Mechanism of action of antifungal drugs

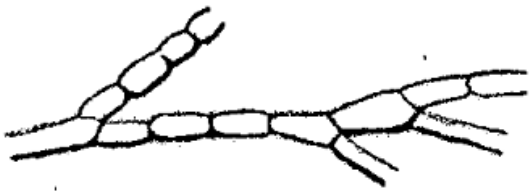
Generalities

What is a fungus

Uni- or pluricellular eukaryotic heterotrophic organism presenting a cell wall rich in chitin
→ Reproduction with spores usually

2 prominent groups of fungi

Filamentous



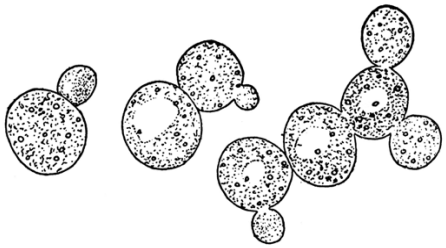
Aspergillus
Microscoporum
Trichophyton …

Dimorphic

Filamentous *in vitro*
Levuriform *in vivo*

Histoplasma
Blastomyces
Coccidioidomyces
Penicillium

Levuriform



Candida
Cryptococcus

Families of antifungal drugs

Polyenes

- Amphotericin B
- (…)

Azoles

- Fluconazole
- Itraconazole
- Voriconazole
- Posaconazole
- Ketoconazole
- (…)

Echinocandins

- Caspofungin
- Micafungin
- Anidulafungin

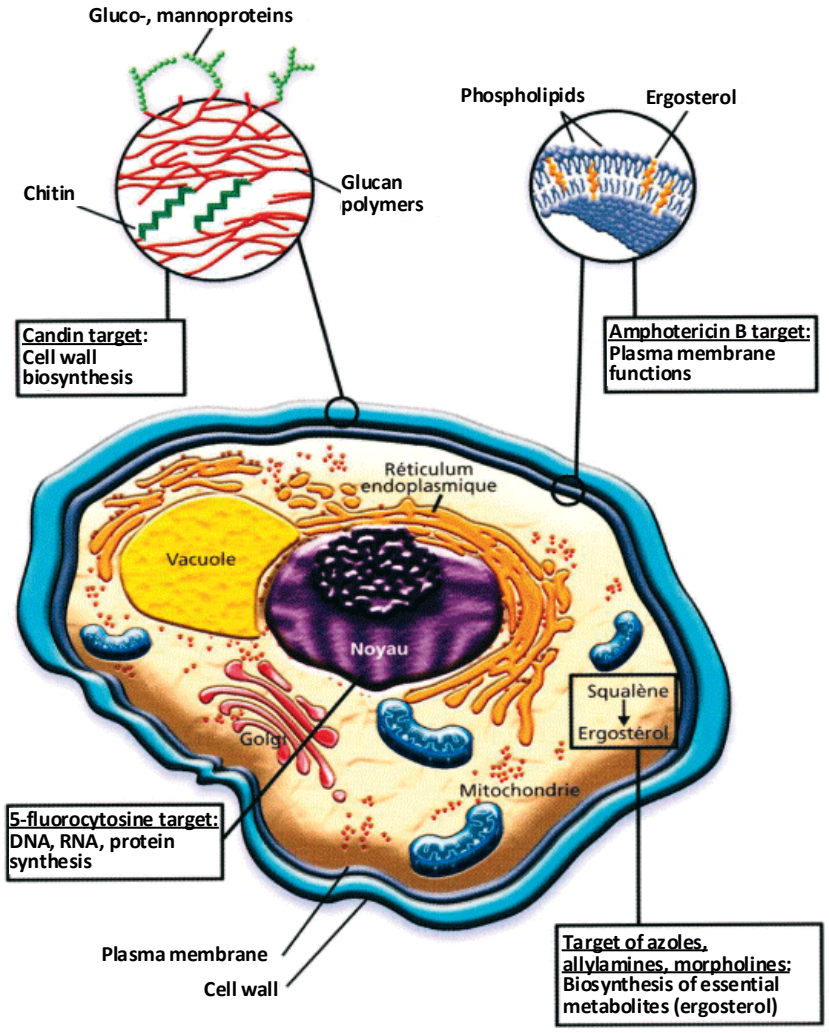
Pyrimidine

- Flucytosine

Allylamines

- Terbinafine

Antifungal drugs

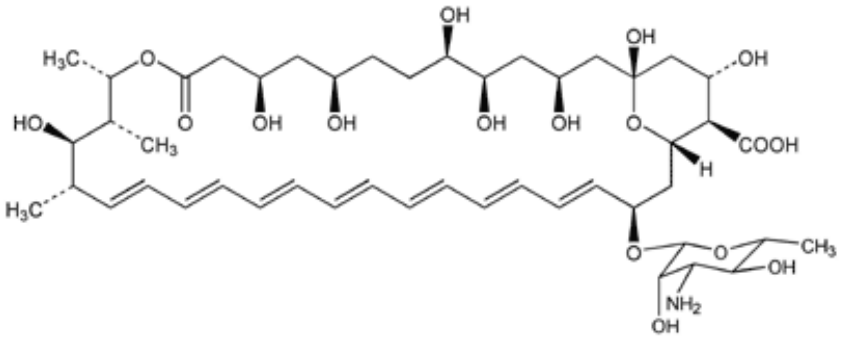


SYSTEMIC ANTIFUNGAL DRUGS

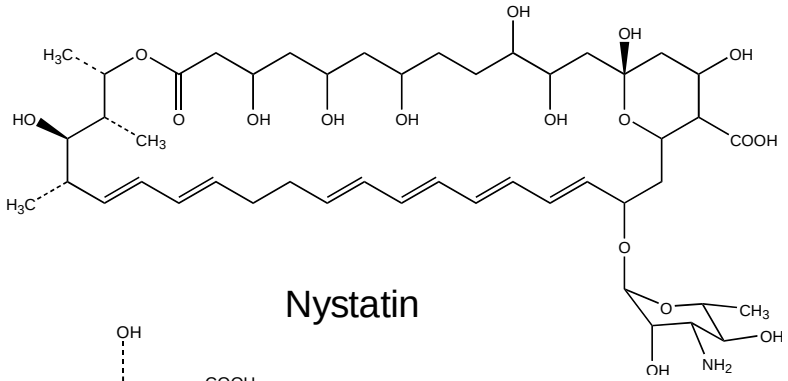
(Treatment of Invasive Fungal Infections (IFI))

POLYENES

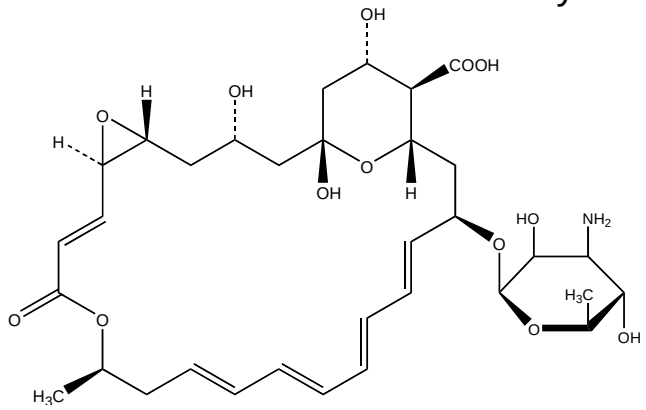
1-Polyenes



Amphotericin B



Nystatin

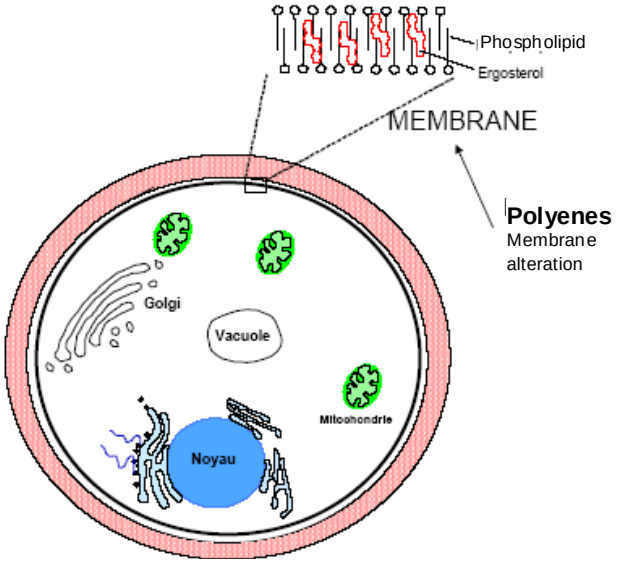


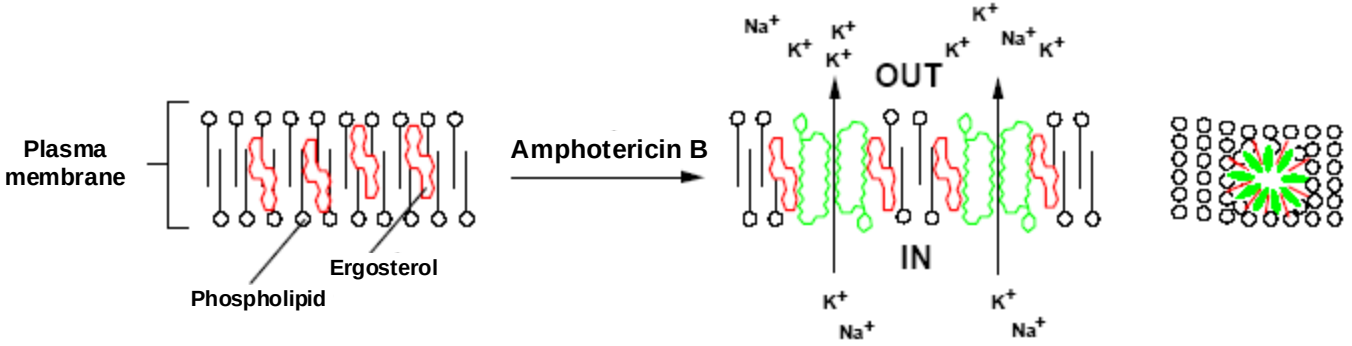
Pimaricin

- Amphiphilic molecules combining with ergosterol to produce holes in fungal plasma membrane

Polyenic antifungals
(Mechanism of action)

- Complex with ergosterol
  → pore formation in the fungal plasma membrane
- ↗ of permeability
  (uncontrolled exchange of electrolytes)
- Cell death





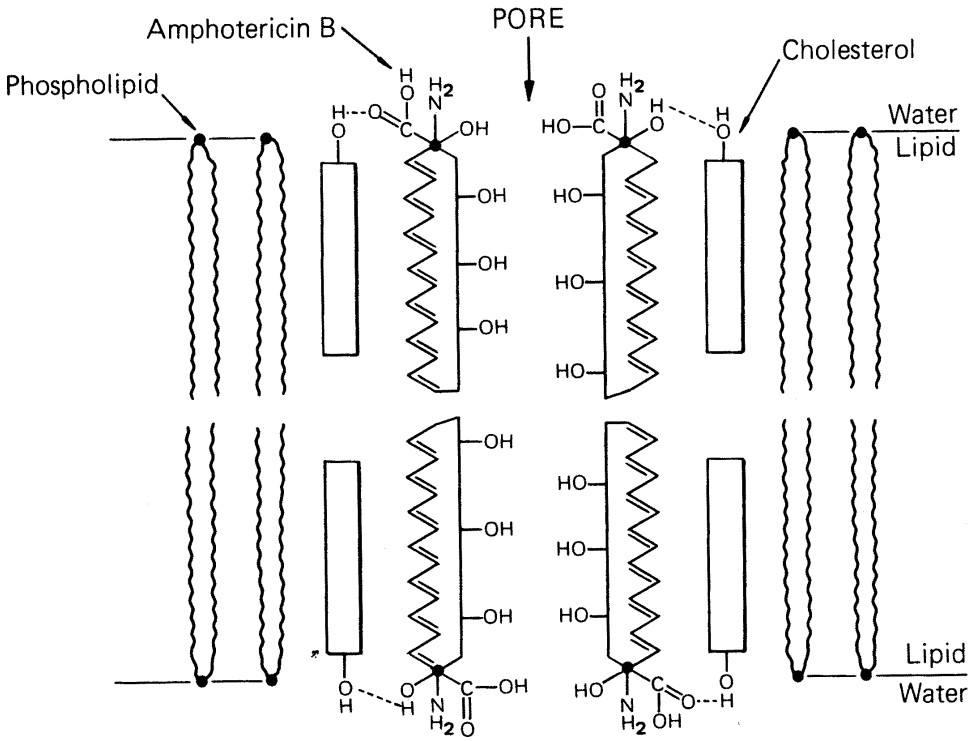
Pore formation allowing the release of monovalent cations leading to cell death

Polyenic antifungals
(Mechanism of action)

→ Fungicide action on yeasts and filamentous fungi

BUT

Also activity on cholesterol of human cells → Toxicity

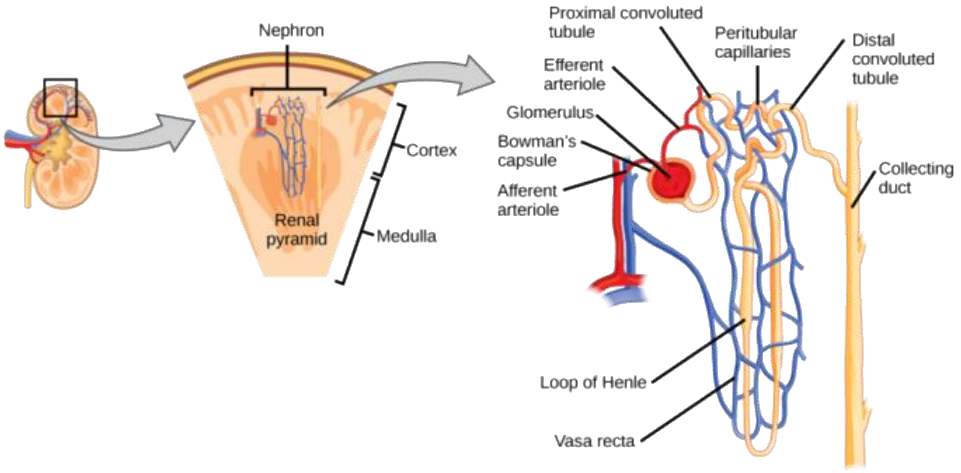


Amphotericin B (AmB)

- Insol. Water and alcohol
- + bile salt for micellisation
- Deoxycholate → Fungizone®
- Not absorbed in the digestive tract
- Weak diffusion in CSF
- IV/or local administration
- **Renal toxicity**

- Slow IV (G5)
- **Creatinine, K+, Mg++**
- **Complete Blood Count (CBC)**

Amphotericin B :



Mechanism of nephrotoxicity

1 – Reversible glomerular toxicity :

- reduction of glomerular filtration
- Renal vasoconstriction
- Contraction of mesangial cells

2 – Tubular toxicity

Formation of membrane pore by AmB oligomers, combining with cholesterol: irreversible…. Chronic renal failure

Mechanism of direct toxicity

Induces production of IL-1 & TNF-α by activating transcription in monocytes, production of PGE2 (hypothalamus): **Fever and shivering**

Fungizone® (for IC
toxicity: renal+++, cardiac++, hematologic+

- **Contraindications**:
- Hypersensitivity to AmB
- Renal failure
- Drugs inducing torsades de pointes (terfenadine, halofanthrine, pentamidine, erythromycin IV, sparfloxacin…), bradycardia, hypo K+, long QT
- **Cautions**:
- Digitalis medicines (hypoK+)
- Drugs inducing hypoK+ (diuretics, gluco- and mineralocorticoids)

- **Cautions (next)**
- Quinidines , amiodarone…
- Monitor ECG (QT), K+,
- **Adverse effects +++**

Fever, anorexia, nausea, vomitting, low blood pressure, diarrhea, pain at the injection point + thrombophlebitis, generalized pain, neurological and hematological (anemia) troubles…

Monitoring: K+, renal function, ECG

Amphotericin B : spectra

- **Large spectra:**
  - **Yeasts**:
  Candida spp. and *Cryptococcus neoformans*

  - **Filamentous** :
  Phaeohyphomycetes (*Alternaria spp.*, …)
  Mucorales
  Aspergillus and other hyalohyphomycetes (*Acremonium spp.*, …)

  - **Dimorphic** :
  Histoplasma, *Blastomyces*, *Coccidioidomyces*, *Paracoccidioidomyces*, *Sporothrix*

Amphotericin B : spectra

- **Lack of activity in the spectra:**
  - *Candida lusitaniae*
  - *Trichosporon sp.*
  - *Aspergillus terreus*
  - *+/- Fusarium sp.*
  - *+/- Scedosporium sp.*
  - Dermatophytes

Fungizone® (for IV),

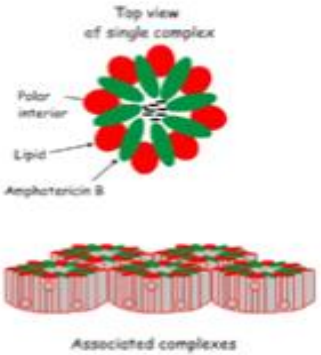
- **Indications**: **was** the drug for systemic mycosis before identification and antifungigram
- **Yeast infections**: *C. albicans, Rhodotorula sp, Cryptococcus neoformans, Histoplasma sp*
- ***Mucorales**: Mucor mucedo, Absidia, Rhizopus*
- ***Invasive aspergillosis** due to A. fumigatus*

- Weak or relative activity : *Candida lusitaniae* (rare), *Trichosporon sp.*
- Active in visceral and muco-cutaneous leishmaniasis (*L. infantum, L. chagasi , L. donovani*)
- Replaced by Liposomal-AmB (AmBisome® and Abelcet), less toxic

AmB formulations: **Abelcet®**, Amphocil®, **AmBisome®**
IV route, less toxic but more expensive

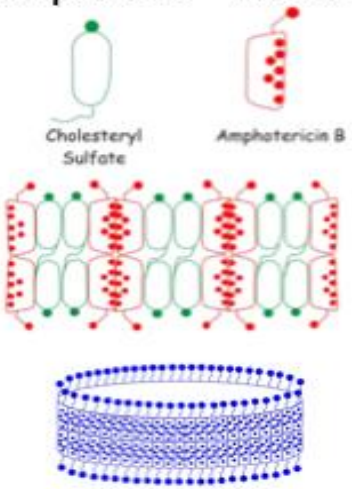
Lipid Amphotericin B Formulations

Abelcet ® ABLC



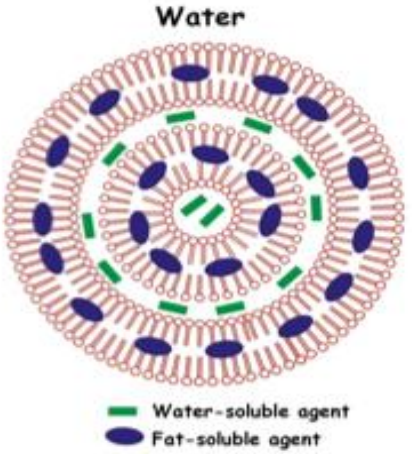
Ribbon-like particles
Carrier lipids: DMPC, DMPG
Particle size (µm): 1.6-11

Amphotec ® ABCD



Disk-like particles
Carrier lipids: Cholesteryl sulfate
Particle size (µm): 0.12-0.14

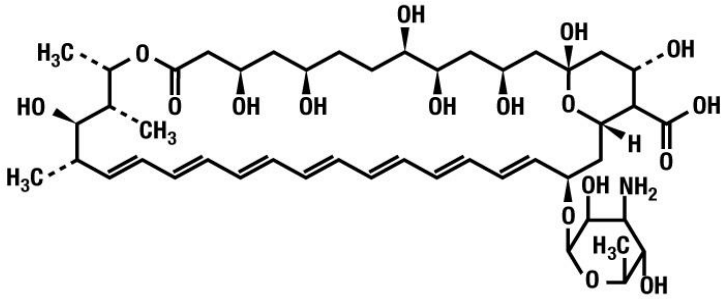
Ambisome ® L-AMB



Unilaminar liposome
Carrier lipids: HSPC, DSPG, cholesterol
Particle size (µm) : 0.08

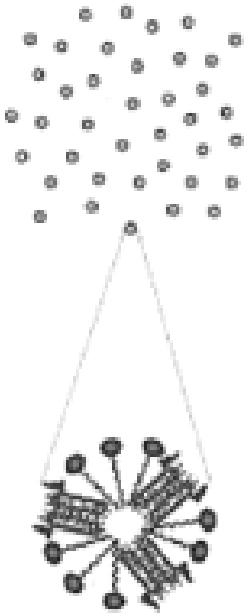
DMPC-Dimyristoyl phospitidylcholine
DMPG- Dimyristoyl phospitidylglycerol
HSPC-Hydrogenated soy phosphatidylcholine
DSPG-Distearoyl phosphitidylcholine

Amphotericin B

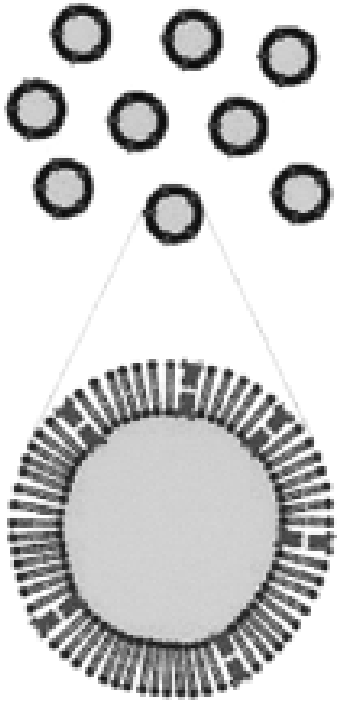


Amphotericin B deoxycholate

Fungizone® (for IV)



Micelle
(DAMB < 10 nm)



Liposome
(LAMB 50-100 nm)

Liposomal amphotericin B

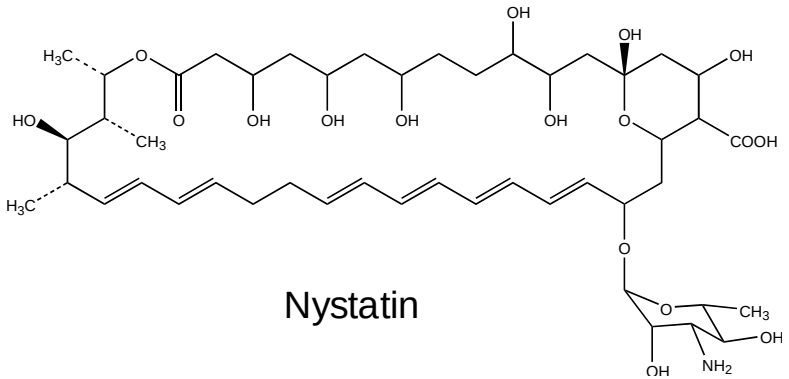
AmBisome®

AmBisome® (Gilead)

- Treatment of invasive mycoses
- Treatment of fungal infections, either presumable or documented
- 3 mg/kg/d in 30 min.
- 3 to 5 mg/kg/d X x week.

- Infantile visceral leishmaniases
- 3 mg/kg/d (D1 to D5 and D10) → 18 mg/kg total
- Expensive
- Monitoring: idem to Fungizone®
- Adapt the posology if renal failure

Antifungal polyene for local administration



Nystatin

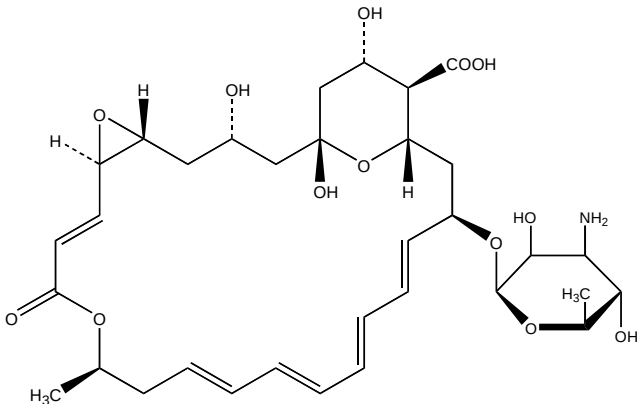
Close to AmB, same spectra, not much absorbed by oral route
Formulations intended for cutaneous and mucosal application, fungal sterilization of the digestive tract (*Candida sp*.)
Mycostatine®, tablets, oral solution

In association with neomycine to treat bacterial and/or fungal mixed local infections: *e. g.* Polygynax Virgo®

Contraindication: allergy to polyenic ATF polyéniques

Usage: digestive candidiases (buccal, pharyngeal) except for oesophageal candidiasis in immunosuppressed (HIV). Sterilization of the digestive tract in case of anal or vaginal cutaneous candidiasis. Prophylaxis of candidiasis in premature baby, immunosuppressed, patients under anticancer chemotherapy

Antifungal polyene for local administration



Pymaricin

Pymaricin, polyene, used for the treatment of keratomycoses. Ophthalmic formulation used in USA at both [C] 2.5% and 5%. The molecule adheres and is active at the basis of the corneal ulcer. Used alone or in association with amphotericin B or topical or systemic azoles (*Aspergillus, Fusarium, Candida*).

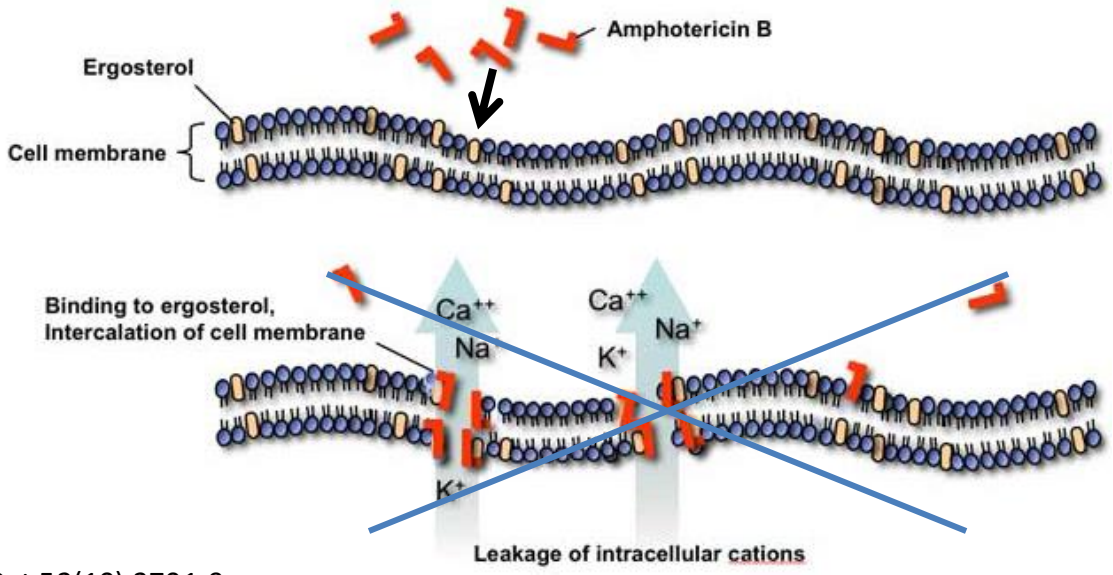
In France, for ocular infection, usage of liposomal AmB in ophthalmic formulation prepared at the pharmacy Hôtel-Dieu, Paris
An ocular formulation Vfend® (voriconazole) is in development

Exceptional acquired resistance to polyenes

➔ System of target disappearance in 2 steps

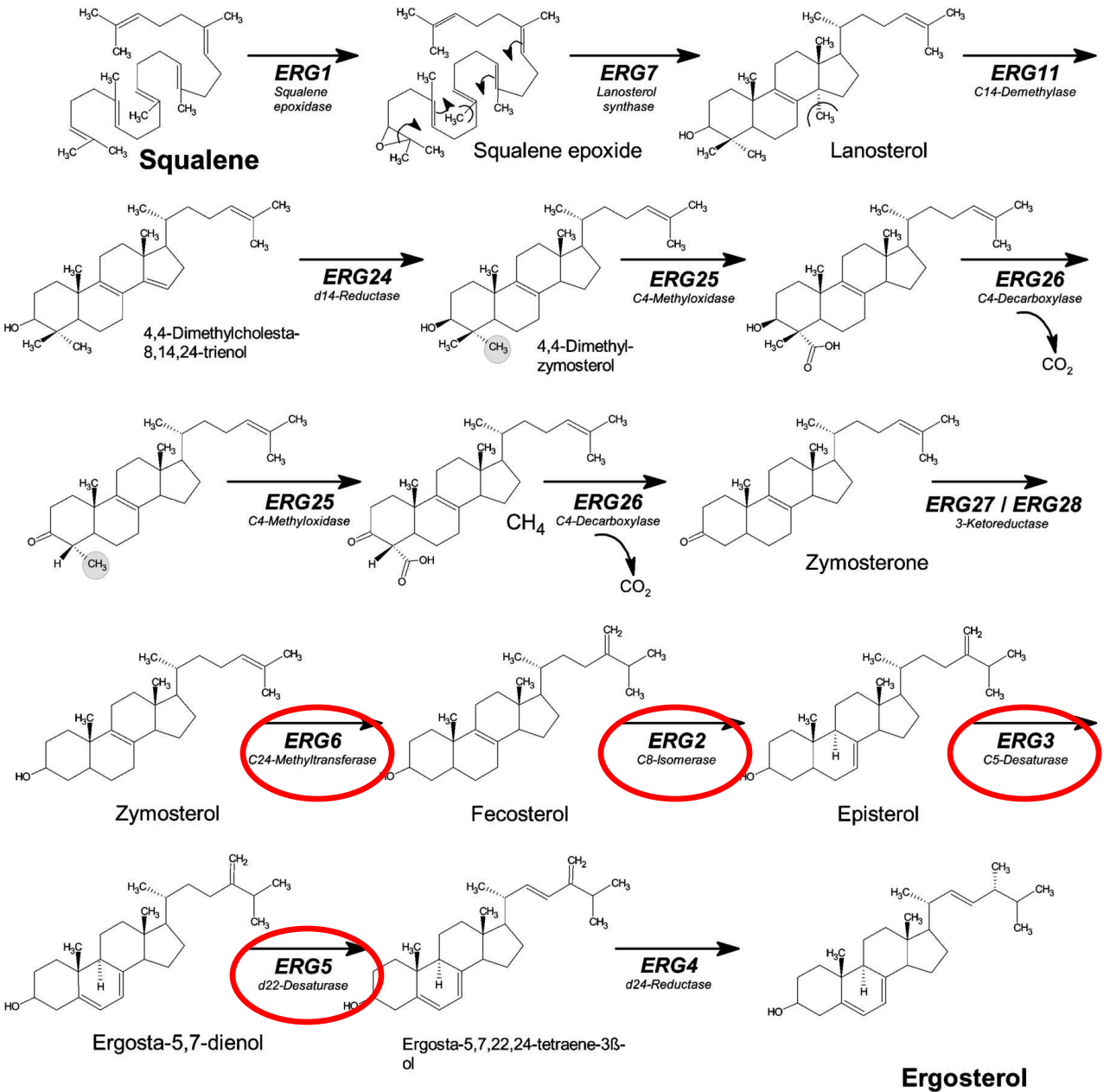
- Target reduction or disappearance (membrane ergosterol) by mutation of ergosterol biosynthesis pathway (*e.g. ERG6* in *C. albicans*)

- Replacement of ergosterol by other « viable » sterols



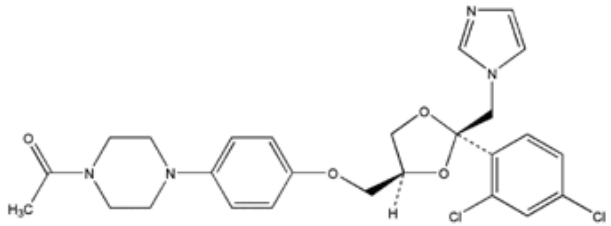
Vandeputte P, AAC 2008 Oct;52(10):3701-9.

Ergosterol biosynthesis pathway

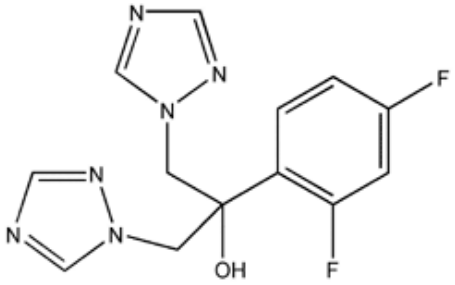


AZOLES

Azoles: di or triazoles



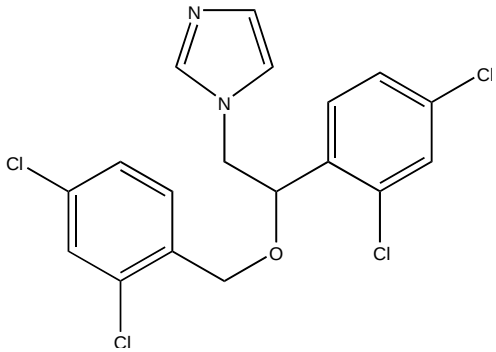
ketoconazole



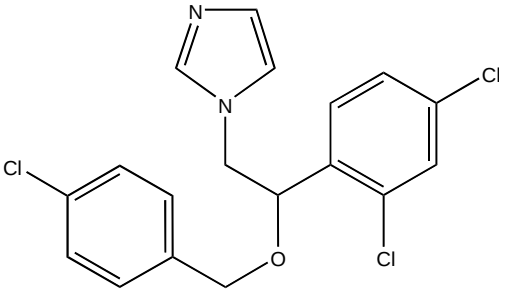
fluconazole

- Prominent antifungal agents: inhibit ergosterol biosynthesis (fungistatic)

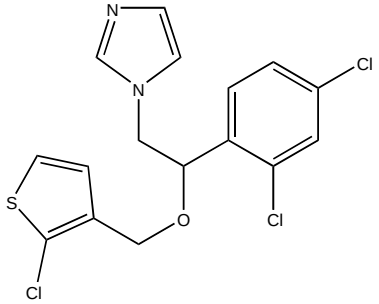
DIAZOLES



Miconazole

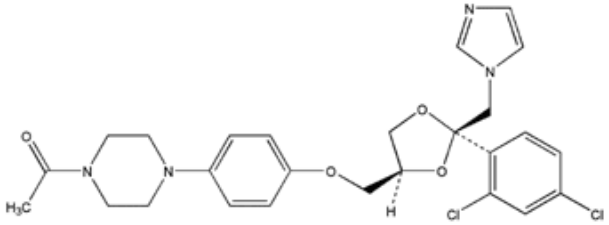


Econazole



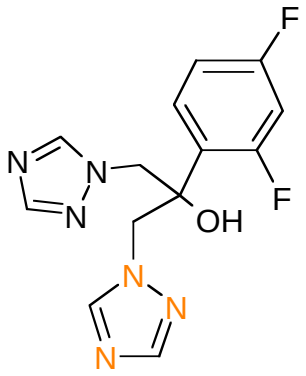
Tioconazole

Local usage



Ketoconazole
(~~Nizoral~~®, Ketoderm®)

TRIAZOLES (1/2)



FLUCONAZOLE TRIFLUCAN ®

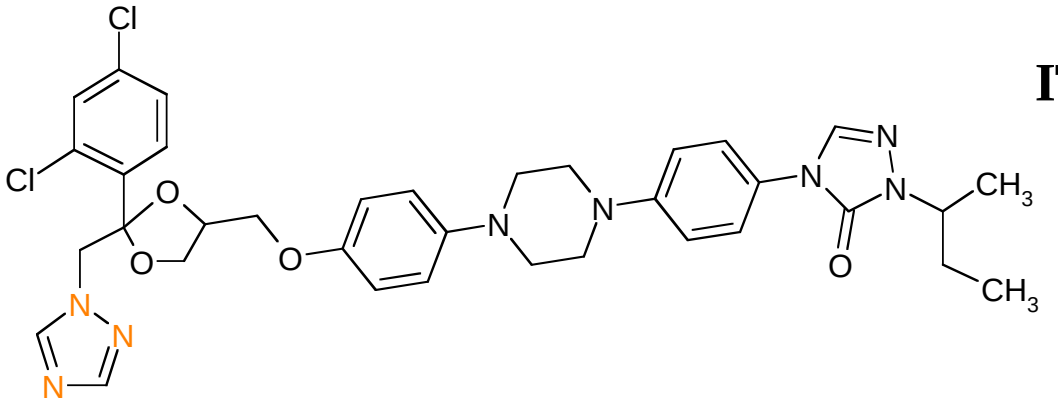
PFIZER 1990

IV form

Good biodisponibility by oral route

Crosses the BBB

Existence of resistances



ITRACONAZOLE SPORANOX ®

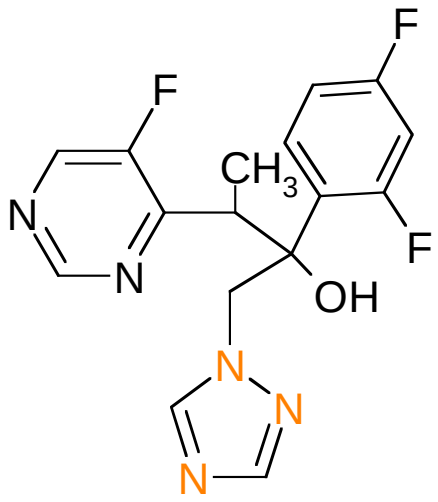
JANSSEN 1992

Larger spectra

Existence of resistances

Evolution of pathogens

TRIAZOLES (2/2)
SECOND GENERATION



VORICONAZOLE VFEND®

PFIZER 2002

Large spectra

POSACONAZOLE: itraconazole analog, more efficient and with a more extended spectra (**Noxafil®**)

RAVUCONAZOLE: fluconazole analog in phase III

ISAVUCONAZOLE: close to posaconazole in phase III

DIAZOLES - TRIAZOLES

- **Econazole (Pévaryl®)**
- **Miconazole (Daktarin®)**
- **Isoconazole (Fazol®)**
- **Bifonazole (Amycor®)**
- **(local usage)**

- **Ketoconazole**

(~~Nizoral®~~, Ketoderm®)

- **Terconazole (Terazole®)**
  → local usage

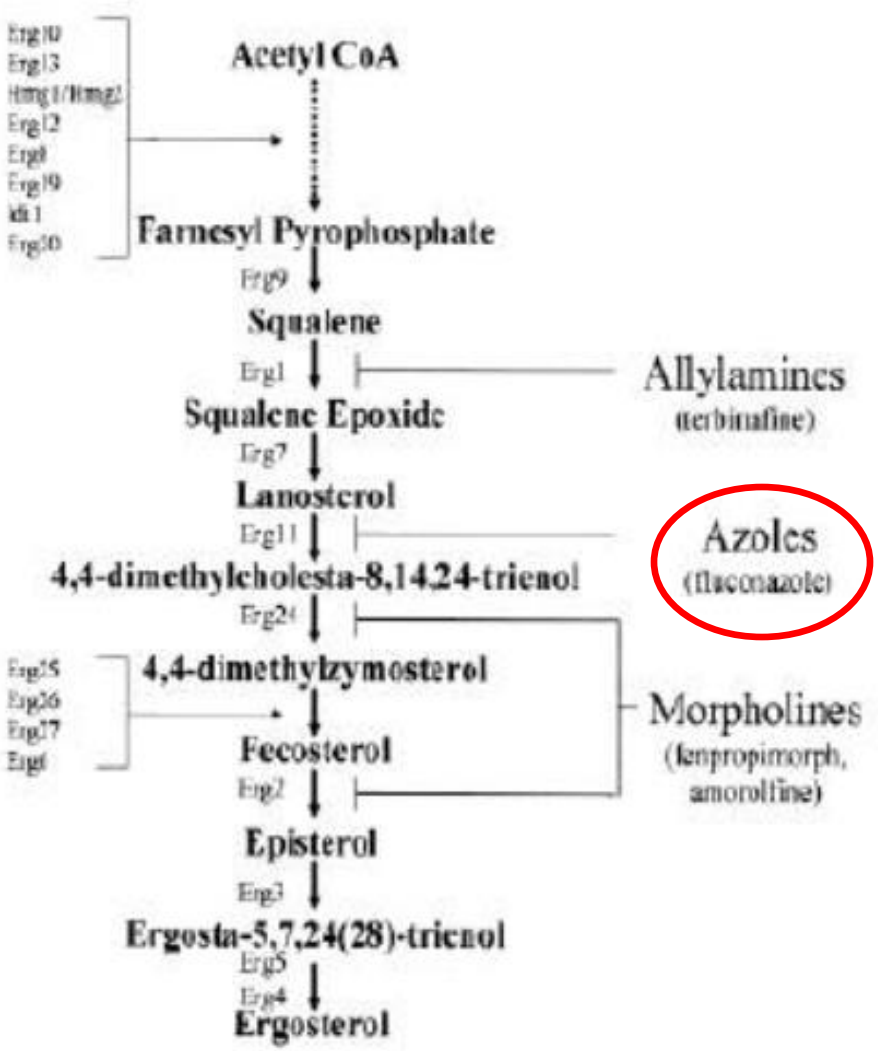
- **Fluconazole (Triflucan®)**
- **Itraconazole (Sporanox®)**

- **Voriconazole (Vfend®)**
- **Posaconazole (Noxafil®)**

Mechanism of action of azoles

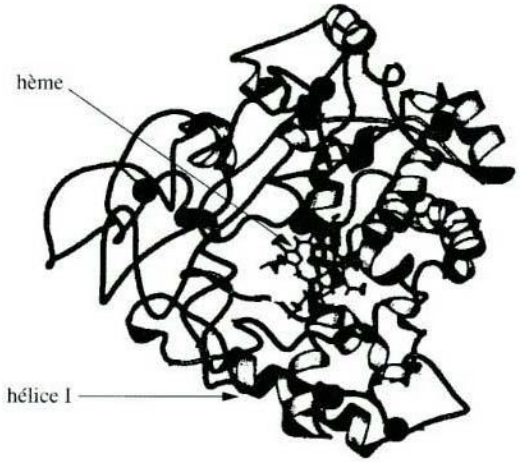
- 14α-demethylase is a cytochrome P450 enzyme [CYP 51; ERG11]: allows the conversion of lanosterol in ergosterol

- Azoles inhibit 14α-demethylase by interacting with its active site

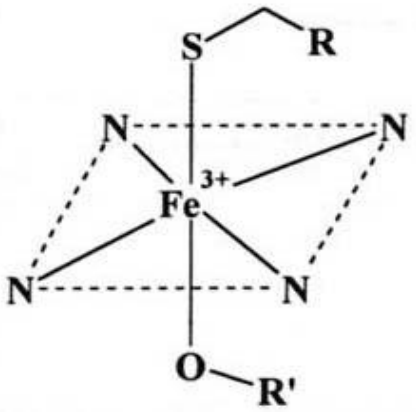


Mechanism of action of azoles

- All azoles inhibit 14α-demethylase, a cytochrome P450 enzyme [CYP 51] ERG11, by preventing the conversion of lanosterol in ergosterol



Molecular structure of 14α-demethylase

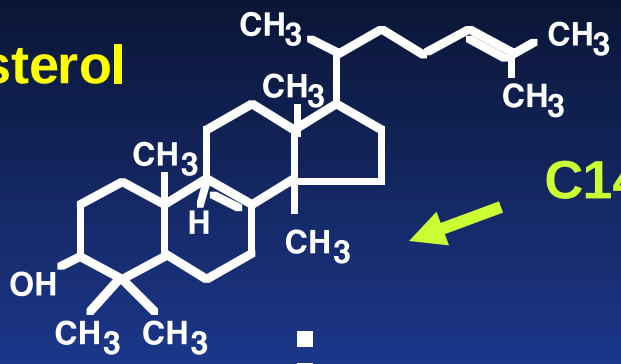


Catalytic site of 14α-demethylase

Traité de chimie thérapeutique vol 5 tome1: Principaux antifongiques et antiparasitaires Ed. Tec& Doc, 1999

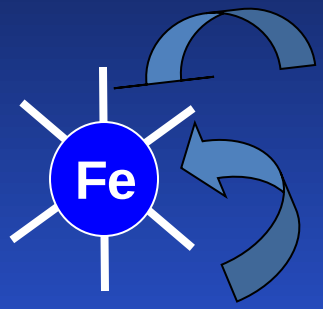
Mechanism of action of azoles

Lanosterol



C14 α demethylase (CYP51, *ERG11* gene)

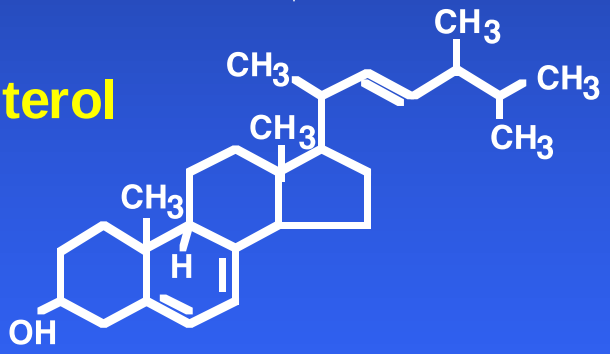
Cytochrome P450-dependent



Interaction with heme

Azoles

Ergosterol



Consequences :

- **Inhibition of ergosterol biosynthesis**
- **Membrane alteration**
- **Accumulation of toxic methylated sterols**

From Cowen LE. Nat Rev Microbiol, 2008; 6: 187-198

METABOLISM

- hepatic by cytochrome P450 isoenzymes from sub-groups 2C9, 2C19 et 3A4
- Polymorphism of 2C19
  - → slow or fast metabolizers
    ratio 2 to 4

Prevalence for slow metabolizers: 5 to 20% depending on ethnia (asians +++ → Vfend® Tx X 4)

Drug interactions by enzymatic inhibition, induction, or both

Azoles : substrates and/or inhibitors of cytochrome P450 enzymes

Table 1. Metabolism of azole antifungals

Enzyme system	Fluconazole	Itraconazole	Posaconazole	Voriconazole
Inhibitor				
2C9	++*	+	–	++
2C19	+	–	–	+++
3A4	++	+++	+++	++
P-glycoprotein	–	Yes	Yes	–
Substrate				
2C9	–	–	–	+
2C19	–	–	–	+++
3A4	–	+++	–	+
P-glycoprotein	–	Yes	Yes	–

**Plus signs* (+) indicate the severity of inhibition or induction.
(*Data from* Wang et al. [11] *and* Saad et al. [62••].)

From Brüggemann et al., 2008, Curr. Fungal Infect. Rep.

PROBLEM

Lack of specificity of the drug

⇩

Cross-inhibition of hepatic cytochrome p450 enzymes acting in sexual hormones and corticoid biosynthesis and in hepatic metabolism

⇩

toxicity of azoles

Azoles : secondary effects

- **Hepatic toxicity**
  Fluconazole < other molecules

- Non specific digestive disorders

- **Cutaneous hypersensitivity**: photosensitivity, prolonged dermatologic observation **with Voriconazole**

- **Drug interactions**

Drug interactions

AmB

- **Un-recommended co-prescription with:**
  - Nephrotoxic drugs (aminosides, ciclosporine)
  - Digitalis
  - Potassium-sparing diuretic
  - Drugs able to induce torsade de pointe

Voriconazole (CYP450)

- **Un-recommended co-prescription with:**
  - Sirolimus
  - Enzymatic inducers able to decrease plasma concentration (rifampicin, carbamazepine, phenobarbital)
  - Drug inducing long QT syndrome
  - Dose of ciclosporine and tacrolimus → to be adapted

Itraconazole (CYP450)

- Interactions close to those of voriconazole

Posaconazole (CYP450)

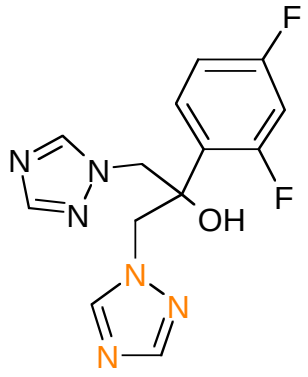
- Less interactions
- Dose immunosuppressors
- Caution with drugs inducing long QT syndrome

Caspofungin

- Few interactions
- With enzymatic inducer: preserve maintenance dose at 70 mg/j

Fluconazole Triflucan®

- Major antifungal drug used against *C. albicans* (mucosal and systemic) and *C. neoformans*
- Possible resistance: antifungigram
- Naturally resistant species: *C. krusei*
- Decreased sensitivity: *C. glabrata*



Itraconazole - Spectra

Good activity on :

→*Aspergillus sp.*

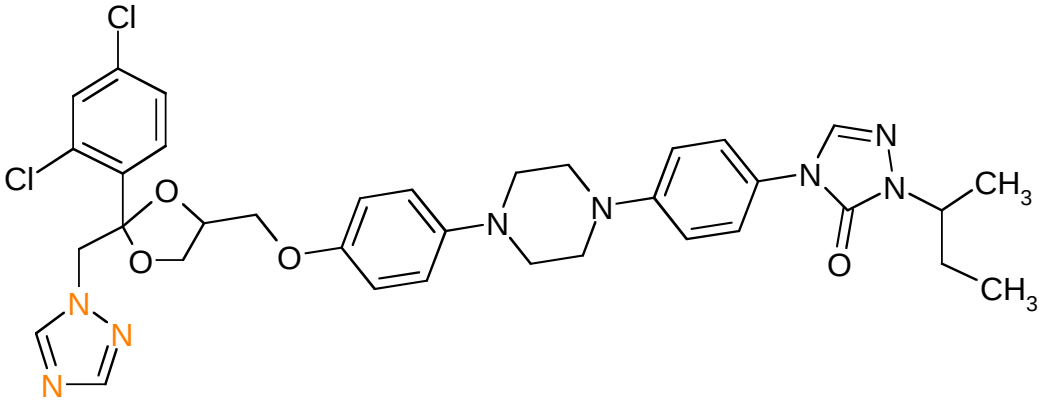
→Dimorphic fungi

→Other phaehyphomycetes (*Altenaria spp.*, …)

→*Malassezia*, *Dermatophytes*

→Yeasts : *Cryptococcus*, *Candida*

Lack of activity on: *Fusarium sp.*, Mucorales



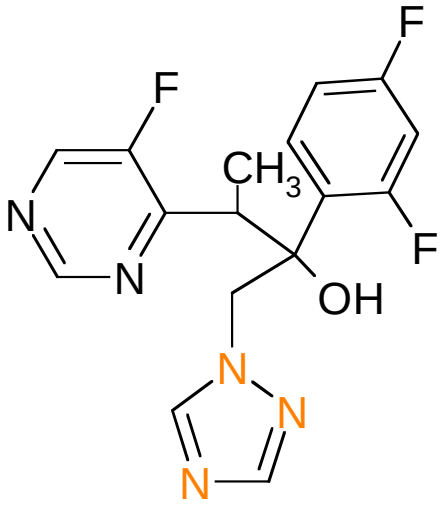
Voriconazole (Vfend®)

- **Pulmonary invasive aspergillosis due to *A. fumigatus***

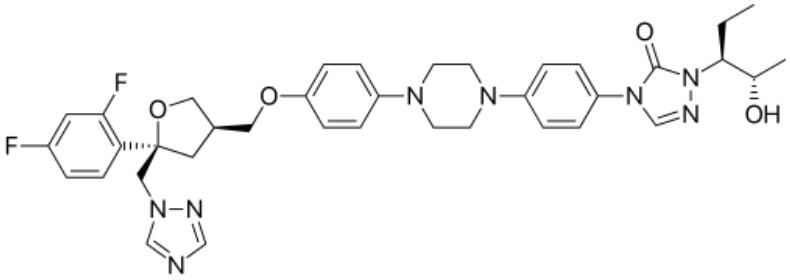
- *Candida* resistant to Fluconazole

  (C. *krusei, C. glabrata, C. guilliermondii*)

- Rare infections:
  - *Fusarium sp, Penicillium marneffei, Scedosporium sp*.
  - Not much active on zygomycetes *(Rhizopus, Mucor*)



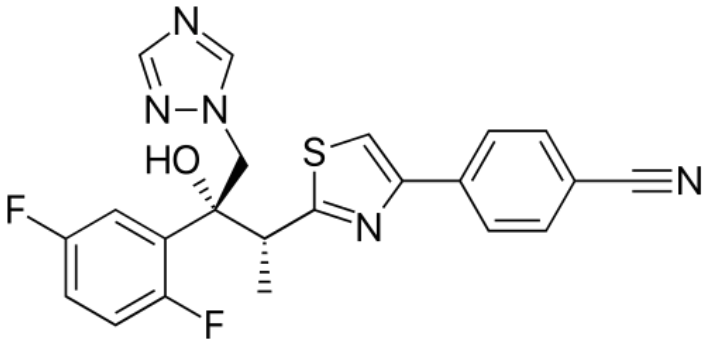
Posaconazole (Noxafil®)



- Close to itraconazole
- Inhibitor of CYP3A4 and CYP51
- PO: fat meal or coca cola
- 600 mg/prophylactic treatment
- D1 and D2 : 200 mg x 4/d (D1) then 400 mg x2/d (D2) curative treatment during a meal
- No modification of posology in patients with renal or hepatic failure
- Dosage +++++++ at D5
- Zygomycetes (*Mucor*),
- Dimorphic fungi
- *Aspergillus fumigatus, Candida sp.*
- *Fusarium sp., Scedosporium*

Isavuconazole

- **Formerly BAL 4815**
- **Large spectra (close to posaconazole)**
- **Can be administrated *per os* or by IV route**
- **Long half-life (4 days)**
- **Good tolerance**



- **Therapeutic option as a first intention treatment for invasive aspergillosis and second intention treatment for mucormycosis in patient where AmB is inappropriate (marketing authorization; AMM)**

Comparison of azoles

International Non-Proprietary Name (INN)	Fluconazole	Voriconazole	Posaconazole	Isavuconazole
Commercial name	Triflucan	Vfend	Noxafil	Cresemba
Half-life	24-30h	6-9h	35h	100h
Biodisp. Food influence	90% No food effect	96 % (fasting) ↘ AUC of 24% with food	Suspension: food and distribution of doses ↗ absorption (+++	95% No food effect
Metabolism Elimination	Predominant renal excretion (unchanged for 80%)	Hepatic metabolism (CYP2C19, CYP2C9, CYP3A4)	Hepatic metabolism (glucuronidation by UDPG)	Hepatic metabolism (CYP3A4)
P450 inhibition	CYP3A4 (+), CYP2C9, CYP2C19	CYP2C19 (++), CYP2C9, CYP3A4	CYP3A4 (+++)	Weak inhibitor of CYP3A4 and P-GP)
Adjust. hepatic insuffisciency	NO	YES (half dose) for weak to moderate insuffisciency	NO for weak to moderate insuffisciency, but non-recommended usage	NO for weak to moderate insuffisciency
Adujst. Renal insuffisciency	YES (half dose if clearance 11 to 50 mL/min)	NO	NO	NO
Presentation	**PO**: 50, 100, 200 mg capsules, powder for drink. sol.: 50, 200 mg/ 5 mL **IV:** perf. Sol (2 mg/ml) 50, 100, 200 mL vials	**PO**: 50, 200 mg tablets, powder drink. sol.: 40 mg/mL **IV (perf)**: 200 mg powder vials	**Drink. sol.**: 40 mg/mL 100 mg **gastro-resistant tablets**	**Capsule** (186 mg isavuconazium sulfate namely 100 mg isavuconazole) **IV solution**
Posology for adults	**Loading**: 800 mg; **Then** 400 mg/d	**Loading** D1: 6 mg/kg (IV) or 400 mg (PO) every 12h, **Then** 4 mg/kg or 200 mg x2/d	**Drink. Sol.**: 400 2x/d; 200 mg 3x/d in prevention **Tablet**: 300 mg x2 D1 then 300 mg 1x/d	**Loading** D1 and D2: 200 3x/d **Then** 200 mg 1x/d

Mechanisms of resistance to azoles

Sanglard et Odds, 2002, The Lancet Infectious Diseases, 2: 73-85

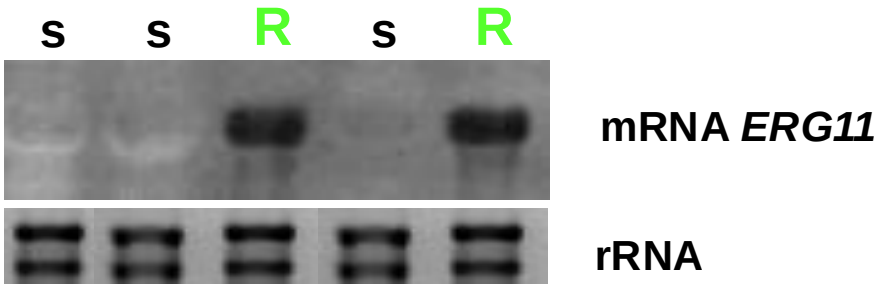
1 - Overproduction of the target CYP51 encoded by the gene *ERG11*

* ↗ of *ERG11* transcription ⟶ overexpression

mRNA increased by a factor 3 to 10

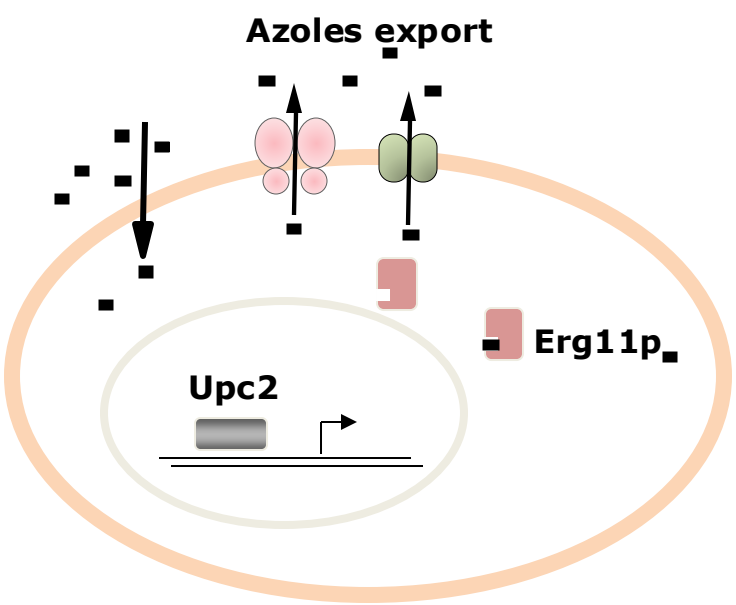
* Gene amplification of *ERG11*

Duplication in several copies ⟶ increase of mRNA

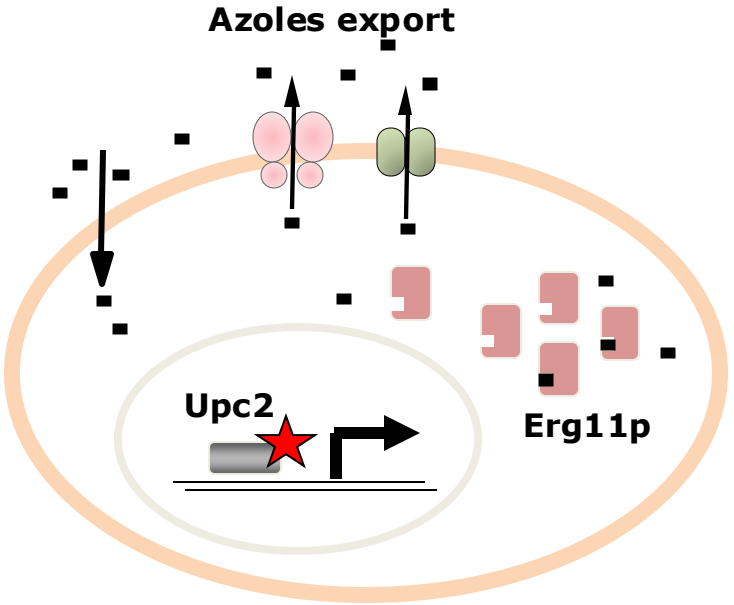


Resistance to azoles by overexpression of the target (Erg11p)

Point mutations in the regulator of *ERG* genes in *C. albicans*



Azole susceptible yeast cell



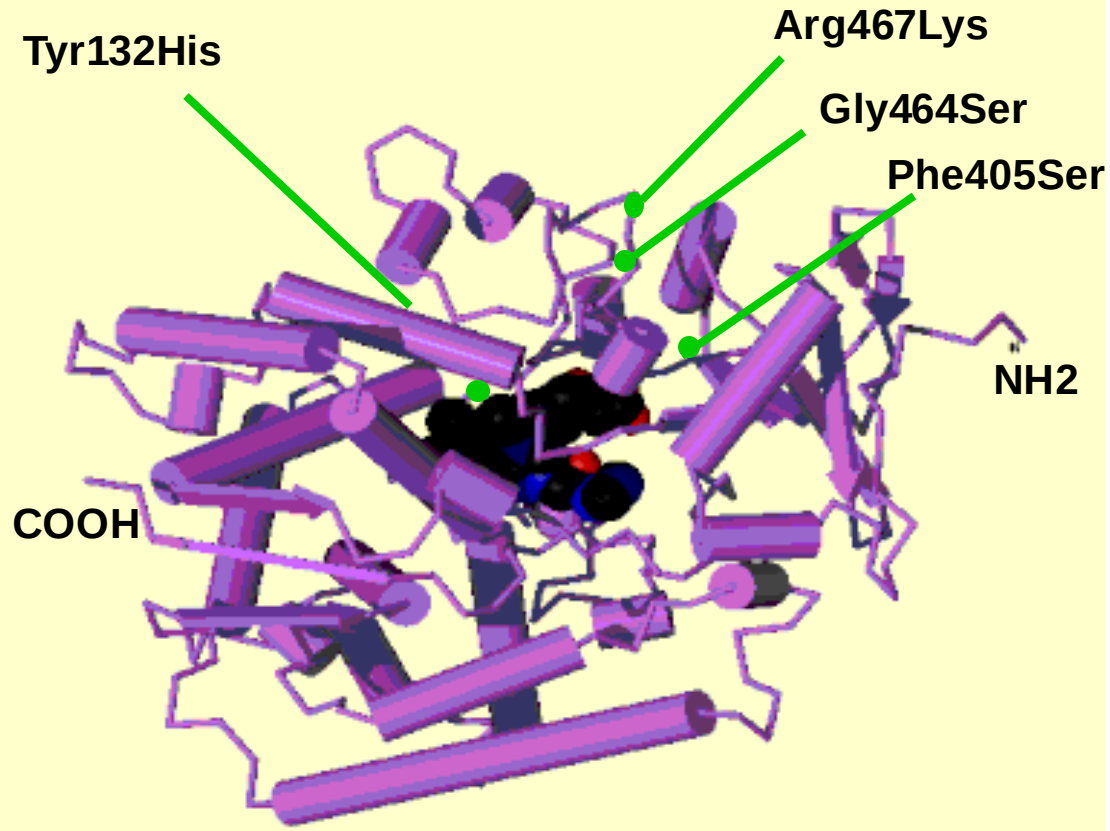
Upregulation of *ERG11*

★ A643V or G648D mutation in the C-terminal part of Upc2 = activation domain

2 - Modification of the target : decrease of affinity for the azole

* Point mutations of *ERG11* gene: substitution of an amino acid

↳ alteration of the CYP51-Azole interaction



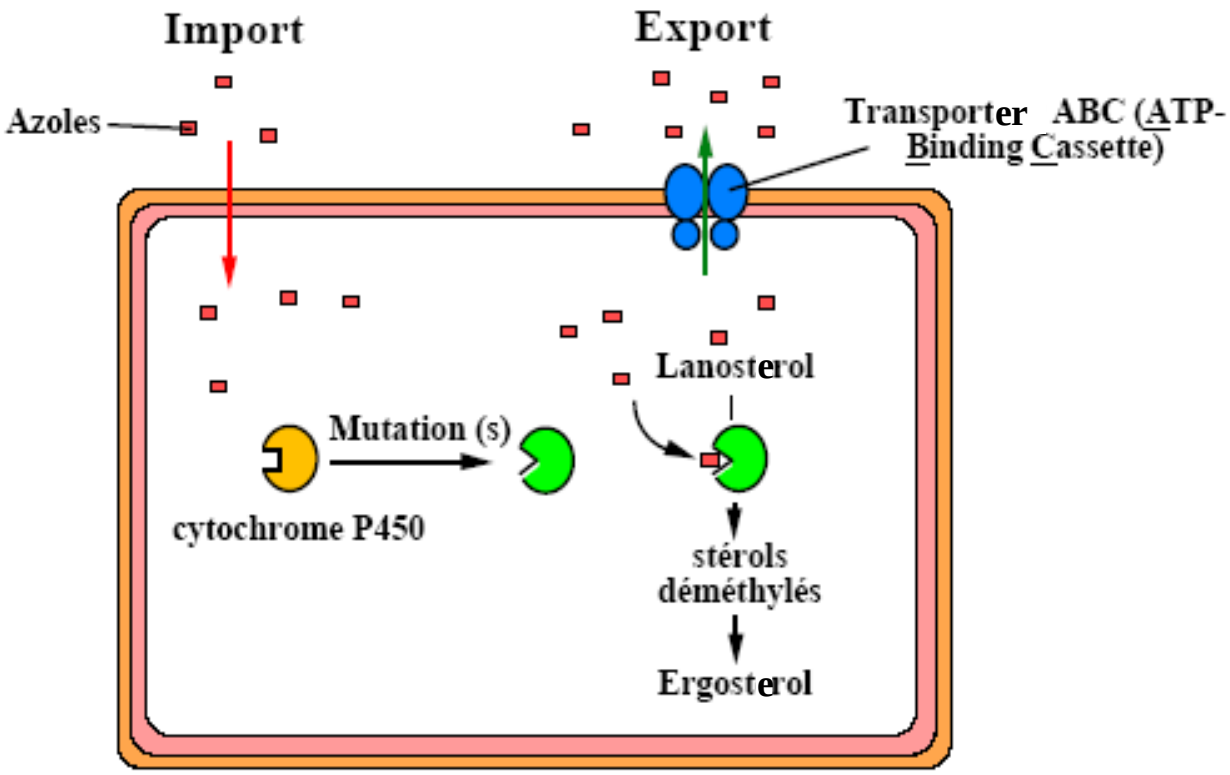
CYP51
Mycobacterium tuberculosis

Podust et al., 2001
PNAS 98: 3068-73

2 - Modification of the target : decrease of affinity for the azole

* **Point mutations in the *ERG11 gene* : substitution of an amino acid**

↳ alteration of the CYP51-Azole interaction



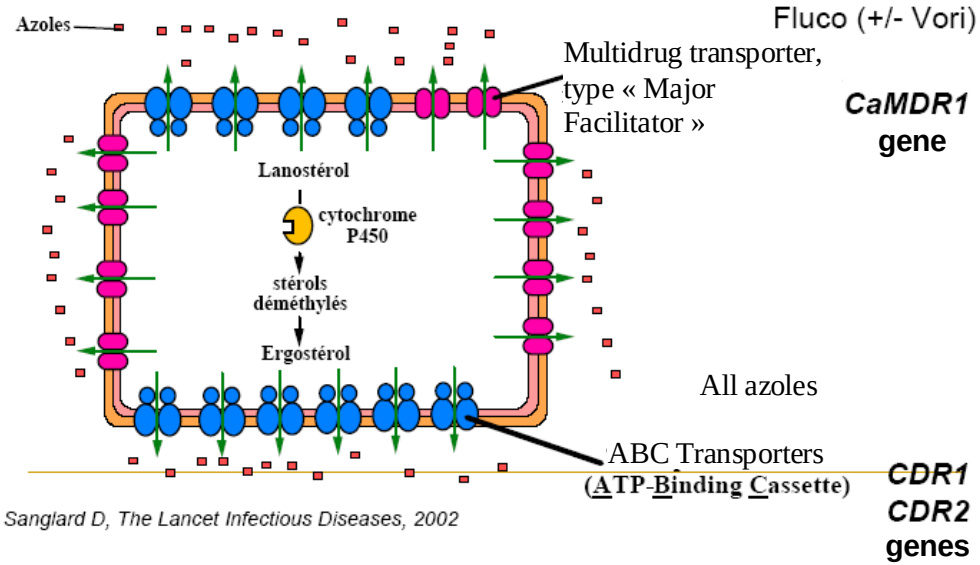
Mechanisms of resistance to azoles

3 – Efflux systems

* Network of membrane transporters (pumps)

  Function : excrete toxic substances

* Systems spread in all cells of all kingdoms



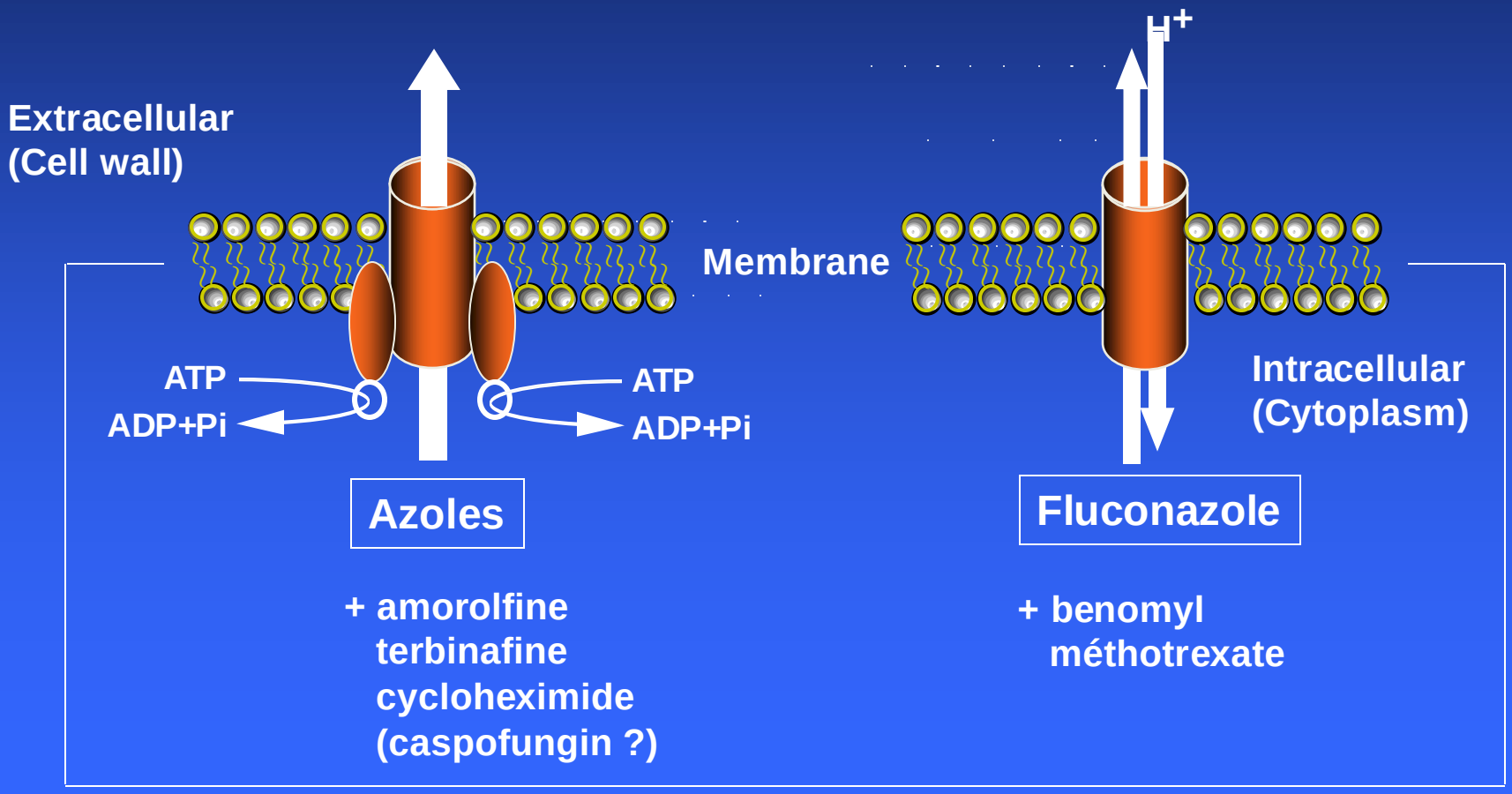
* In yeasts, 2 transporter families involved azole resistance. Can be distinguished by:
  - → the energy necessary for their function
  - → their spectra for antifungals

+ Regulator genes family ⟶ quantity of transporters

Efflux pumps

ABC Pumps
(ATP-Binding Cassette)
Genes *CDR1, CDR2, etc.*

MF Pumps
(Major Facilitator)
Genes *MDR1 (FLU1)*



+ amorolfine
terbinafine
cycloheximide
(caspofungin ?)

+ benomyl
méthotrexate

Genes regulating
The expression
of CDR et MDR genes

Receptor-ligand

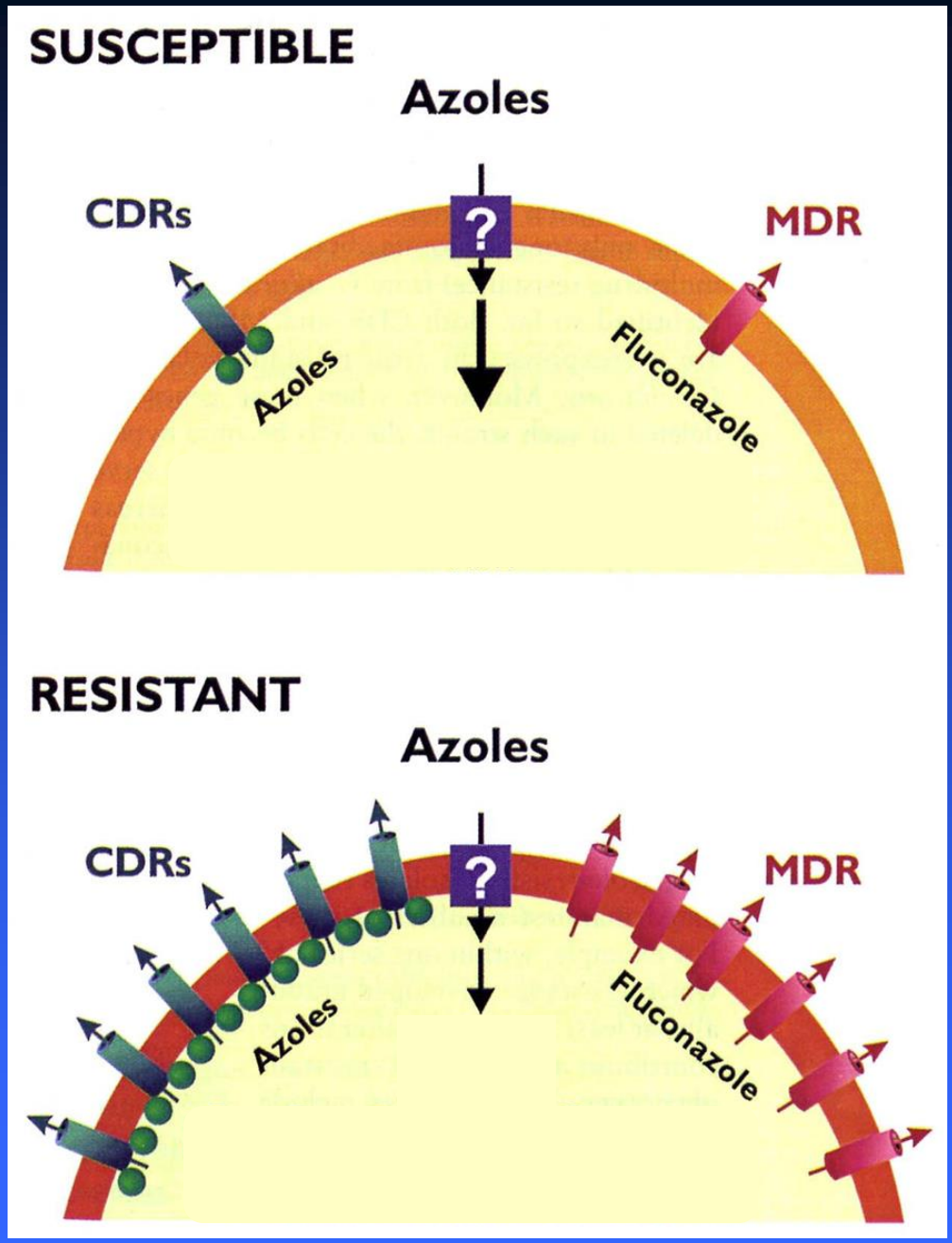
CDR *MDR*

Activation of transcription

mRNA

Transporters

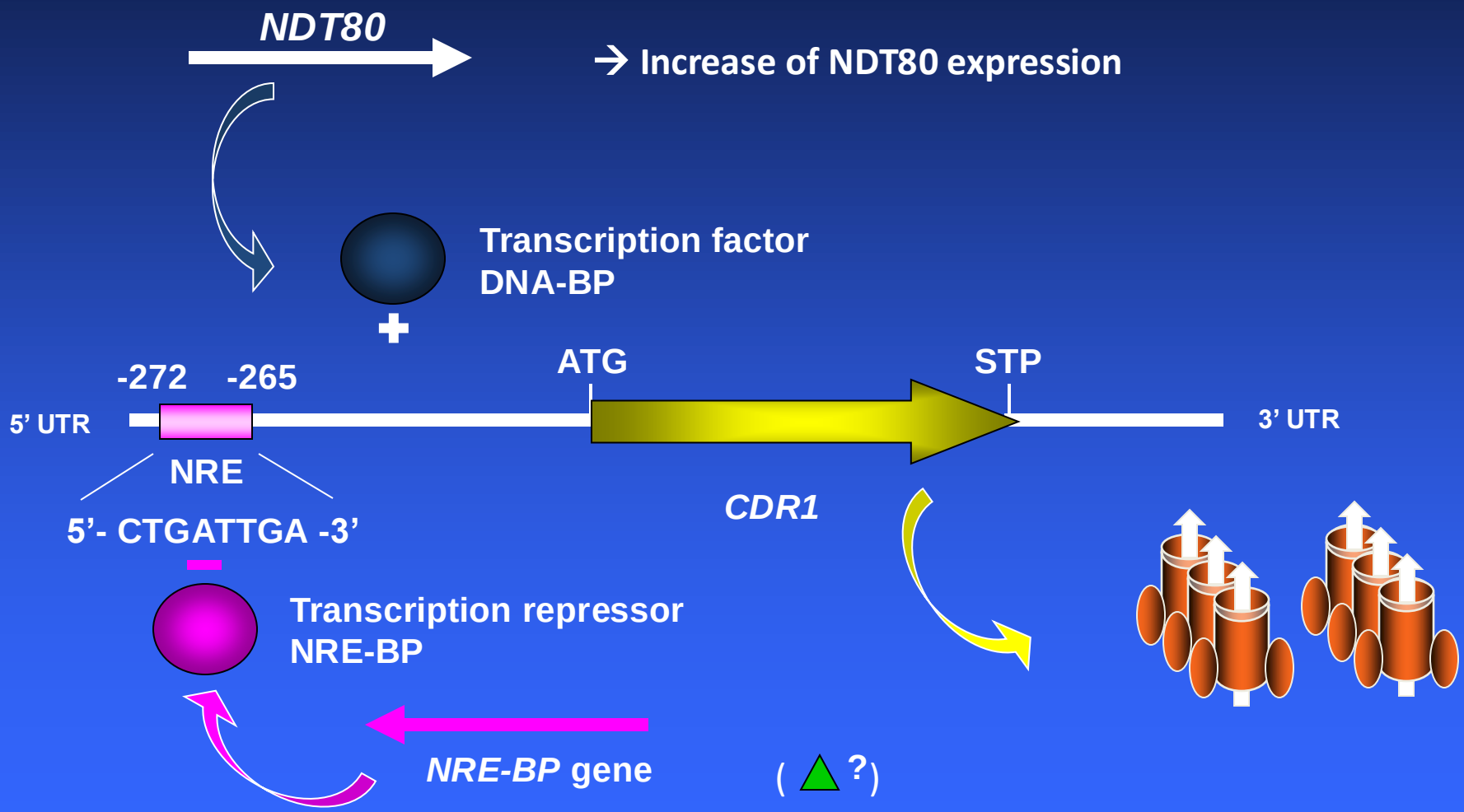
White et al., 1998, CMR 11, 382-402



Transcription activators of *CDR* genes

C. albicans

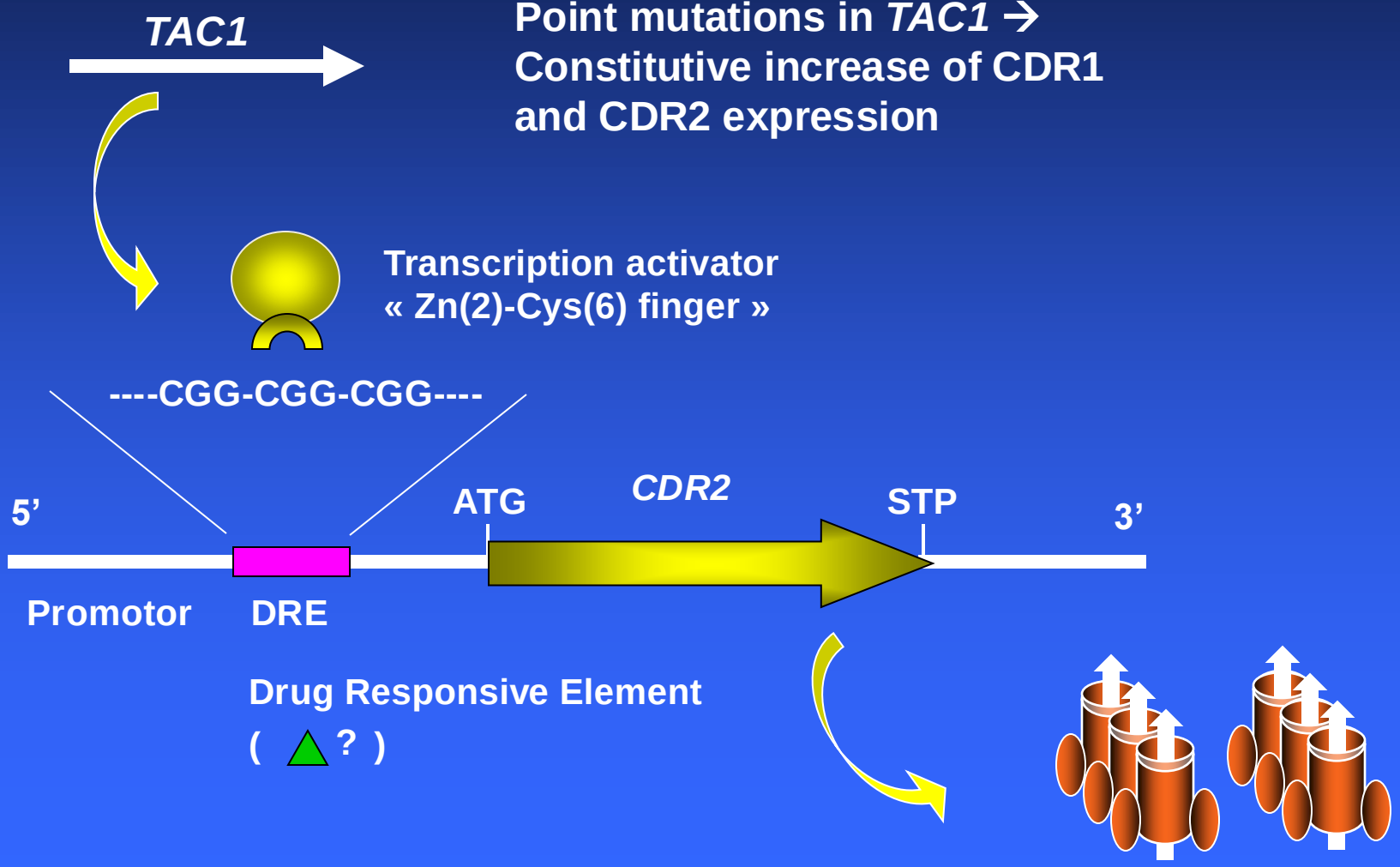
Chen et al., 2004,
AAC 48: 4505-12



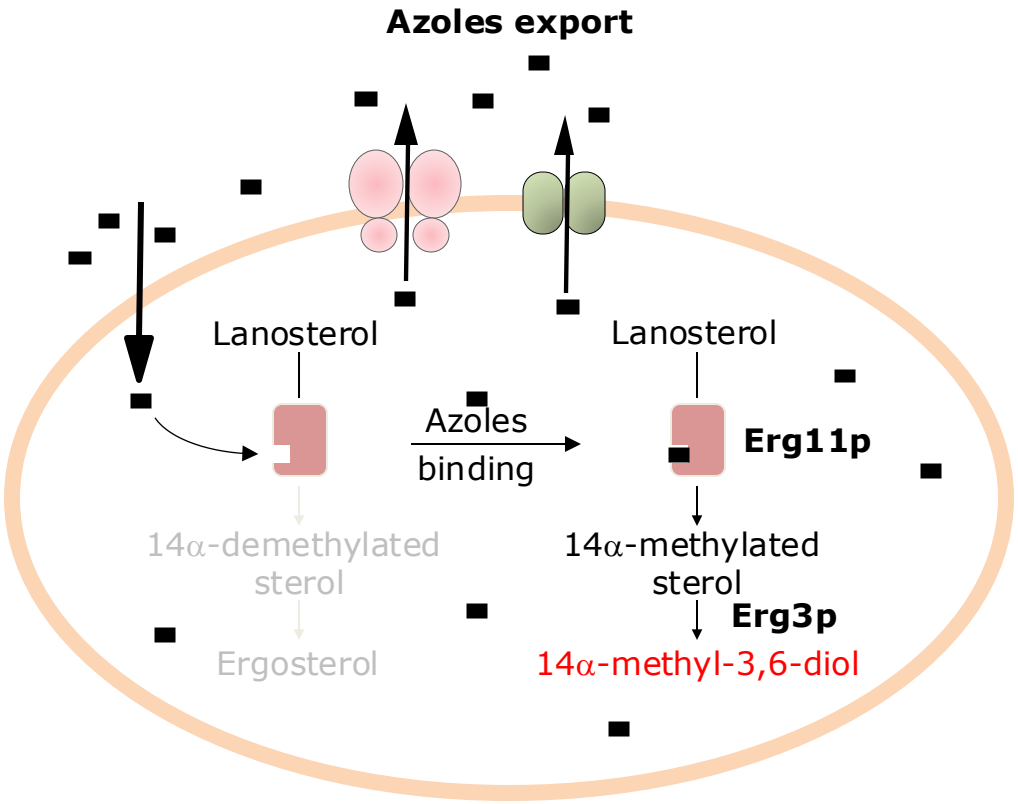
Transcription activators of *CDR* genes

C. albicans

Coste et al., 2004, Eukaryotic Cell 3: 1639-52



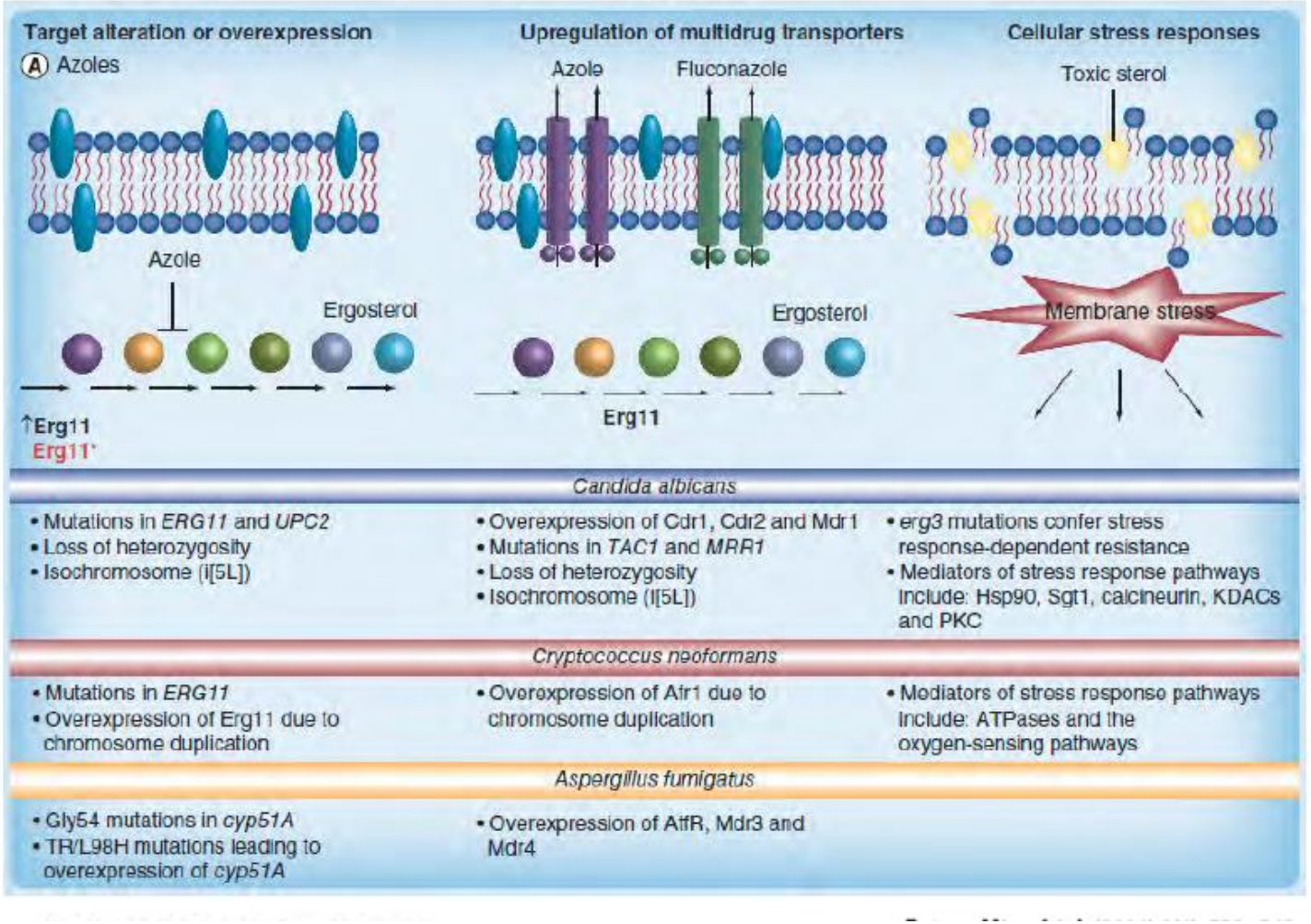
4- Resistance to azoles by modification of ergosterol biosynthesis



Alteration in ergosterol biosynthesis

- Rare mechanism, only detected in clinical isolates of *C. albicans.*
- Leads to cross resistance in all azoles

Summary of mechanisms of resistance to azoles

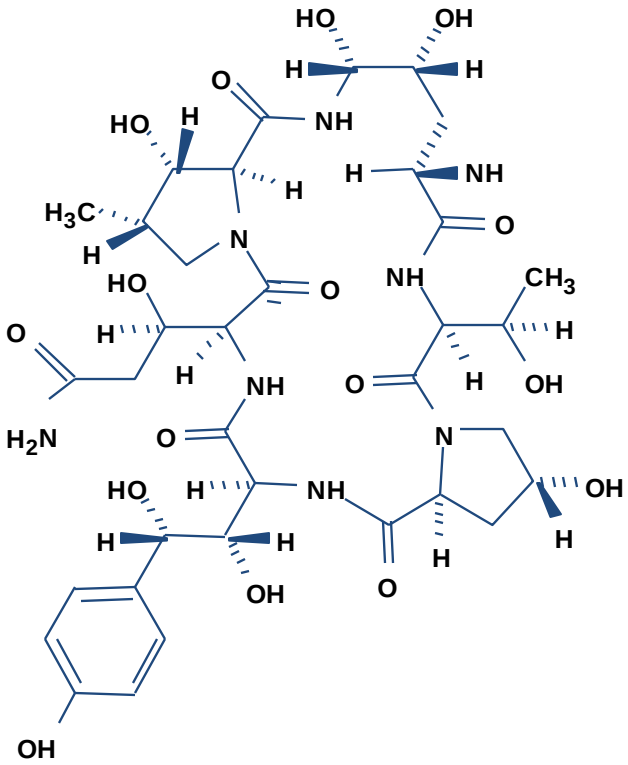


Xie et al., 2014, Future Microbiol. 9(4): 523-542

ECHINOCANDINS

Echinocandins

Echinocandin "backbone"

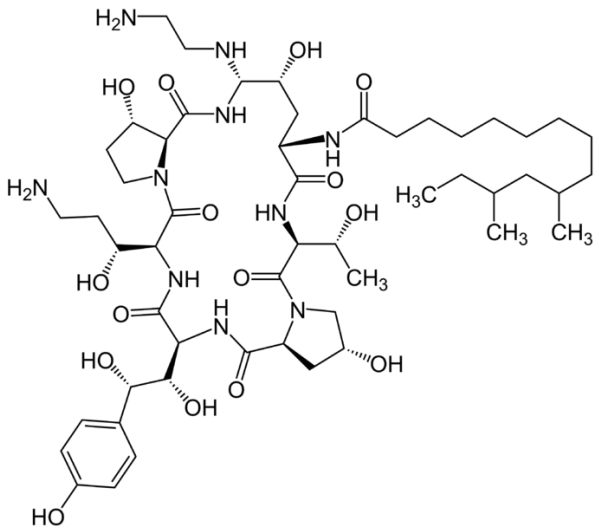


- Cyclic lipopeptids inhibiting 1,3 -β–D glucane synthase encoded by FKS1/FKS2
  - Heterodimeric 210 kDa protein integrated in the fungal plasma membrane
  - Maximal activity: branching and extremities of filaments (growth region)
  - Does NOT exist in HUMAN

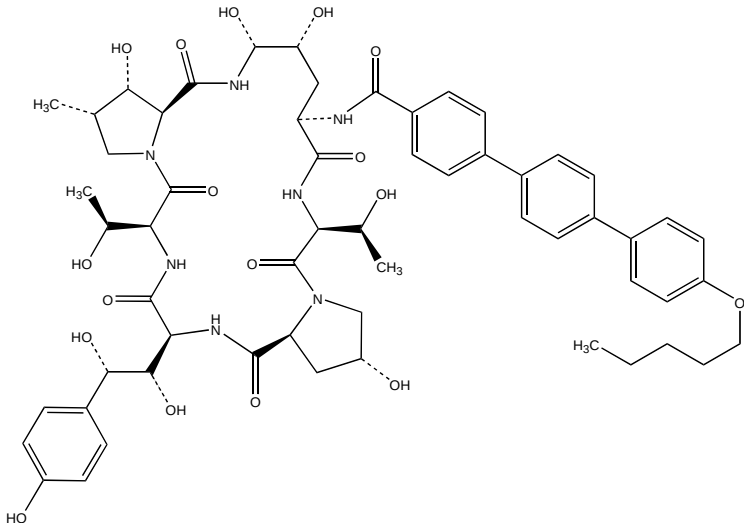
- Three echinocandins
  - **Caspofungin (Cancidas ®) i.v.**
  - **Micafungin (FK463) (Mycamine®)**
  - **Anidulafungin (VER 002) (Eraxis®)**

  - Resistance to one= resistance to others → no difference of spectra between molecules
  - No activity on fungi without 1,3 -β–D glucane in the cell wall : *C. neoformans*, Zygomycetes

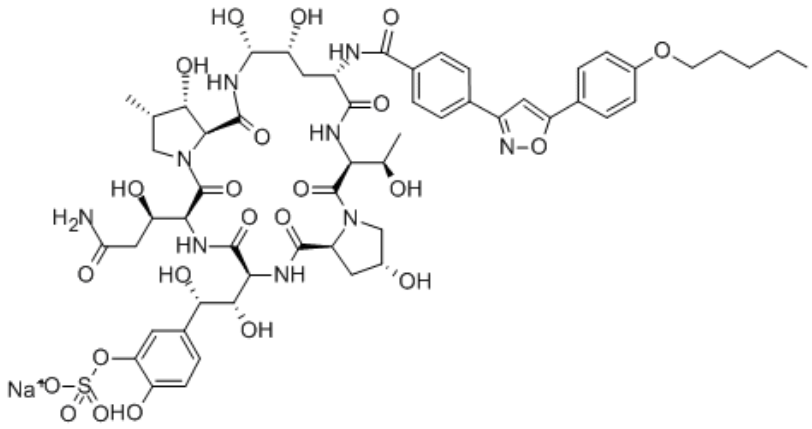
Echinocandins



Caspofungin, (Cancidas®)



Anidulafungin, (Eraxis®)

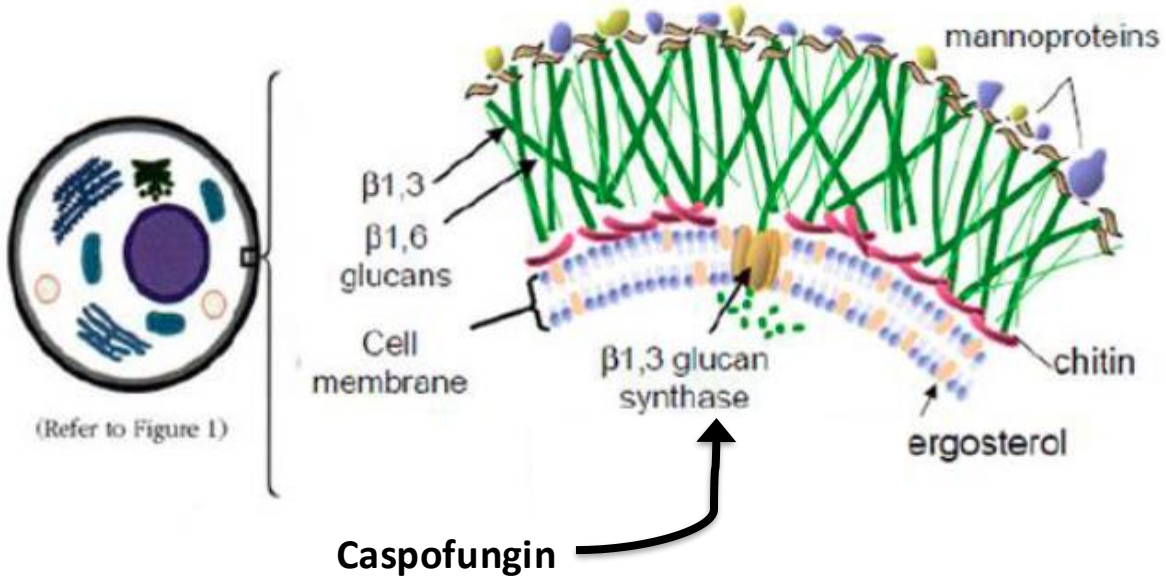


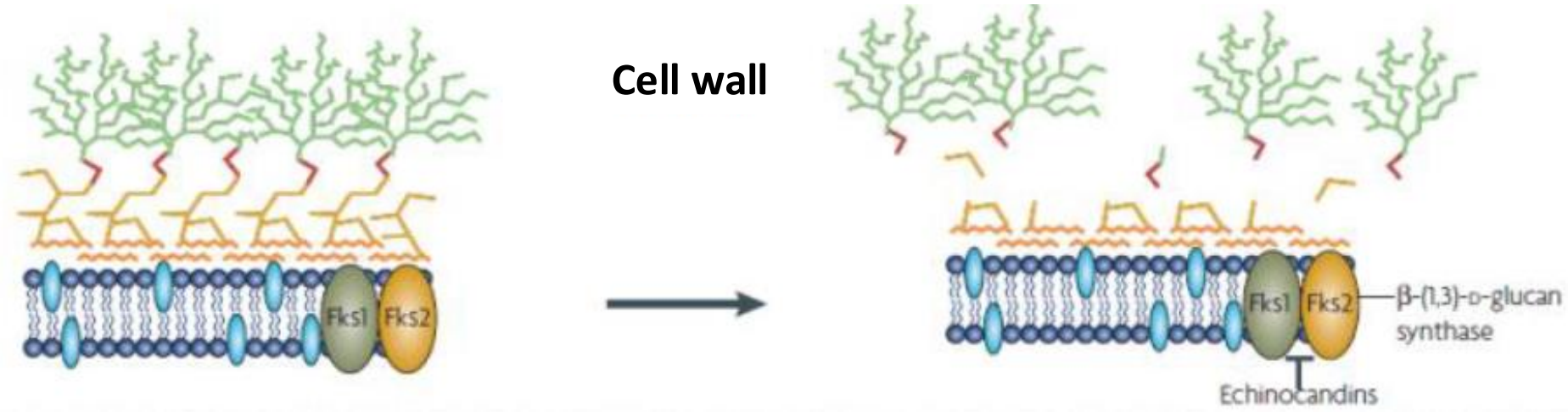
Micafungin, (Mycamine®)

Mechanism of action of echinocandins

→Competitive inhibition of the 1,3-β-D glucane synthase
→ Destabilization of the fungal cell wall

→ Anti-biofilm action





From Cowen, 2008, Nat. Rev. Microbiol., 6: 187-198

Spectra of echinocandins vs. yeasts

- Fungicide vs. *Candida* spp.

  Including *Candida* resistant to fluconazole

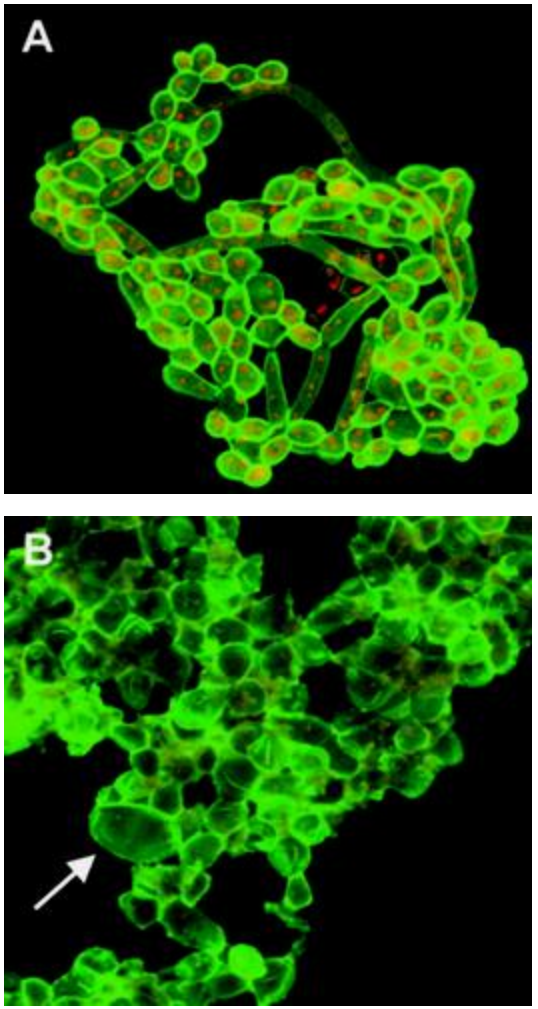
  – *C. albicans = C. tropicalis = C. glabrata = C. krusei*

  – Active on biofilms

  – *No activity on*

  Cryptococcus neoformans,

  Candida paraspilosis, Mucor (FKS1 unsusceptible?), Fusarium, Trichosporon



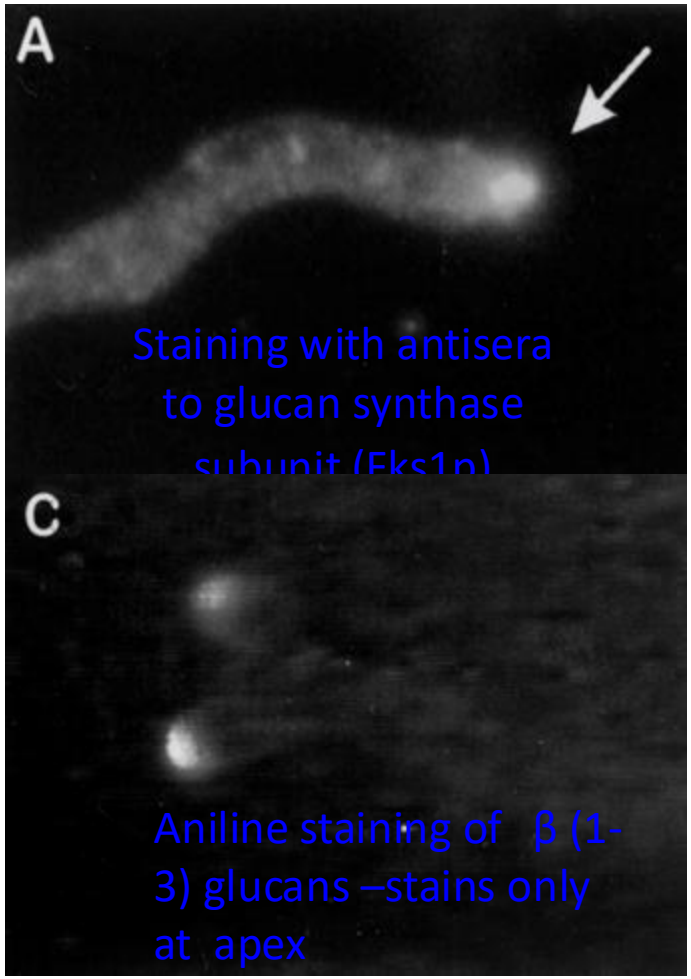
Kuhn et al. *Antimicrob Agent Chemother* 2002;46:1773-80.

Spectra of echinocandins vs. filamentous

- Active on *Aspergillus* sp

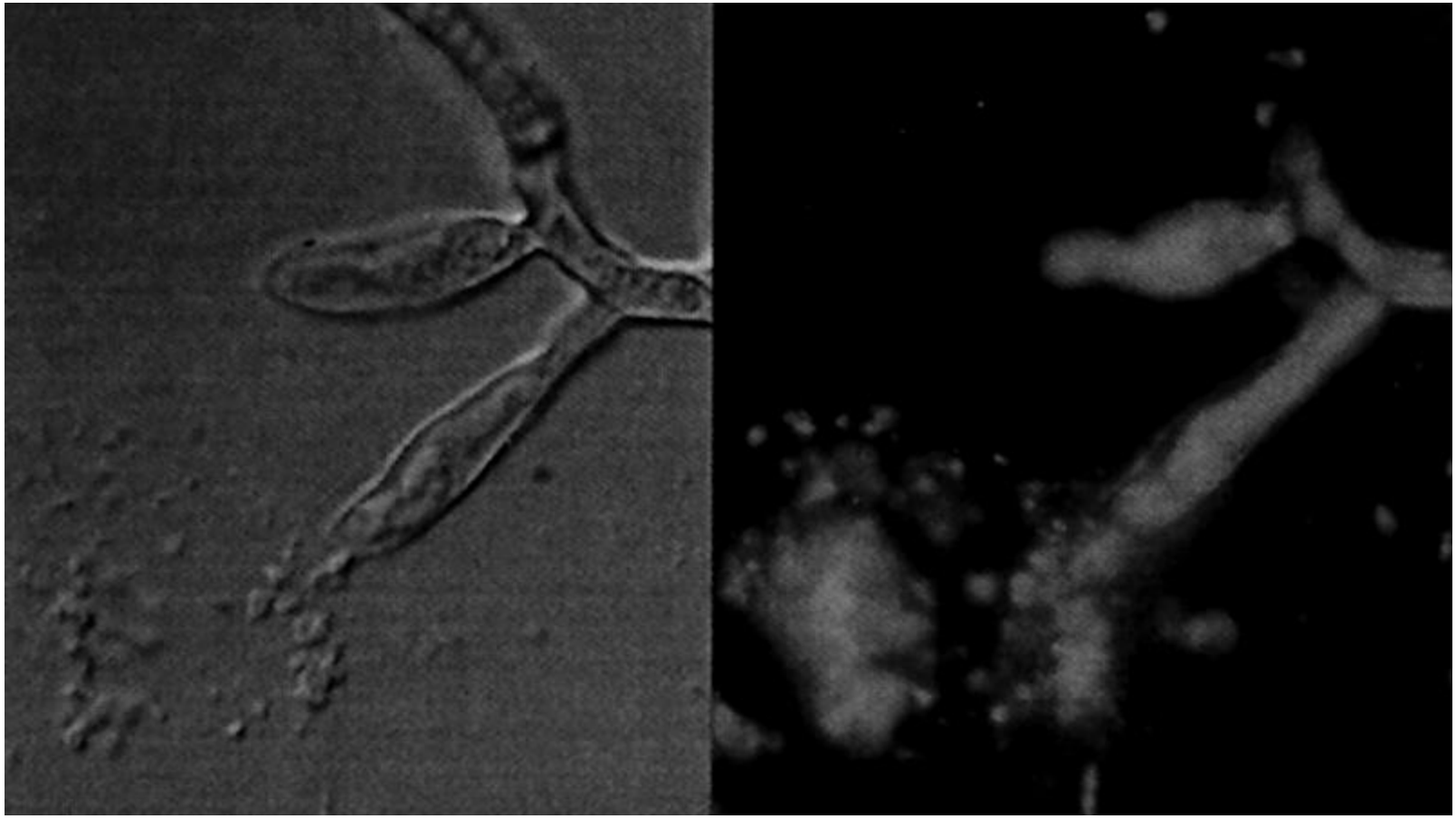
(including AmB R strains)

  - Glucane synthase localized at the apical extremity
- Activity on other moulds is less described and more variable
- Do not use in dimorphics (***Histoplasma sp.***,… inactive on yeast forms)



Beauvais et al. J. Bacteriol 2001;183:2273-79

Echinocandins act at the apical extremity of *Aspergillus sp.* hyphae



Bowman et al. *Antimicrob Agent Chemother* 2002;46:3001-12

Comparison of marketing authorizations of echinocandins

INN	Caspofungin	Anidulafungin	Micafungin
Commercial name	Cancidas	Ecalta	Mycamine
Oesophageal candidiasis			YES for patients (> 16 years old) for whom an IV treatment is appropriate
Treatment of invasive candidiasis	YES in neutropenic and non neutropenic **adults**	YES in non neutropenic **adults**	YES **for all ages** (included newborn)
Treatment of invasive aspergillosis	YES in **refractory adults** (no improvement in 7 days) or **intolerant** to AmB and/or itraconazole		
Empiric treatment for febrile neutropenic patients	Empiric treatment of presumed fungal infections (notably Candida and Aspergillus) in **febrile neutropenic adults**		
Prevention			Prevention of Candida infections in patient (**all ages**) receiving an **allograft of hematopoietic cells** or where a **neutropenia is expected** (PNN<500/µL) for at least 10 days

Echinocandins : adverse effects

- Disturbance of hepatic balance
- Excellent general tolerance
- Not much drug interaction

Caspofungin, Cancidas®

- Can replace liposomal amphotericin B for the treatment of systemic fungal infections before fungal identification in febrile neutropenic patients.

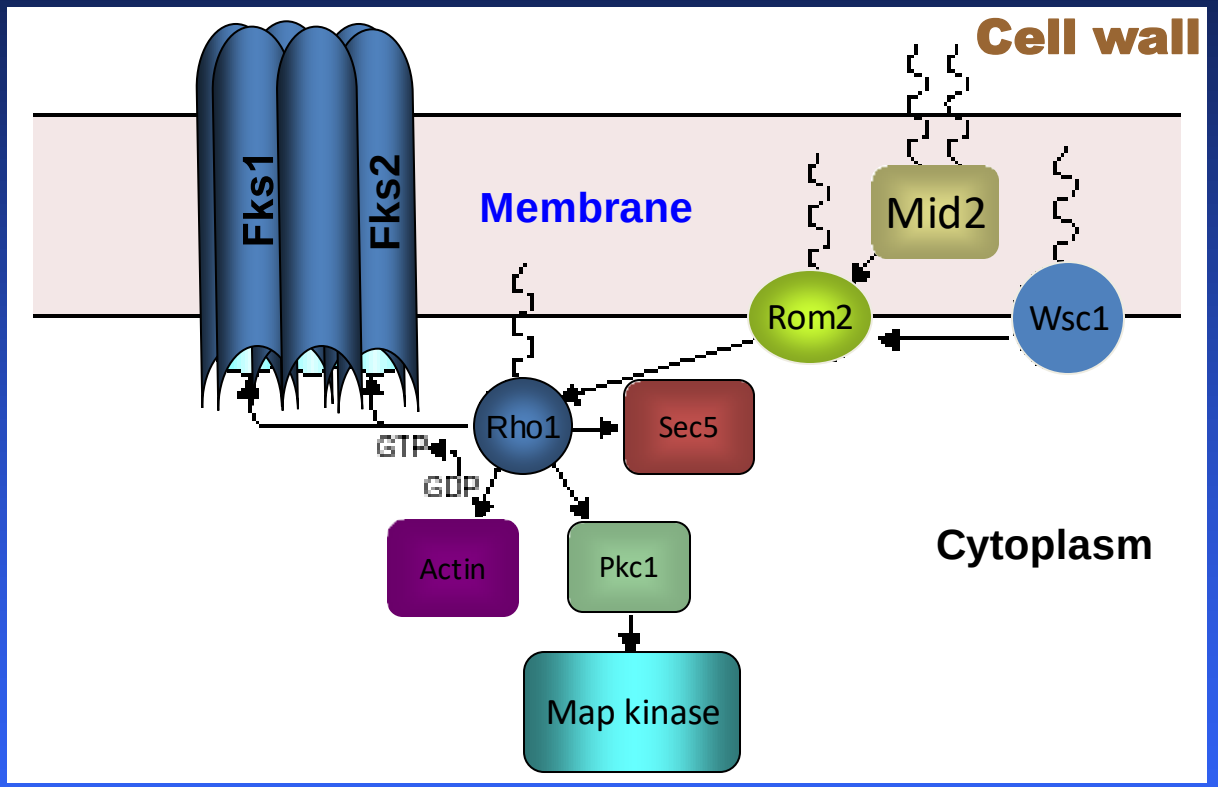
- Documented invasive candidiasis (adults) with R to fluconazole
- Drawback: very expensive (800 €/d) x 15 days minimum

Mechanisms of caspofungin resistance

Denning, 2003, Lancet 362: 1142-51

Glucane synthase complex
- Membrane sub-units
  Fks1, Fks2
- Cytoplasmic sub-unit
  Rho1

Regulation by interaction
- Sensors (ex. Mid2)
- MAP kinase



Resistance
- Mutation in gene encoding for Fks1 sub-unit (*Δfks1* resistant)
- Overexpression of glucane synthase via Mid2-Rho1-MAP kinase cascade
- Caspofungin substrate of Cdr2 (ABC transporter)

Ohyama et al., 2004, AAC 48: 319-22
Schuetzer et al., 2003, Mol Microbiol 48: 225-35

Mechanisms echinocandin resistance

The appearance of resistances to echinocandins start to be observed in:

- *C. glabrata* ++ and in constant increase
- *C. albicans* +
- *C. kefyr* +
- *Aspergillus fumigatus* +

Antifungal spectra

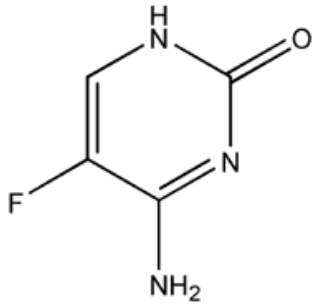
Main fungi: *C. albicans, Aspergillus spp, C. neoformans*

	Polyènes	Fluco	Itraco	Vorico	Posaco	Candines
C. albicans	+	+	+	+	+	+
C. krusei et glabrata	+	-	-	+	+	+
Cryptococcus neoformans	+	+	+	+	+	-
Aspergillus spp.	+	-	+	+	+	+
Zygomycetes spp.	+	-	-	-	+	-
Fusarium spp.	+	-	-	+/-	+/-	-

PYRIMIDIN ANALOG : 5-FLUOROCYTOSINE (ANCOTIL®)

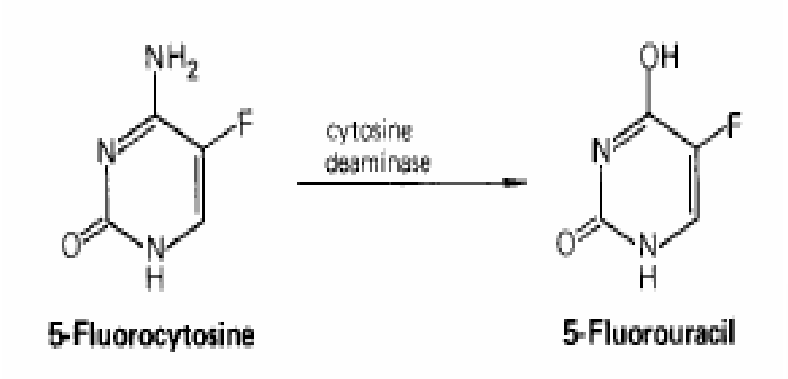
Pyrimidine analog:
5-FLUOROCYTOSINE (ANCOTIL®)

- PO and (IV)
- Diffuse in CSF LCR, CNS, eye
- Oral biodisponibility : 90%
- **Frequent resistances**
- **Always associated with**:
  - AmB+++ / Fluconazole (**meningitis due to *C. neoformans***)
  - Or Caspofungin (endocarditis)
  - Or Itraconazole (Chromomycosis)
- **Serious infections due to *Candida sp.* (bone, heart, brain)**
- Activity/Toxicity by deamination → conversion in 5FU

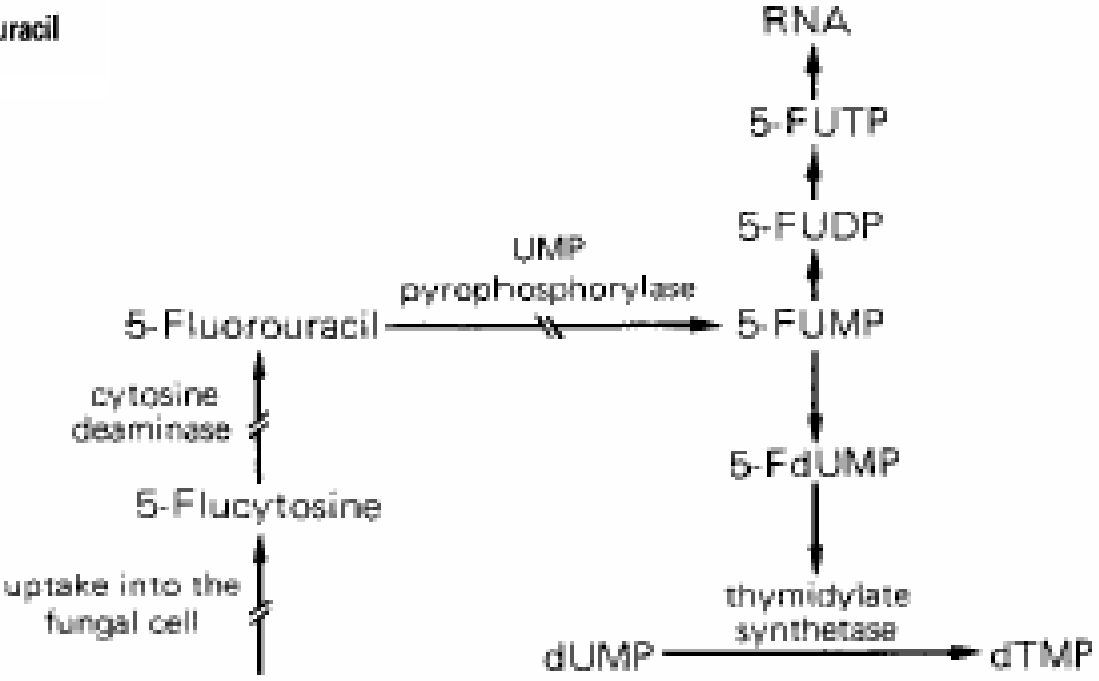


flucytosine

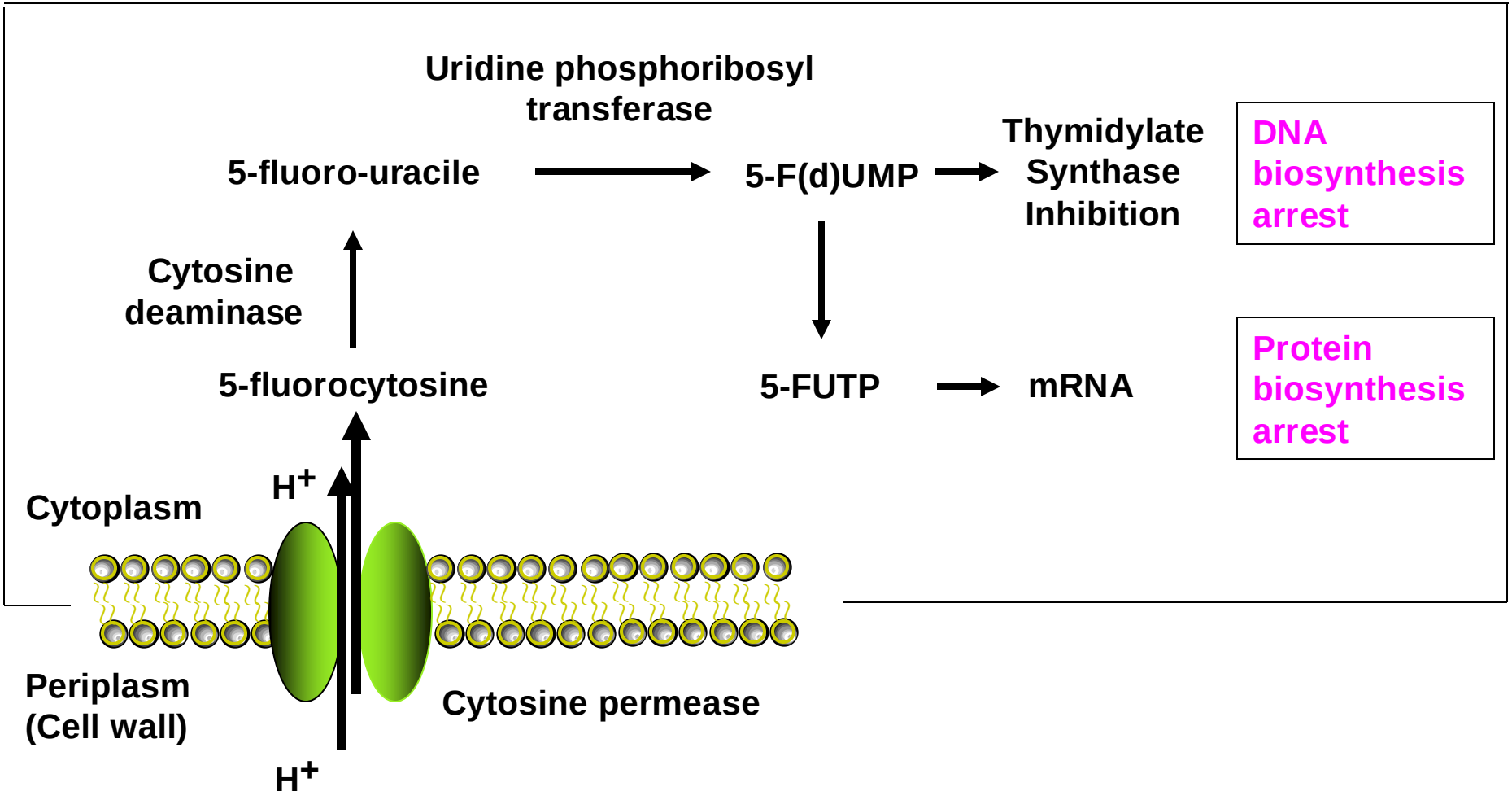
Mechanism of action: Flucytosine



Membrane crossing
Deamination in 5-FU
Competition with uracile (mRNA)
Inhib. Thymidylate synthase



Mechanism of action of Flucytosine



5-FLUOROCYTOSINE : adverse effects

- **Gastro-intestinal toxicity**
  - Gastric pains
  - Ulcero-membranous enterocolitis
  - (Perforation)

- **Hepatic toxicity** (if concentration > 100 mg/l): cytolysis

- **Hematologic toxocity** (if concentration > 100 mg/l): bone marrow
  - leucopenia, agranulocytosis
  - pancytopenia

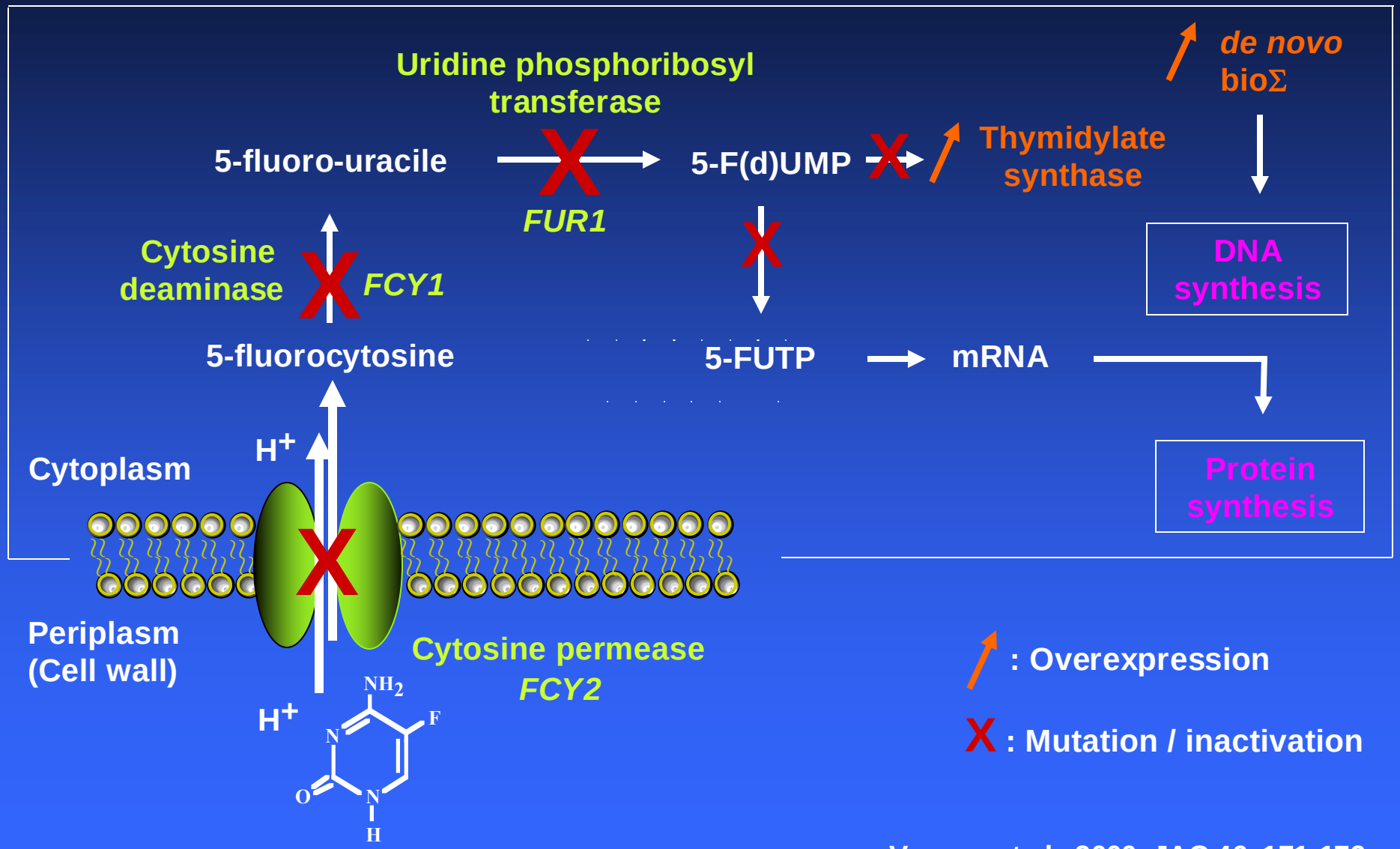
5-FLUOROCYTOSINE : mechanisms of resistance

- Rare natural resistance

- Acquired resistance
  →Linked to monotherapy
  →Appears rapidly

- Mechanisms
  →Penetration defisciency (permease activity)
  →Metabolism defisciency (cytosine deaminase or UMP pyrophosphorylase activity)
  →Overexpression of thymidylate synthase or *de novo* biosynthesis pathway of pyrimidines

Mechanisms of flucytosine resistance



Vermes et al., 2000, JAC 46, 171-179

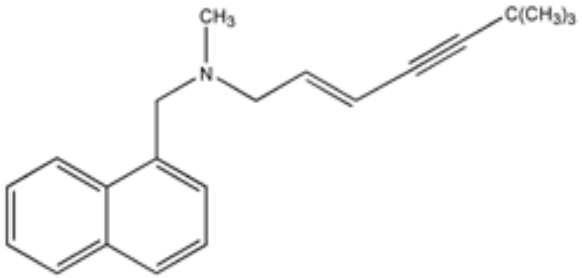
OTHER ANTIFUNGALS FOR LOCAL USAGE

(Treatment or cutaneous and mucosal infections)

ALLYLAMINES

- **Terbinafine (lamisil®)**
  po/local (cream 1%)
- **Replace griseofulvin**
- Dermatophytosis
- 250 mg/day po

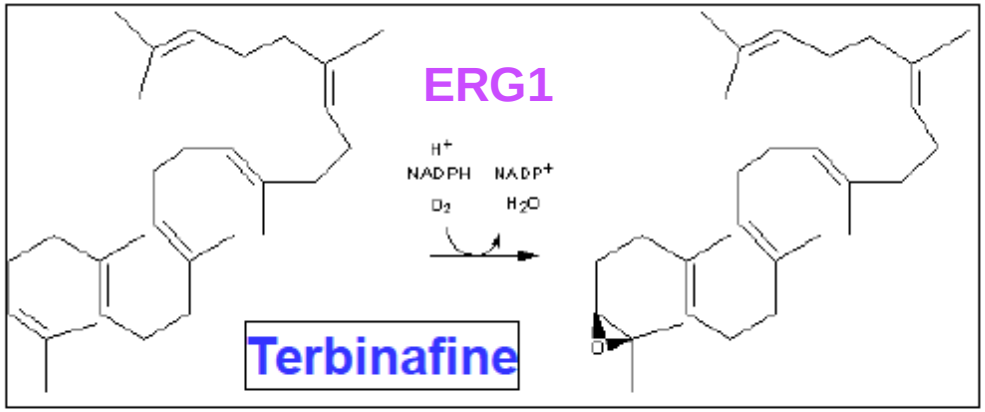
- Inhib. squalene epoxidase (non P450) → accumulation of intracellular squalene



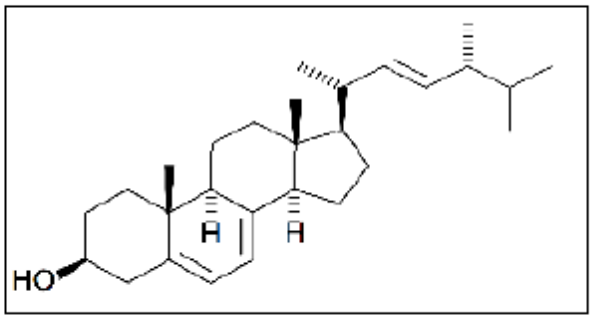
terbinafine

Allylamines : mechanism of action

- **Inhibition squalene epoxidase (*ERG1*)**
- **Accumulation of toxic squalene**
- **Inhibition of ergosterol synthesis**

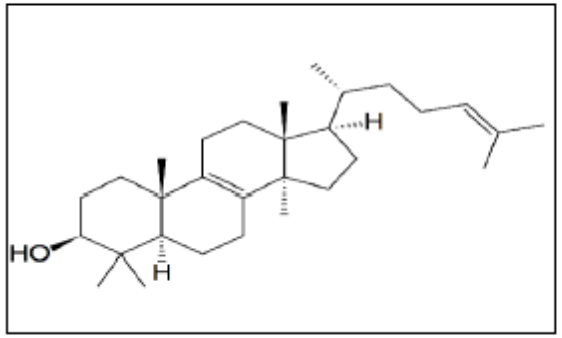


Squalene ⟹ **Squalene epoxide**



Ergosterol

⟸



Lanosterol

Terbinafine (Lamisil®)

- Replace gradually griseofulvin on dermatophytes (Adults)
- More efficient
- Nails 2-3 months
- Skin 4 weeks
- Adverse effects:

  → frequent (>10%) digestive, cutaneous

  → Rare (0.1 à 0.01%) mixed hepatitis

- *Per os* : inactive on pityriasis and vaginal candidiasis
- Absolute contraindication for severe hepatic or renal insuf.
- Relative contraindication during pregnancy, breastfeeding
- No **authorization marketing** in pediatry

If prescription of Terbinafine:

- INFORM the patient:
- Risk of cutaneous allergy +++ necessitating treatment arrest and possible agueusia (rare)
- For prescription > 3 months:
- Control at 6 weeks of ALAT, ASAT, PAL and complete blood count
  - Do not attribute a toxicity to the drug without link/certitude: not much ATF available
  - If multi-drug treatment, control every month

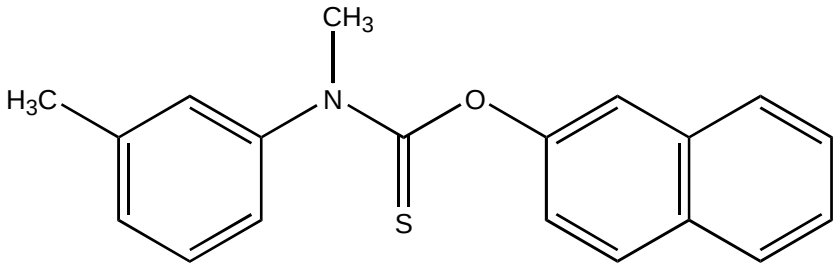
Terbinafine : mechanism of resistance

→ Mutations in squalene epoxidase confering a resistance to terbinafine (Cannon et al., 2009, Clin Microbiol. Rev., 22: 291-321)

Species	Mutations	Ref
T. rubrum	L393F F397L	Osborne et al., AAC, 2006
A. fumigatus	F389L	Rocha et al., AAC, 2006

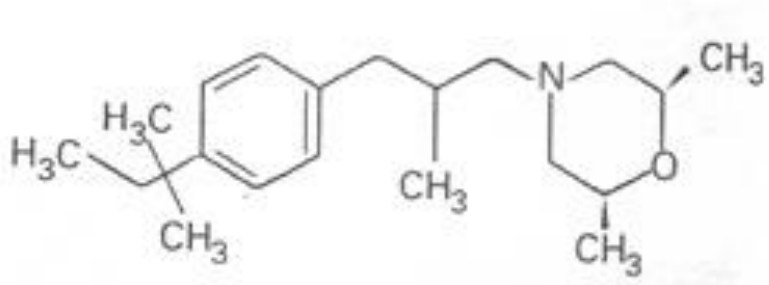
Tolnaftate (Sporiline®)

- Similar mechanism of action to allylamines
- Lotion 1%
- Treatment or secondary treatment of dermatophytoses (cutaneous or nail infections)
- Adverse effects: irritations, burns



AMOROLFINE (Loceryl®)

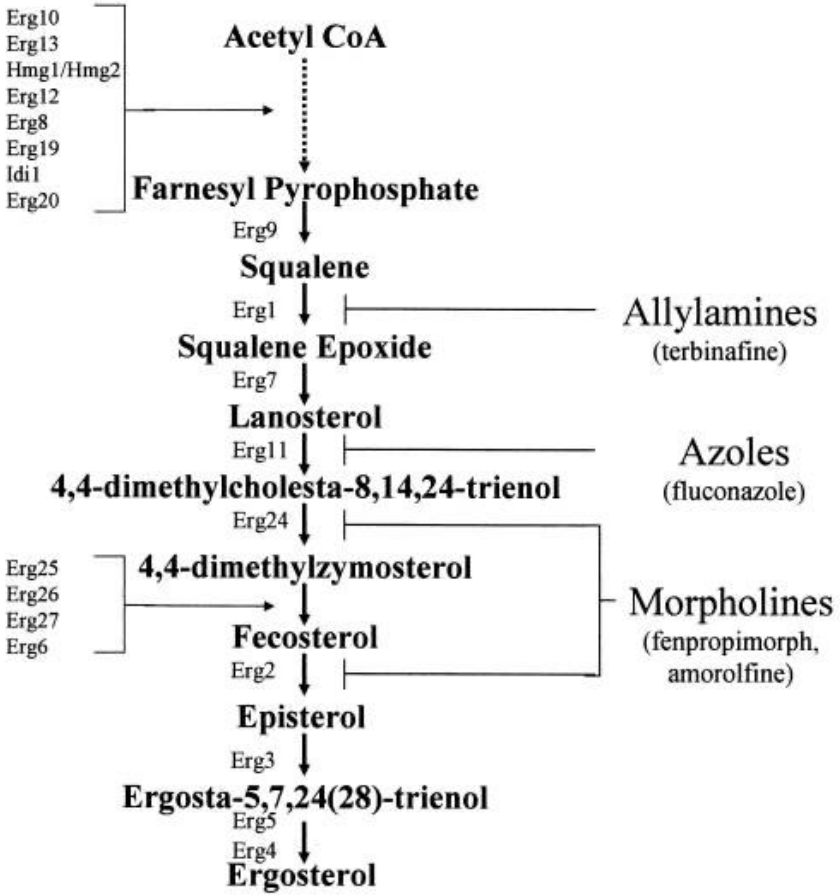
- Family of morpholines
- Film-forming solution at 5% (varnish)
- Treatment of dermatophytoses (onychomycoses)
- 1 application 1 to 2 x / week for 6 to 9 months
- Secondary effects: breaking/ uncolored nails, burns



Amorolfine

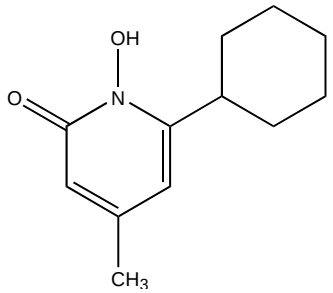
AMOROLFINE (Loceryl®)

- Mechanism of action: inhibition of C-8 sterol isomerase (Erg24) and C-14 sterol reductase (Erg2) in the ergosterol biosynthesis pathway



PYRIDONE

Family of **pyridones**, active on *Candida sp., Pityrosporum sp*. and dermatophytes (*Trychophyton, Epidermophyton, Microsporum*) (MIC 1 to 4 µg/ml)



Ciclopirox, MYCOSTER®

Diffuse well in epidermis and sebaceous follicle, but poorly in deep layers of the derm. Very weak percutaneous absorption. 1 to 2 % of the dose administrated found in the urine.

Mycoster® 1% ointment for cutaneous lesions, powder 1% for intertrigo,
Mycoster® filmogenic solution 8% for the the treatment of onyxis

<u>Mechanism of action</u>: inhibition of absorption or chelation of polyvalent cations (Al^{3+}, Fe^{2+}), phosphates and K^{+}
→ inhibition of metal-dependent enzymes degrading peroxides
→ redox imbalance → fungal deah

<u>Adverse effects</u>:
Burnings, erythema, itching (skin 2% of cases)
Hypersensivity reaction (vesicules) → treatment arrest