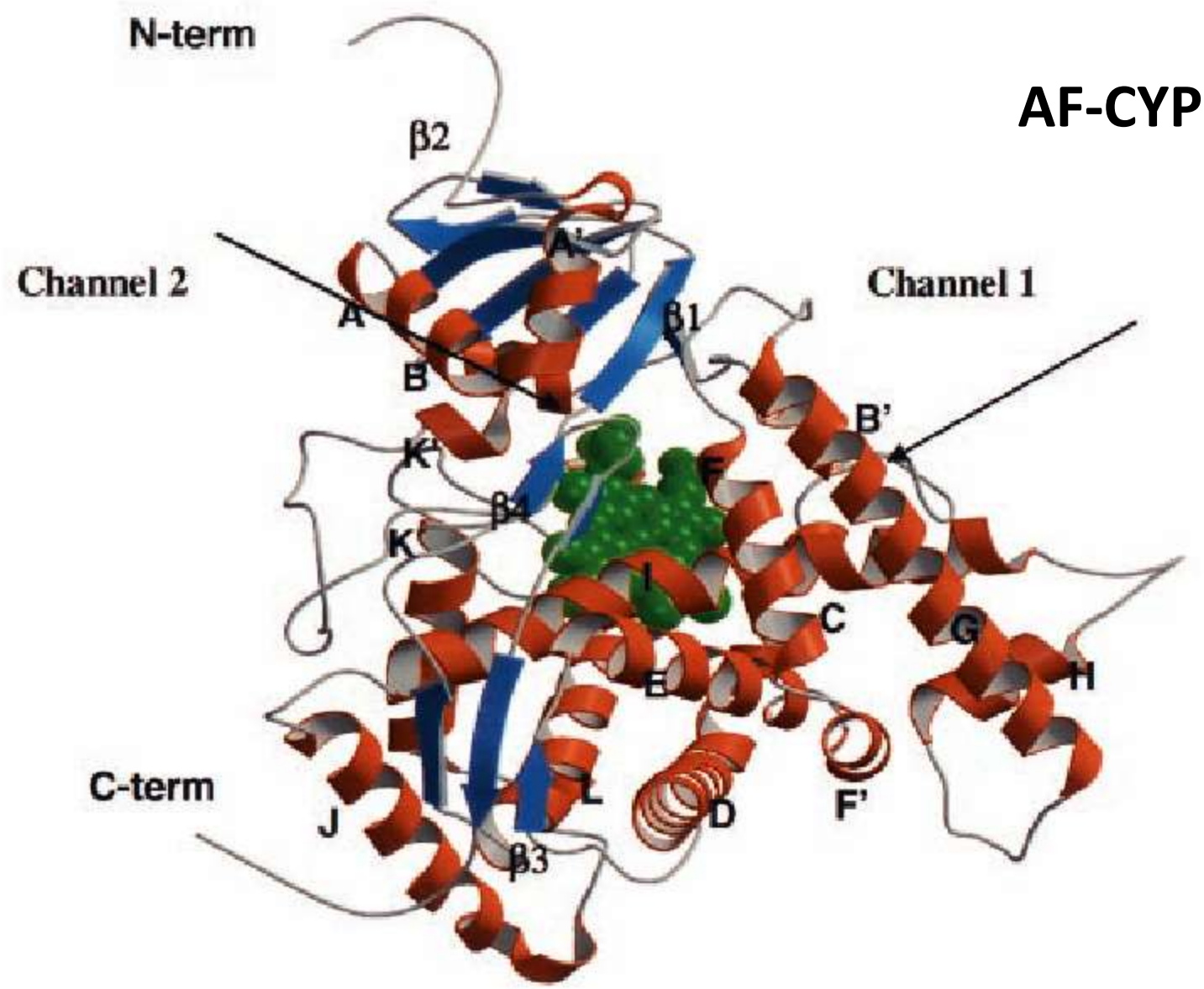
International expert opinion on the management of infection caused by azole-resistant *Aspergillus fumigatus*



Management of patients with clinical suspicion of IPA in regions with environmental resistance of ≥10%.

Verweij PE, et al. 2015. Drug Resist Updat 21-22:30-40.

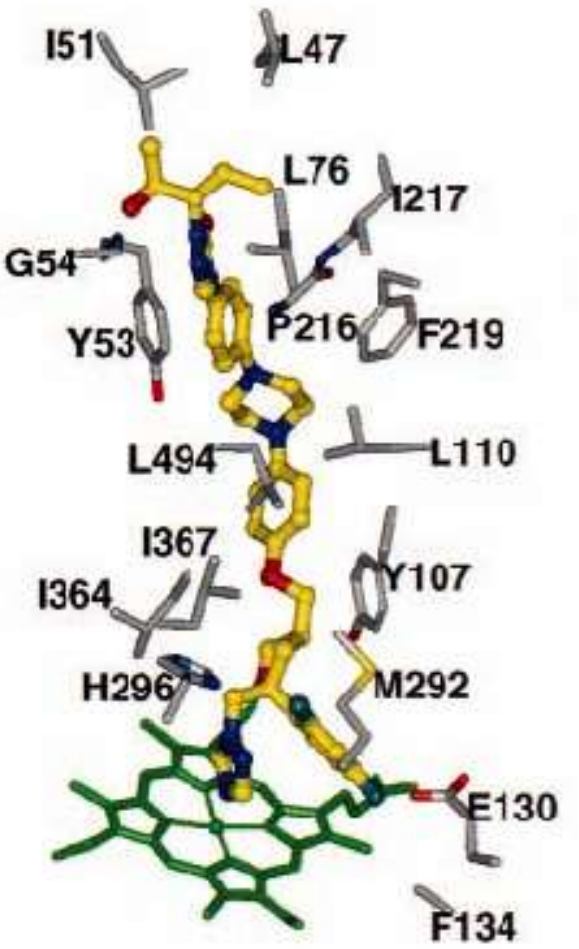
Azolés – Mode d'action



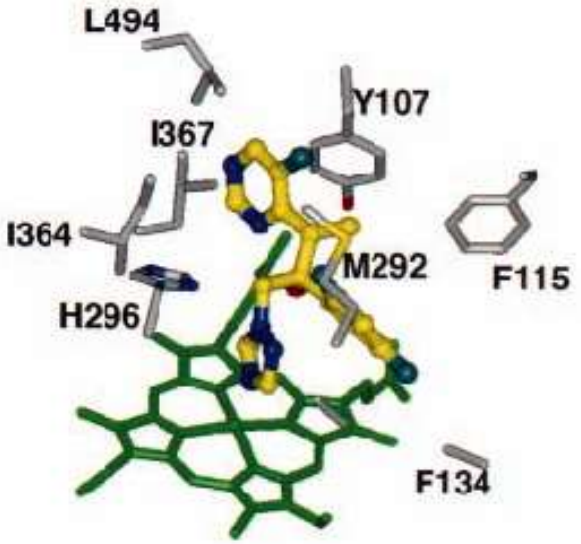
AF-CYP51A

Xiao, L., V. et al 2004. Antimicrob Agents Chemother 48:568-74.

Azolés – Mode d'action



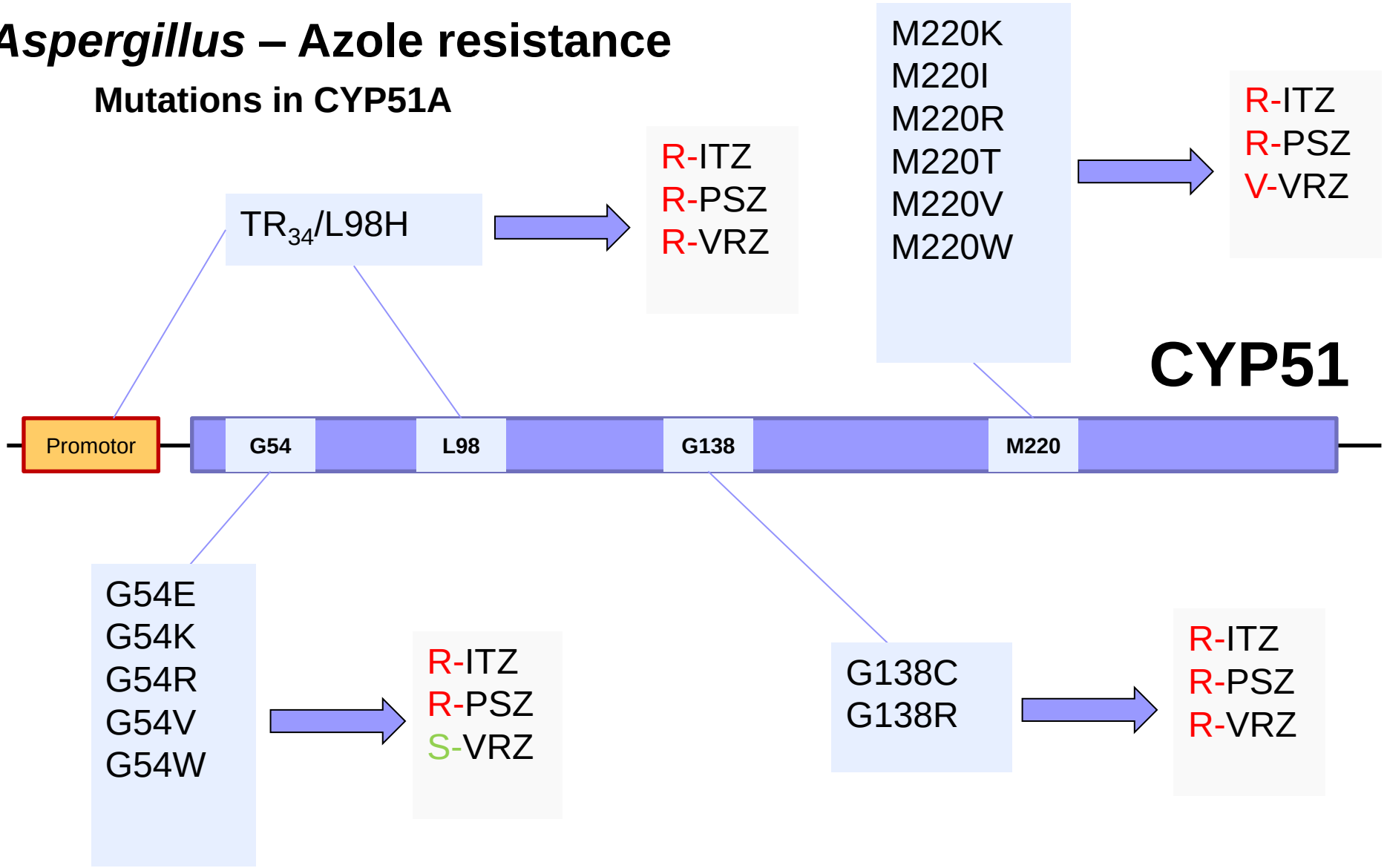
AF-CYP51A



Xiao, L., V. et al 2004. Antimicrob Agents Chemother 48:568-74.

Aspergillus – Azole resistance

Mutations in CYP51A



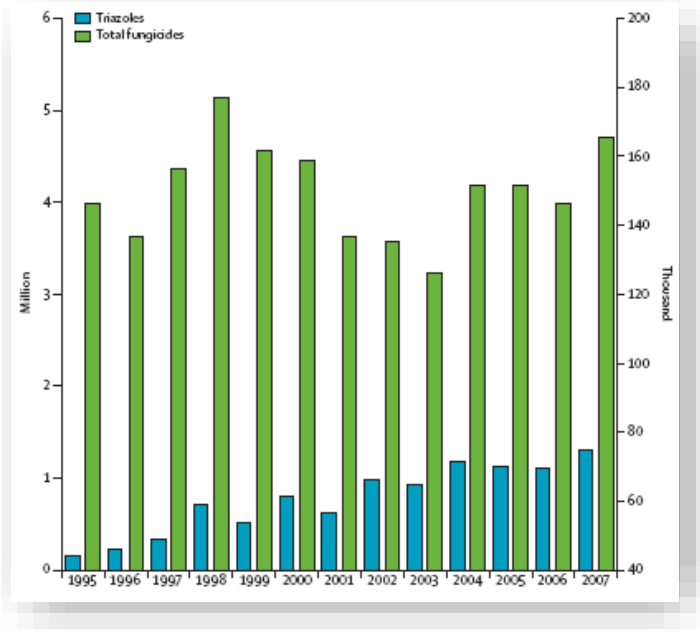
Mellado, E., et al. 2007. AAC 51:1897-904
Snelders, E., et al. 2008. PLoS Med 5:e219
Howard, S. J., et al. 2009. EID 15:1068-76
Snelders, E., et al. 2010. AAC 54:2425-30
Bueid, A., et al. 2010. JAC 65:2116-8
Morio, F., et al. 2012. JAC 67:1870-3
Burgel, P. R., 2012.. AAC 56:869-74

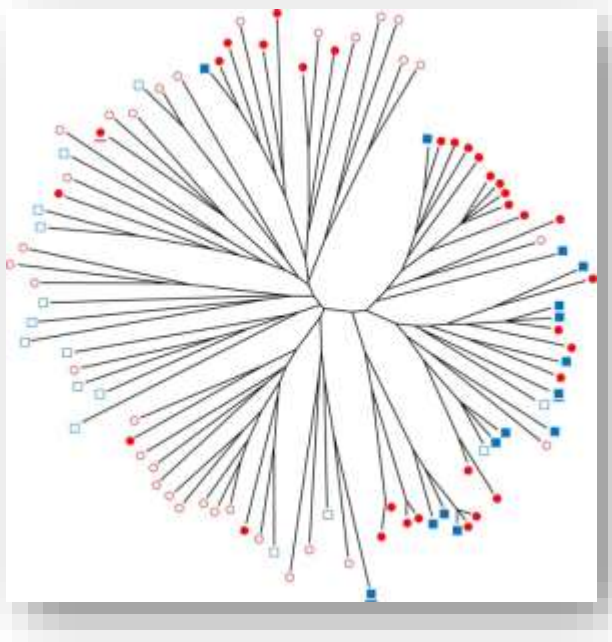
Antifongiques : résistance

Farm Fungicides Linked to Resistance in a Human Pathogen

Farmers' friend. Azoles are used to protect a wide variety of crops from fungi.

www.sciencemag.org SCIENCE VOL 326 27 NOVEMBER 2009



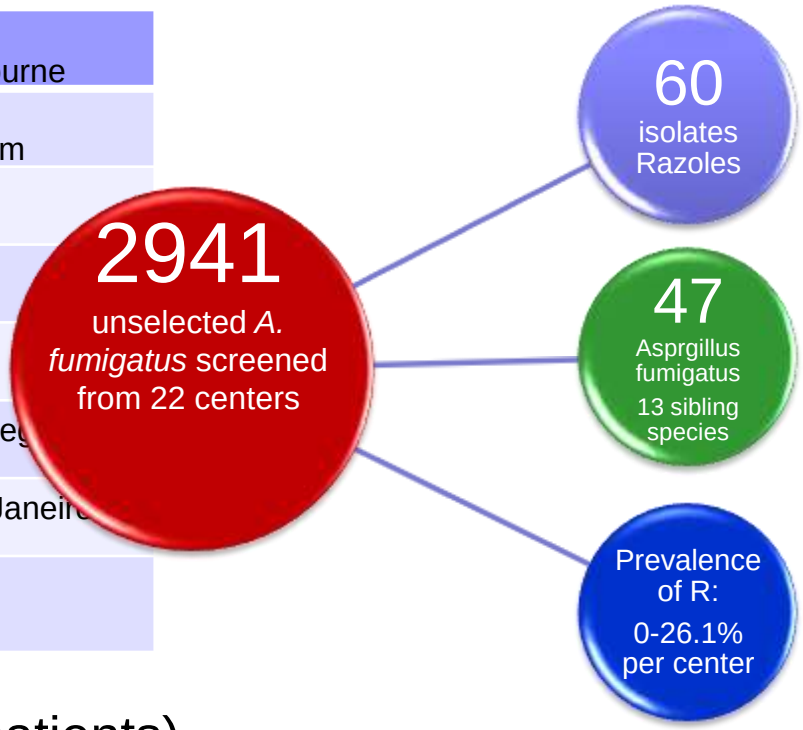


- Clinical RITZ
- Environ RITZ

Verweij, PE., et al. 2009. Lancet Infect Dis 9: 789; **Snelders E.** et al. 2009. Appl. Environ. Microbiol. 75:4053; **Enserink M.** 2009. Science 326:1173

Prospective international surveillance of azole resistance in *Aspergillus fumigatus*. SCARE-Network

Netherlands - Nijmegen	**Italy - Milano**	Australia - Melbourne
Denmark - Copenhagen	Greece - Athens	USA - Birmingham
UK - Manchester	Turkey - Ankara	USA - Madison
Belgium - Leuven	Norway - Oslo	China - Peking
France - Grenoble	Sweden - Stockholm	
France - Paris -HEGP	Poland - Gdansk	Brazil - Porto Ale
Germany - Münster	Austria - Innsbruck	Brazil - Rio de Janei
Switzerland - Lausane	Spain - Madrid	Russia - Saint-Petersbourg

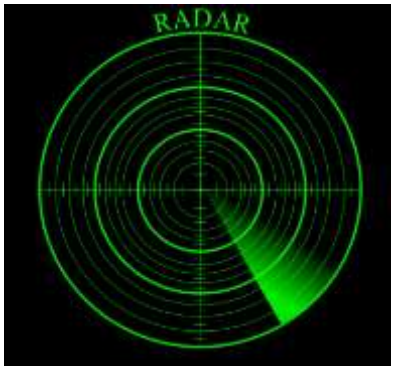


- ❑ Overall prevalence of R: 3.2% (patients)
- ❑ TR_{34}/L98H predominant mutation (48.9%)
- ❑ Presence of mutants in patients with IA (no fitness-cost)

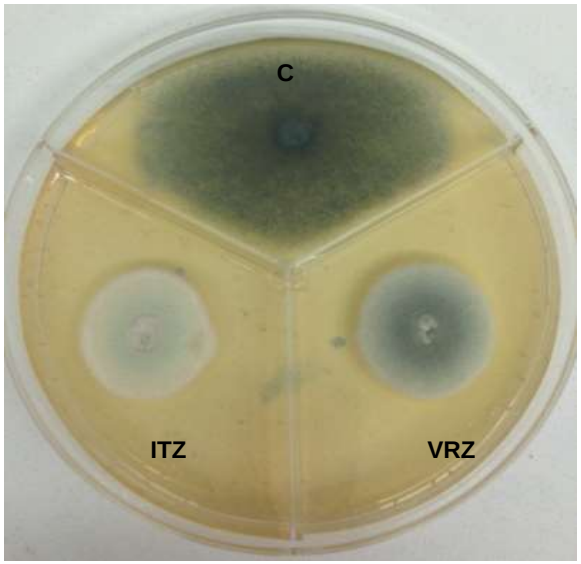
van der Linden JW. 2015. Emerg Infect Dis 21:1041-1044.

Aim of the study

The RADAR **prospective** study



Risk in
Aspergillosis:
Detection of
Azole
Resistance



- Surveillance study – Evaluation of prevalence and mechanisms
- Prospective - Multicenter
- All *Aspergillus* spp. screened on azole-containing plates

Results

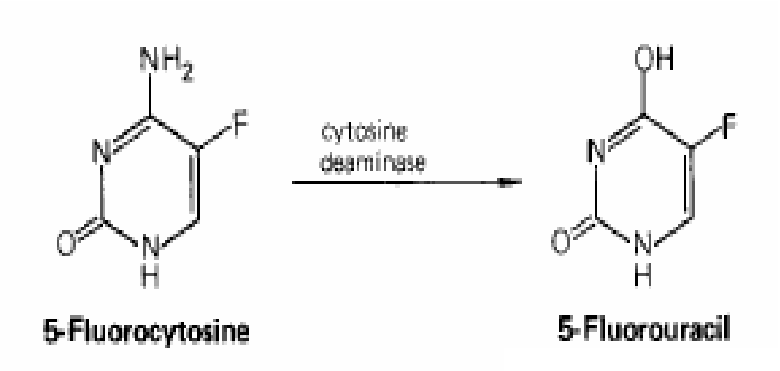
The RADAR prospective study

108 *A. fumigatus* resistant isolates

Cyp51 mutation	n	Cyp51 mutation	n
TR_{34}/L98H	66	G448S	1
Pas de mutation	19	G54W	1
G54R	5	M220V	1
TR_{34}/L98H/H147Y	5	TR_{34}/L98H/S297T/F495I	1
H285Y	3	TR_{46}/Y121F/T289A	1
M220I	2	Y121F	1

- Various underlying diseases
  - ✓ CF
  - ✓ SOT
  - ✓ Kc
  - ✓ Hematol malignancies
  - ✓ …
- Various aspergillosis
  - ✓ Colonization
  - ✓ ABPA
  - ✓ IA
  - ✓ Aome
  - ✓ …

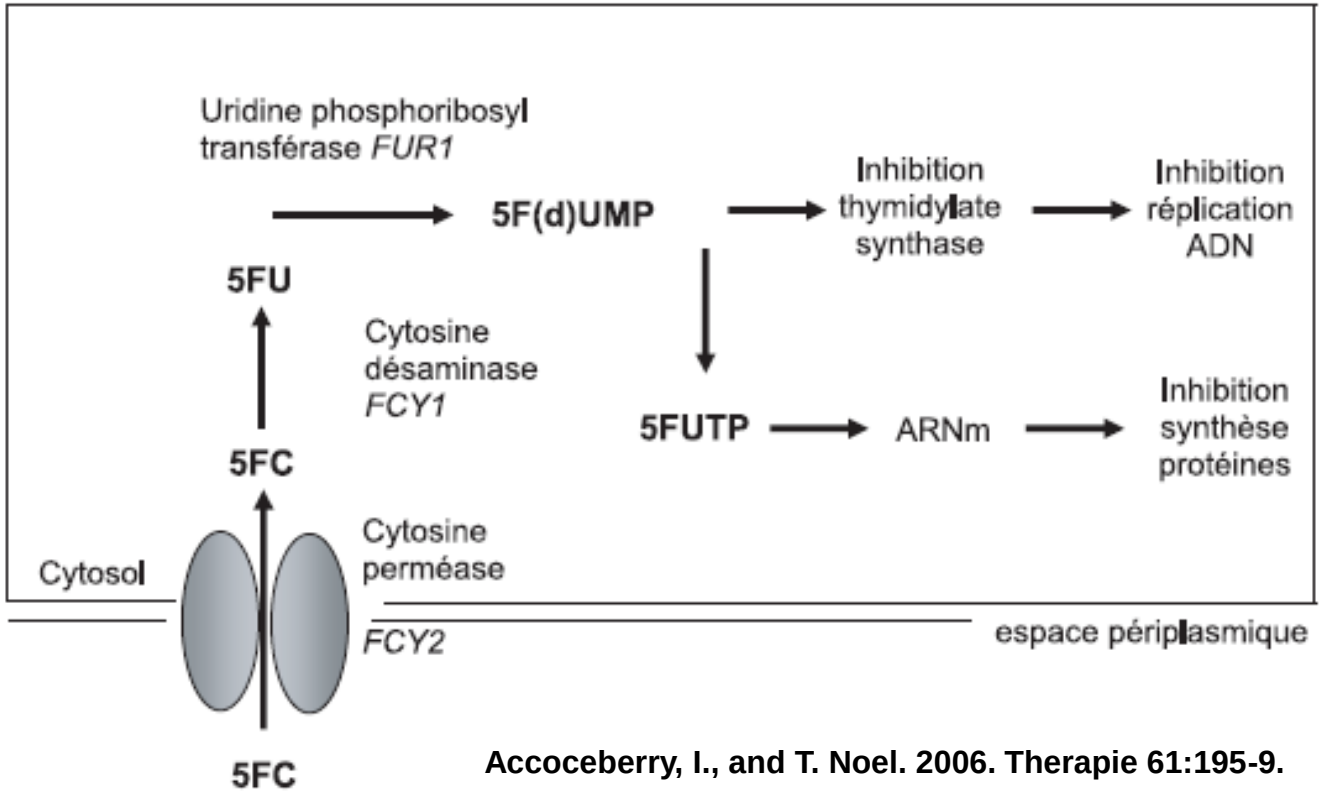
5-Fluorocytosine



Fluorinated pyrimidine related to flurouracil.

Fig. 1. Mécanisme d'action de la flucytosine et principaux gènes et enzymes impliqués dans son métabolisme.

5FC : Flucytosine ou 5-fluorocytosine ; 5FU : 5-fluoro-uracile ; 5F(d)UMP : 5-fluoro-desoxy-uridine monophosphate ; 5FUTP : 5-fluoro-uridine triphosphate.



Accoceberry, I., and T. Noel. 2006. Therapie 61:195-9.

Pharmacocinétique

PK parameter	AMB (AMB Lipo)	5FC	FCZ
Biodisponibilité orale, %	<5	80	95
Voie d'administration	IV	IV/PO	IV/PO
Liaison protéines	>95	4	10
½ vie (h)	100-150	3-6	31
TDM	non	oui	non

Table 2. The major PD characteristics by fungal drug class

Drug class	Concentration dependent	Prolonged PAFE	PD index predictive of efficacy
Polyene	Yes	Yes	C_{max}/MIC
Flucytosine	No	No	$T >$ MIC
Azoles	No	Yes	AUC/MIC
Echinocandins	Yes	Yes	C_{max}/MIC or AUC/MIC

Dodds Ashley et al. 2006. Clin Infect Dis 43:S28-S39.

5-Fluorocytosine

- Spectre d'activité restreint

Monothérapie : plus utilisée

- Candidoses
- Cryptococcoses

} **En association avec amphotéricine B ou fluconazole**

5-Fluorocytosine

- Résistance acquise

  > liée à monothérapie

  > d'apparition rapide

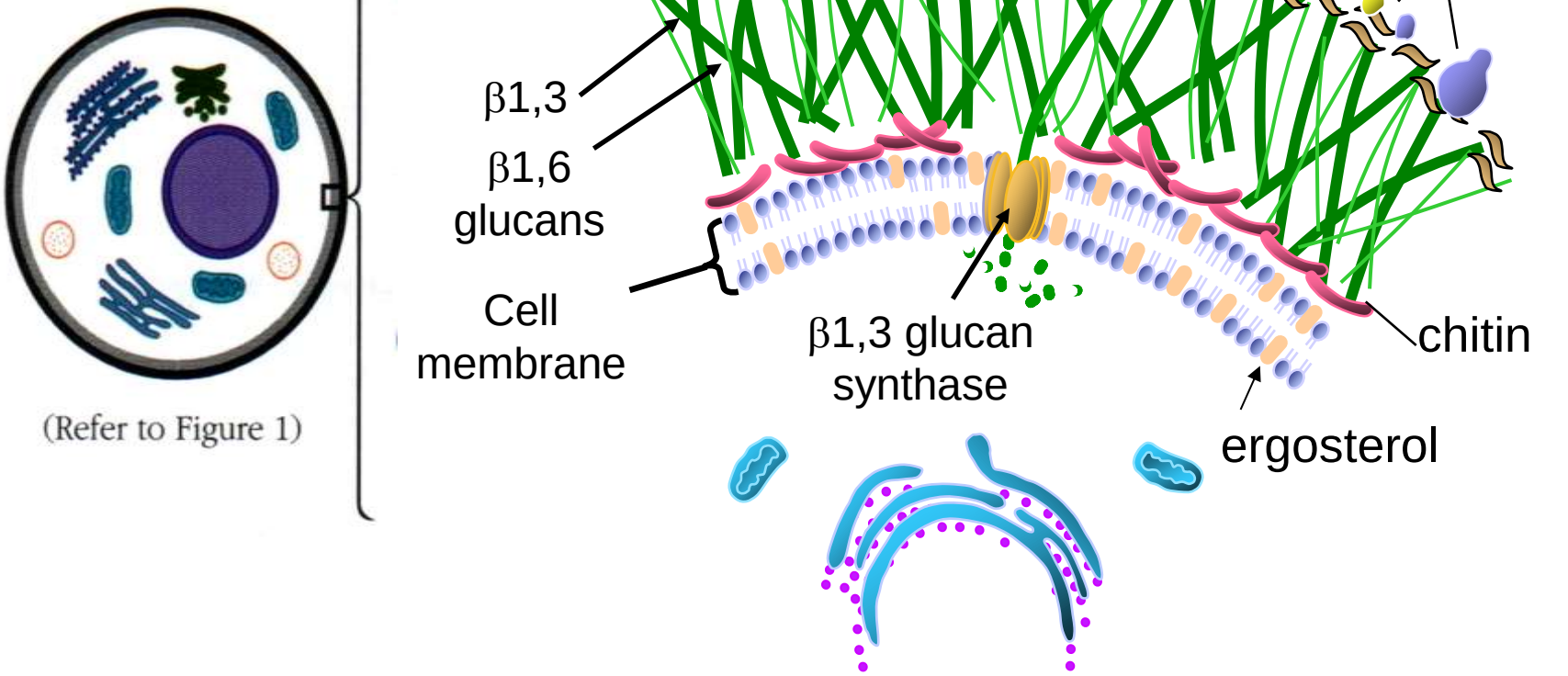
Mécanismes:

1) Déficit de pénétration (activité perméase)

2) Déficit du métabolisme (activité cytosine deaminase ou UMP pyrophosphorylase)

Mécanisme d'action des echinocandines

The Fungal Cell Wall

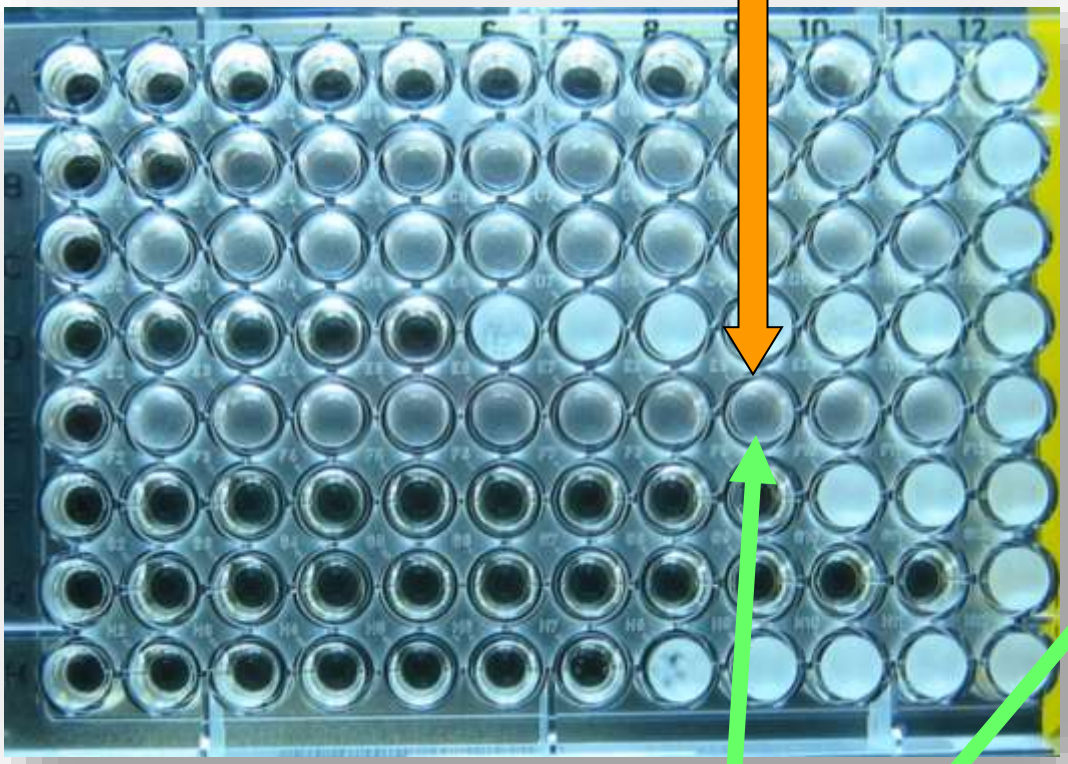


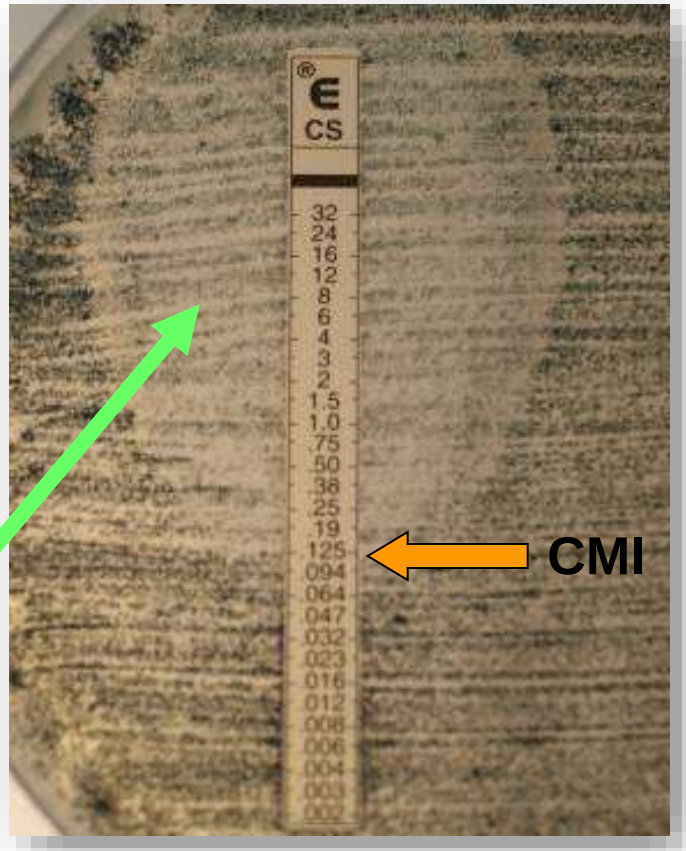
Atlas of fungal Infections, Richard Diamond Ed. 1999
Introduction to Medical Mycology. Merck and Co. 2001

Echinocandines : EUCAST, Etest

CMI

Aspergillus spp.



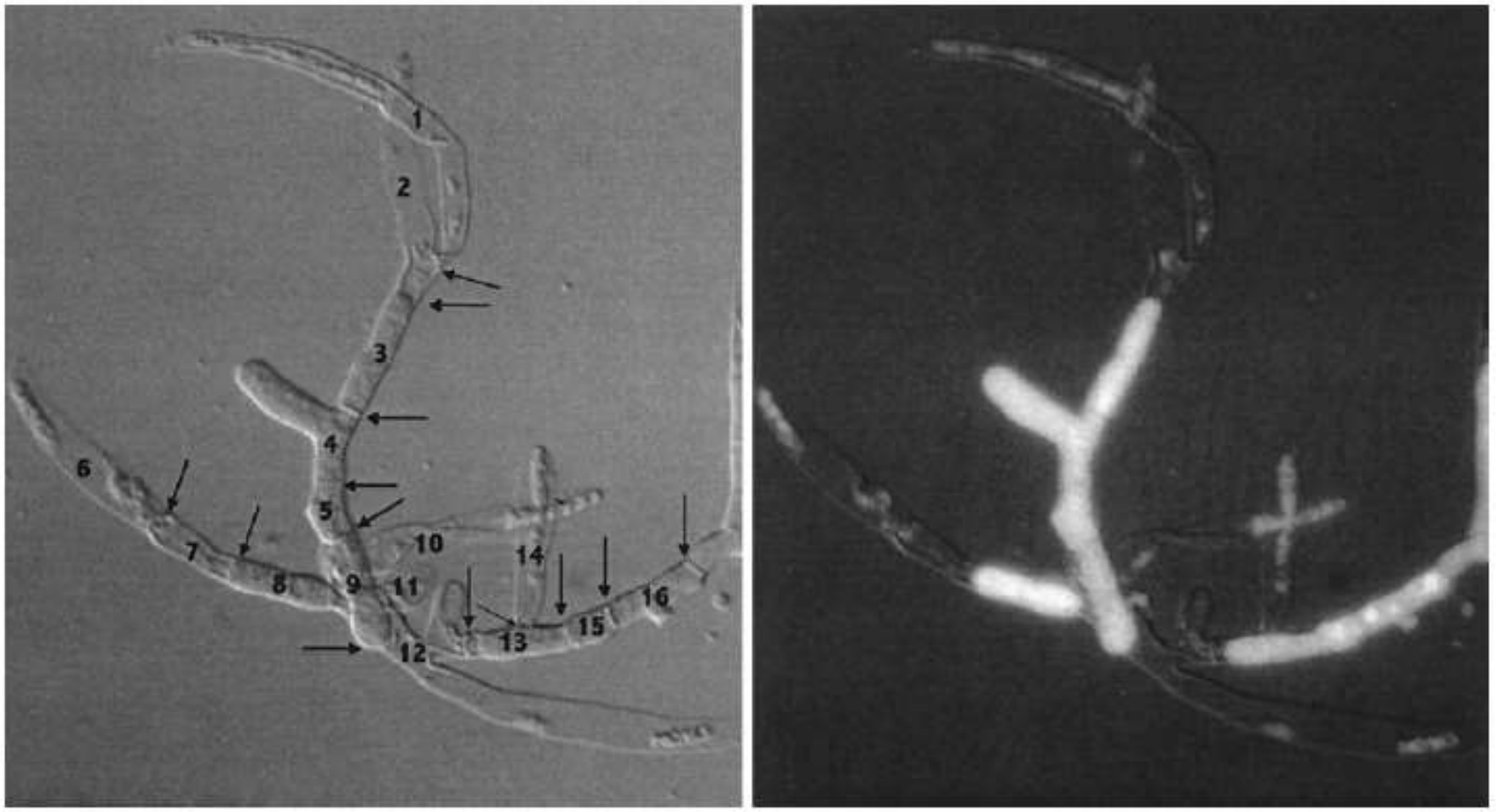


Inhibition partielle

Activité des antifongiques : Echinocandines

Bright field

CFDA



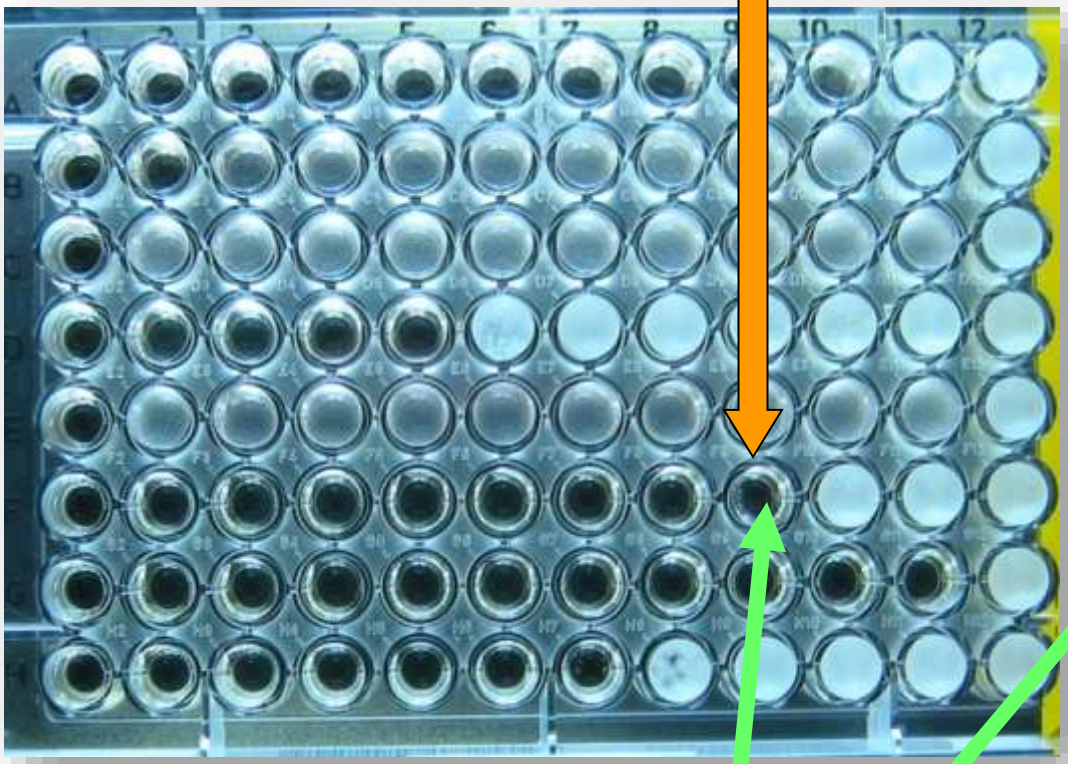
Fungistatique sur *Aspergillus fumigatus*

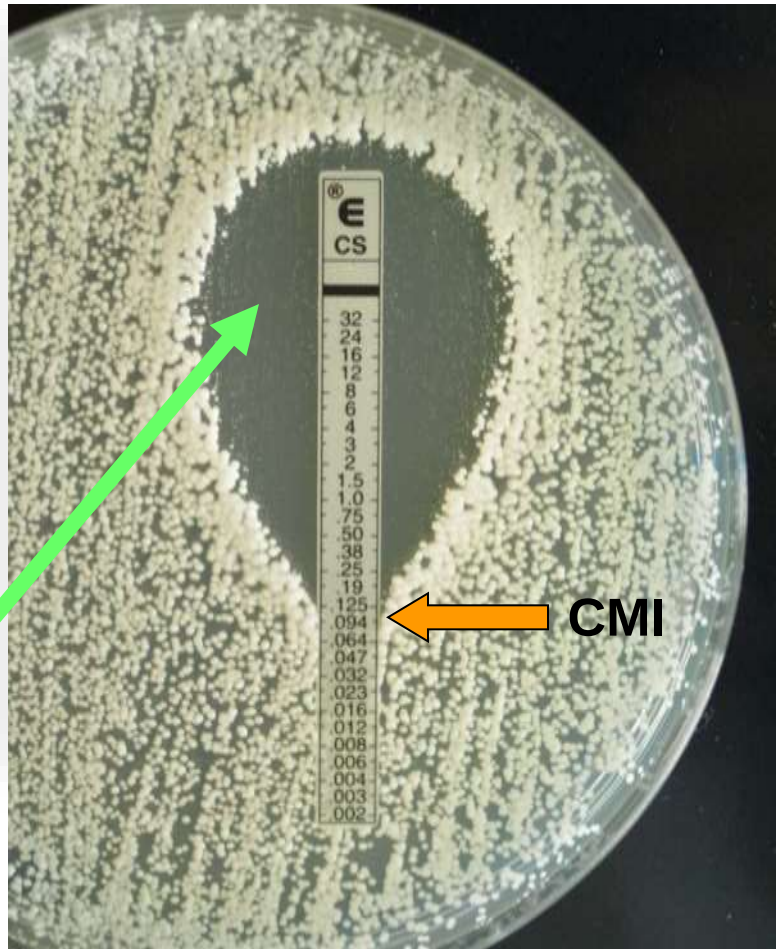
Bowman, J. C., et al 2002. Antimicrob Agents Chemother 46:3001-3012.

Echinocandines : EUCAST / CLSI, Etest

CMI

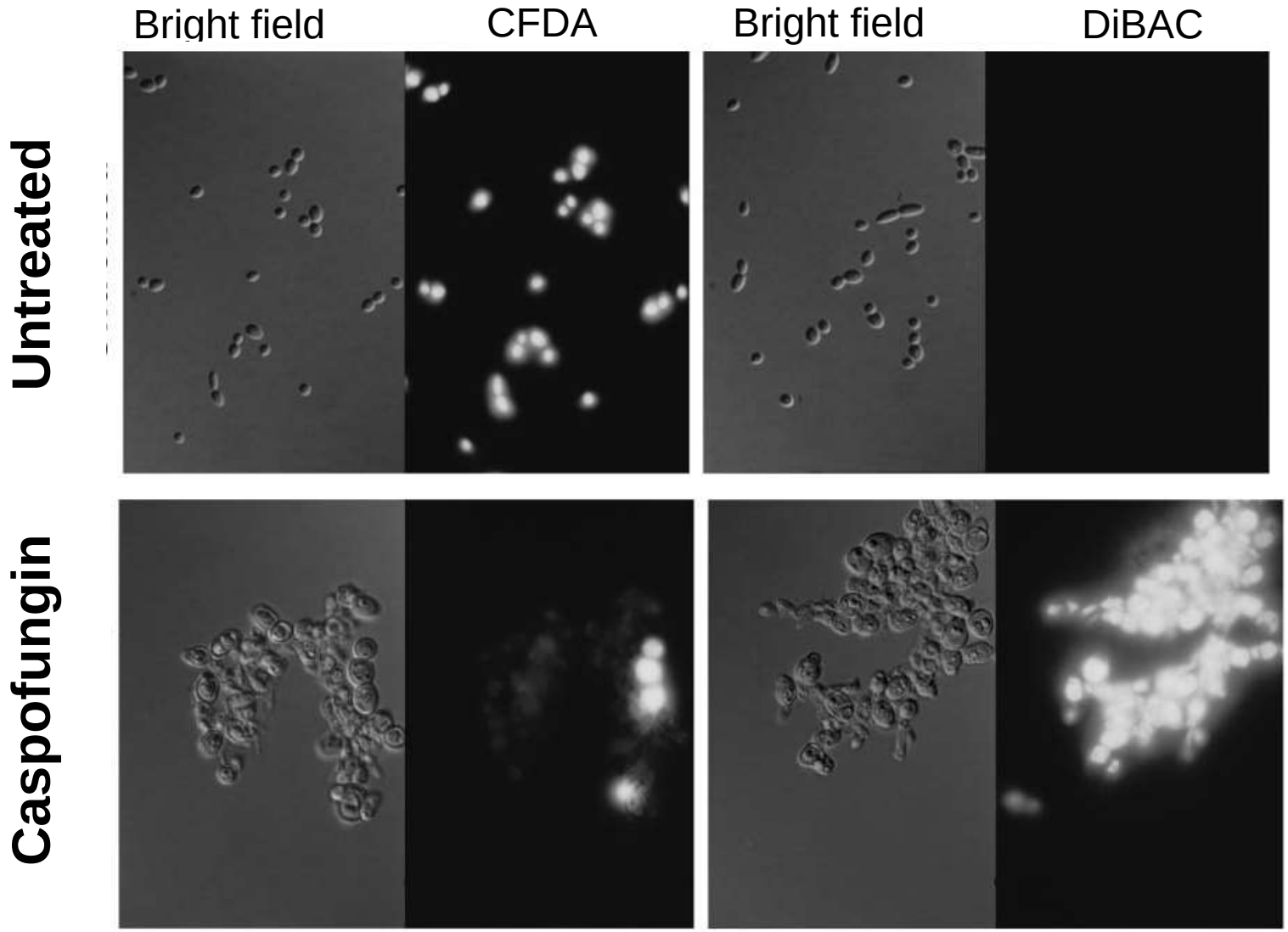
Candida spp.





Inhibition complète

Activité des antifongiques : Echinocandines



Fungicide sur *Candida albicans*

Bowman, J. C., et al 2002. Antimicrob Agents Chemother 46:3001-3012.

PK/PD

PK parameter	CAS	MIC	ANI
Biodisponibilité orale, %	<5	<5	<5
Voie d'administration	IV	IV	IV
Liaison proteines	97	99	84
½ vie (h)	30	15	26
TDM	non	non	non

Table 2. The major PD characteristics by fungal drug class

Drug class	Concentration dependent	Prolonged PAFE	PD index predictive of efficacy
Polyene	Yes	Yes	C_{max}/MIC
Flucytosine	No	No	$T >$ MIC
Azoles	No	Yes	AUC/MIC
Echinocandins	Yes	Yes	C_{max}/MIC or AUC/MIC

Caspofungine - spectre

- *CANDIDA*: *C. albicans, C. dubliniensis, C. glabrata, C. kefyr, C. krusei, C.lipolytica, C. lusitaniae, C. tropicalis*
  - *C. parapsilosis, C. guilliermondii, ???*

  Caspofungine est fongicide sur *Candida* spp

- *ASPERGILLUS*: *A. fumigatus, A. flavus, A. niger, A. nidulans, A. terreus*
  - Caspofungine est fongistatique sur *Aspergillus* spp

- Activité sur les autres champignons moins bien définie

- Pas d'activité contre *Cryptococcus neoformans*, *Trichosporon*, Mucorales

Levures, *Candida, Crypto*
Champignons filamenteux *Aspergillus, Fusarium, Scedo*, Zygo

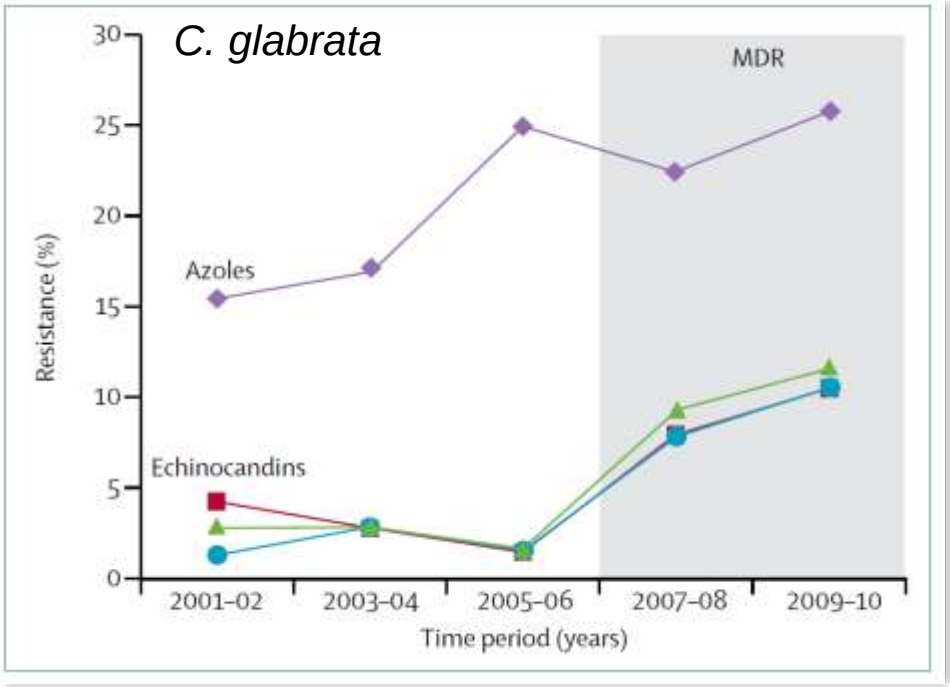
	Echino
Candida albicans	
Candida tropicalis	
Candida parapsilosis	
Candida krusei	
Candida glabrata	
Candida lusitaniae	
Crypto neoformans	

	Echino
Aspergillus fumigatus	
Aspergillus terreus	
***Fusarium* spp.**	
Scedo. apiospermum	
Scedo. prolificans	
***Rhizopus* spp.**	
***Absidia* spp.**	
***Mucor* spp.**	

Changing epidemiology: emergence of resistance

Echino resistance / *Candida*

- In patients

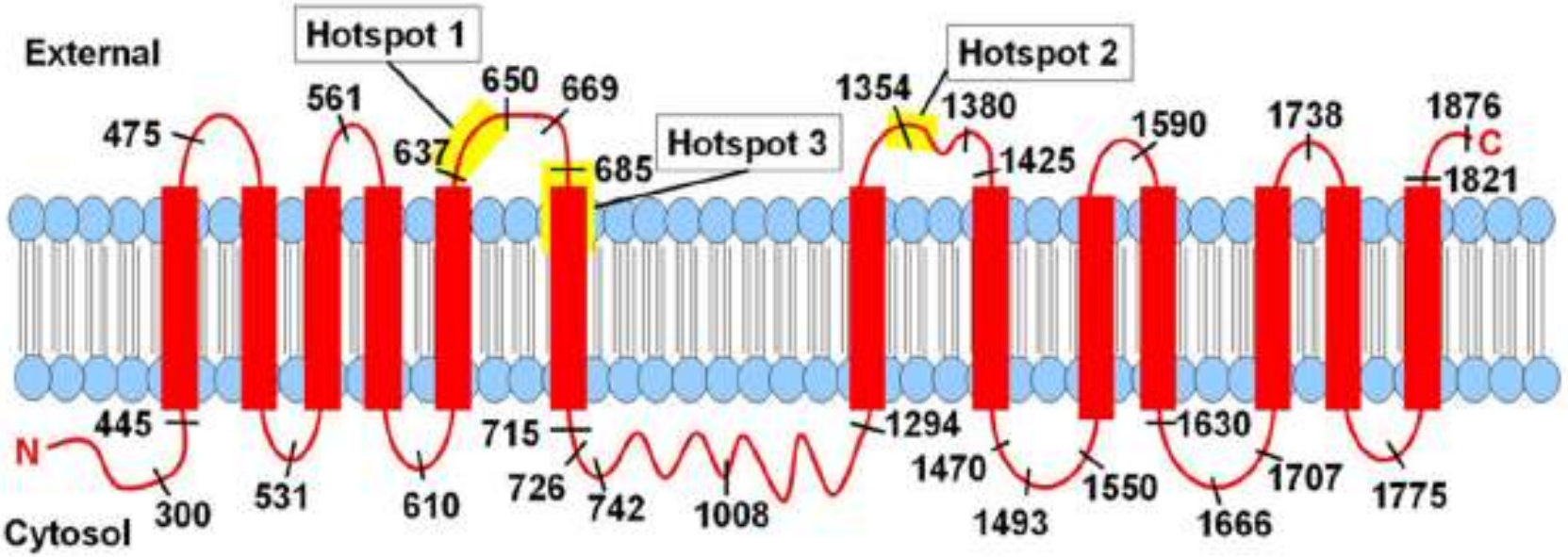


Risk factors:

- ✓ echino treatment
- ✓ intra-abdo reservoirs

Dannaoui E, 2012. EID 18:86-90
Alexander BD, et al. 2013. CID 56:1724-1732.
Perlin DS, et al. 2017. Lancet ID 17:e383-e392

Schematic diagram of *Fks1* hot-spots associated with reduced susceptibility to caspofungin

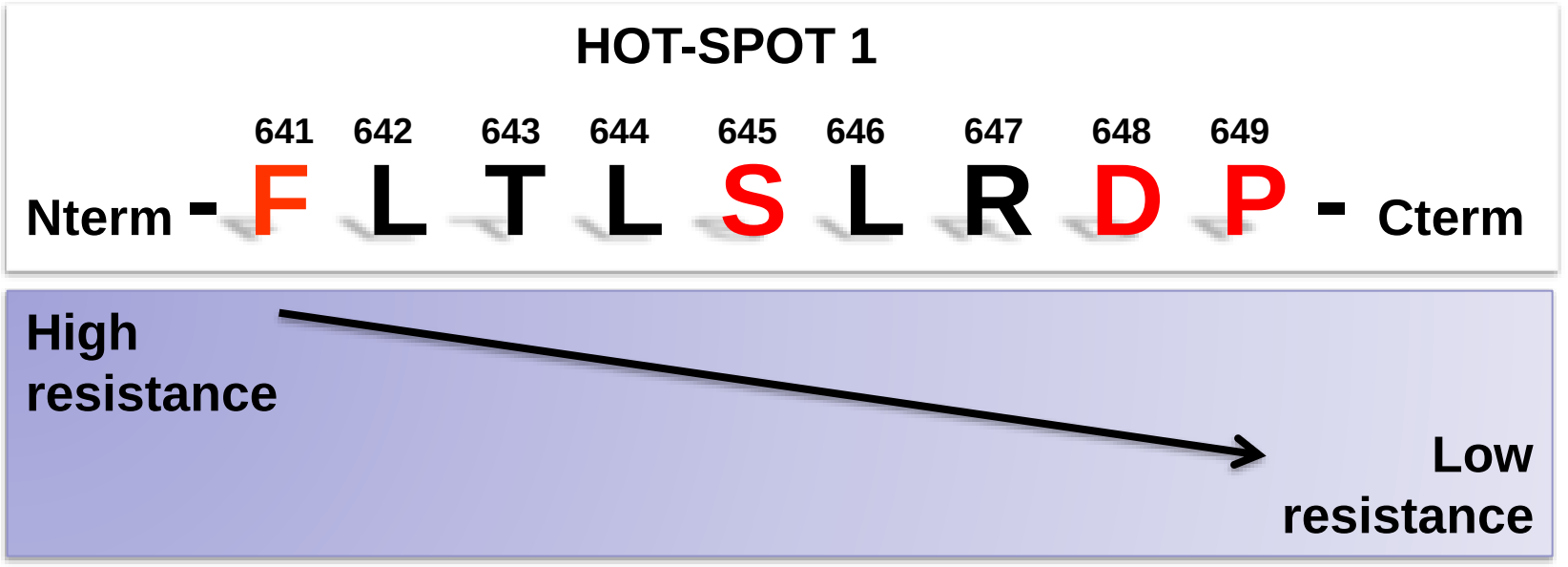


Johnson, M. E., and T. D. Edlind. 2012. Eukaryot Cell 11:952-60.

Les résistances acquises

Mutations des gènes Fks

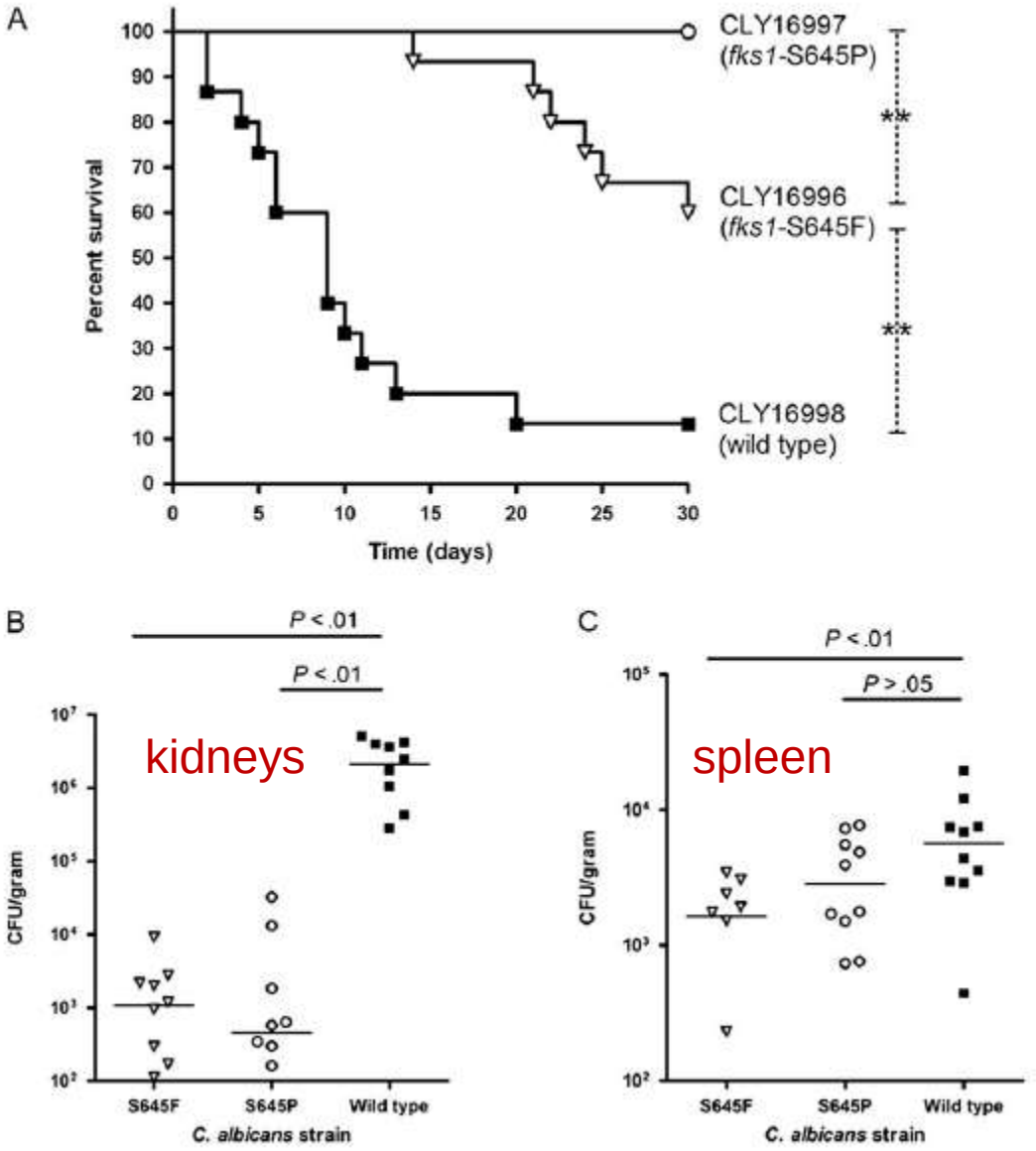
- Certaines positions préférentielles
- Phénotype (MIC) dépends du type de mutation



G. Garcia-Effron and D. Perlin. **Echinocandin drugs resistance**. TIMM 2009. **W11.2**

Antifongiques : résistance

Fitness and virulence costs of *Candida albicans* FKS1 hot spot mutations associated with echinocandin resistance



Ben-Ami, R., et al. 2011. J Infect Dis 204:626-35.

Les résistances naturelles

- *C. parapsilosis*[1]
  - ✓ CMI plus élevées
  - ✓ Toutes les souches
    - ❑ mutation naturelle HS1 (P660A) dans Fks1
    - ❑ GS moins sensible aux echinocandines (équivalent aux souches *C. albicans* Rcas mutés)
    - ❑ Transformation de *S. cerevisiae* avec cette mutation induit la résistance

- *C. guilliermondii*[2]
  - ✓ Mutation naturelle HS1 (Y641) dans Fks2 responsable de la résistance

- *Fusarium solani, Scedosporium prolificans*[2,3]
  - ✓ Mutation naturelle HS1 (Y641) dans Fks1 responsable de la résistance
  - ✓ Mutation non présente chez *A. fumigatus*

1. Garcia-Effron, G., et al. 2008. AAC **52:**2305-2312.
2. Katiyar, S., et al. 2006. AAC **50:**2892-2894.
3. Katiyar, S. K. and T. D. Edlind. 2009. AAC **53**: 1772-8.