ANTIFUNGAL AGENTS

Alison Clode, DVM, DACVO

Port City Veterinary Referral Hospital
Portsmouth, New Hampshire

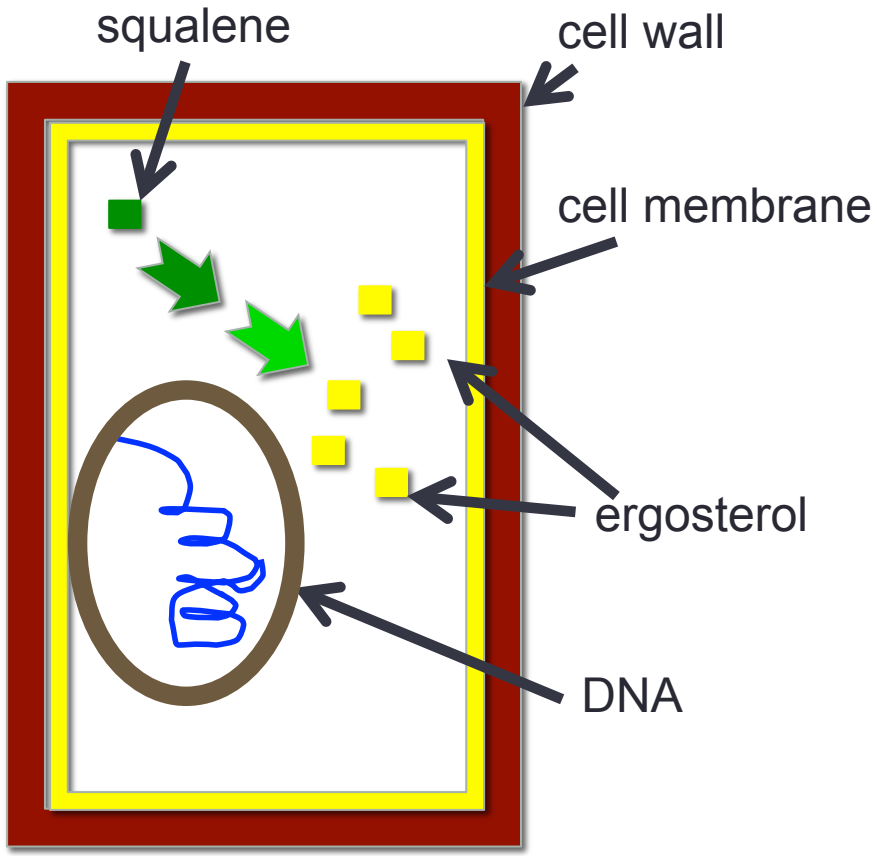
New England Equine Medical and Surgical Center
Dover, New Hampshire

Overview

- Fungal organisms
- Mechanisms of resistance
- Antifungal mechanisms of action
- Specific antifungal agents

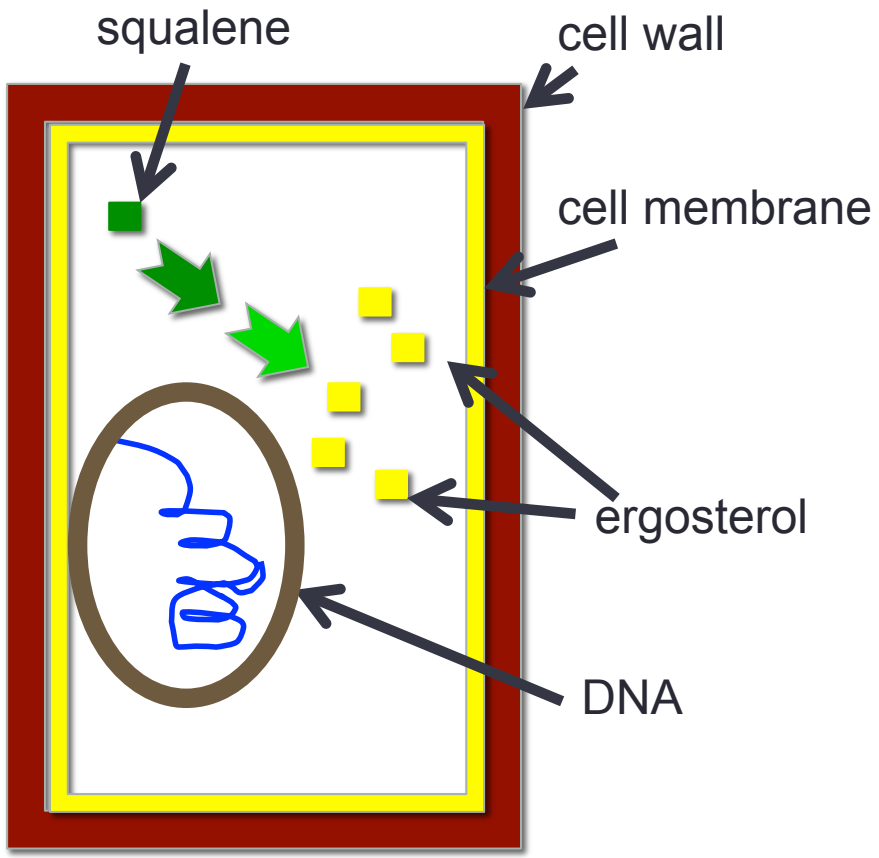
Fungal Organisms

- Eukaryotic
  - Internal membranes
  - Cell wall = glycoproteins + polysaccharides
  - Polysaccharides = glucan + chitin
  - Cell membrane = ergosterol
- Forms
  - Yeast
  - Mold (filamentous)
  - Dimorphic



Fungal Organisms

- Virulence factors:
  - Protease production
  - Toxin production
  - Cytokine production
  - Exploitation of host defenses
  - Capsule production

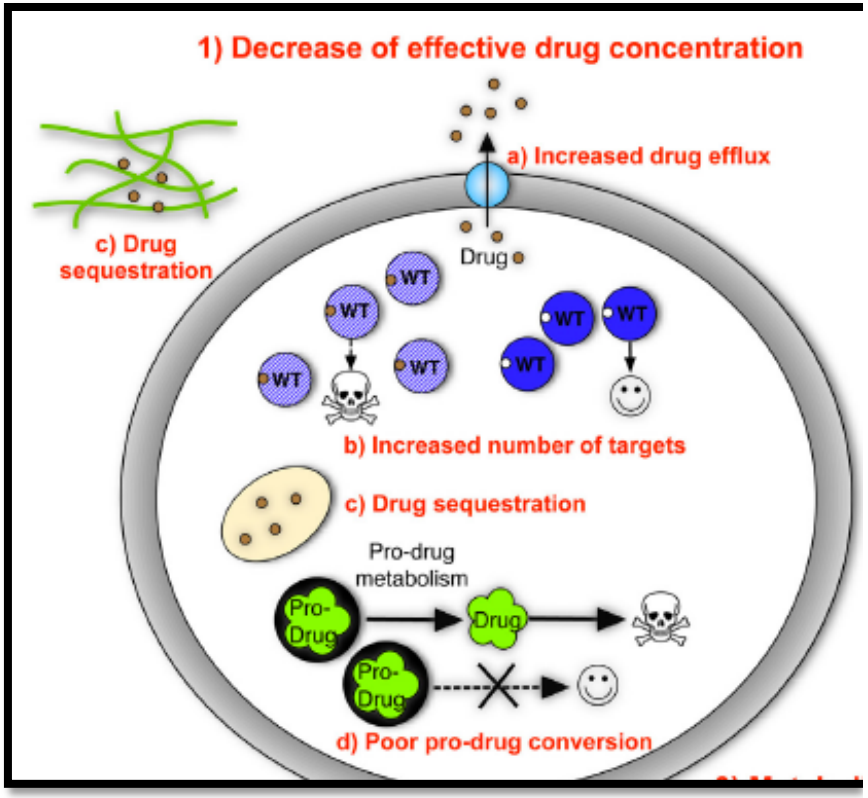


Fungi – Resistance

1. Decreased effective drug concentration

- **Efflux pumps**
  - ABC transporter systems
  - MFS transporter systems

- Well-described at a genomic level within fungi
- Specificity to fungal genus and antifungal agent



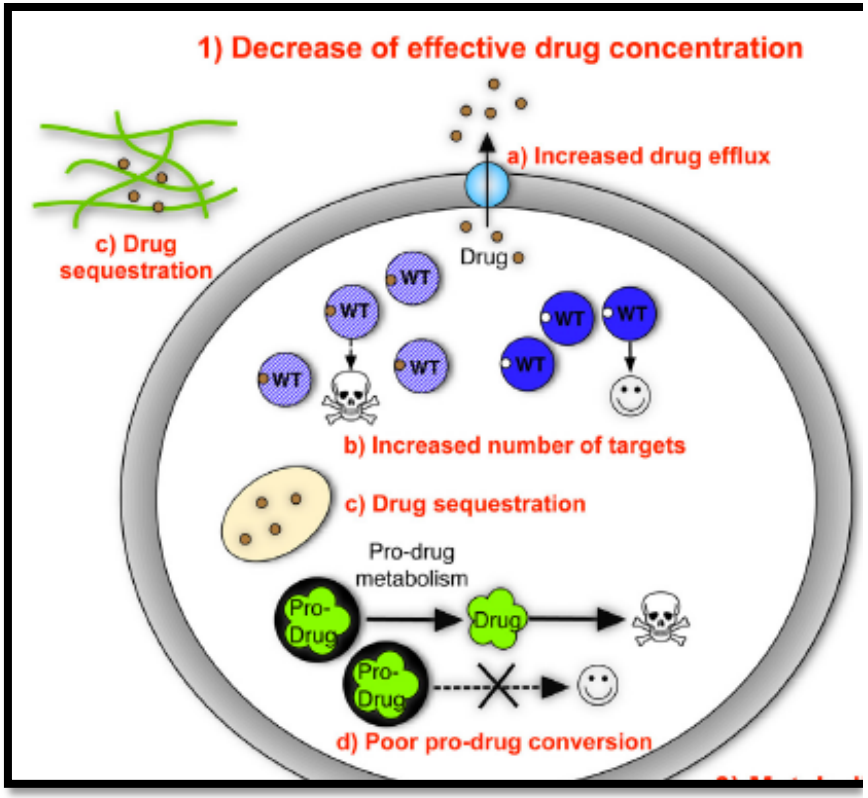
Sanglard, Frontier in Med, 2016

Fungi – Resistance

1. <u>Decreased effective drug concentration</u>

- **Overexpression of drug target**
  - Greater number of drug targets → requires increased drug concentrations to saturate target

- Well-described at a genomic level within fungi
- Specificity to fungal genus and antifungal agent

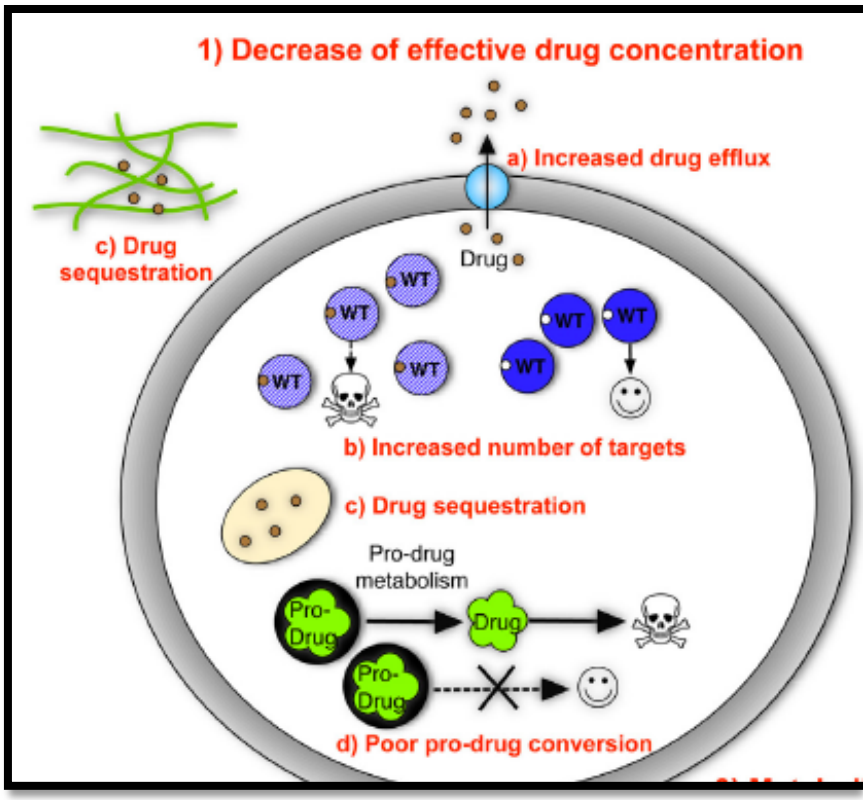


Sanglard, Frontier in Med, 2016

Fungi – Resistance

1. Decreased effective drug concentration

- **Sequestration of drug (intra- or extra-cellularly)**
  - Extracellular biofilm matrix (*Candida* and *Aspergillus*)
  - Intracellular vesicles?



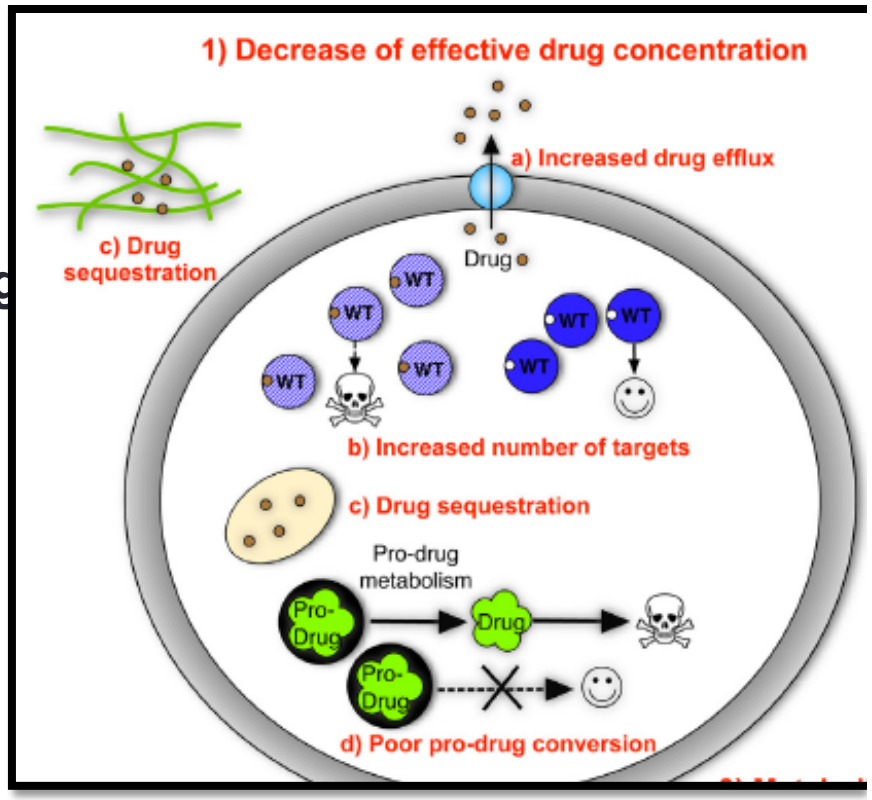
- Well-described at a genomic level within fungi
- Specificity to fungal genus and antifungal agent

Sanglard, Frontier in Med, 2016

Fungi – Resistance

1. Decreased effective drug concentration

- **Poor conversion of pro- to active drug**
  - 5-FC → 5-FU
  - Conversion occurs intracellularly
  - Deficient conversion → decreased effective drug

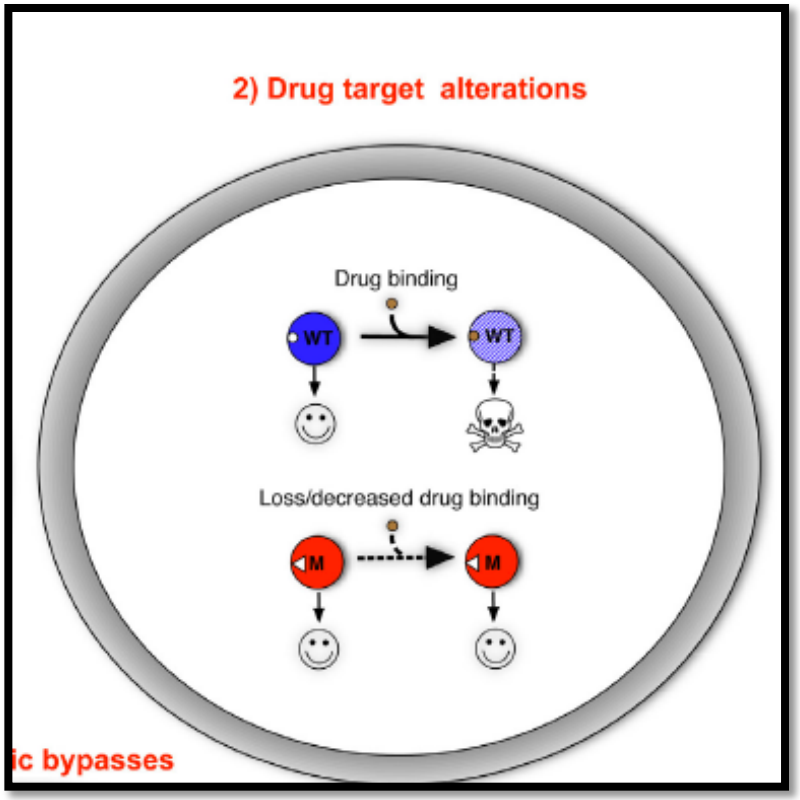


- Well-described at a genomic level within fungi
- Specificity to fungal genus and antifungal agent

Sanglard, Frontier in Med, 2016

Fungi – Resistance

2. Alterations in target enzyme
   - 14α lanosterol demethylase
   - β- 1,3 glucan synthase
   - Altered target → decreased affinity of drug for target
   - Well-described at a genomic level within fungi
   - Specificity to fungal genus and antifungal agent

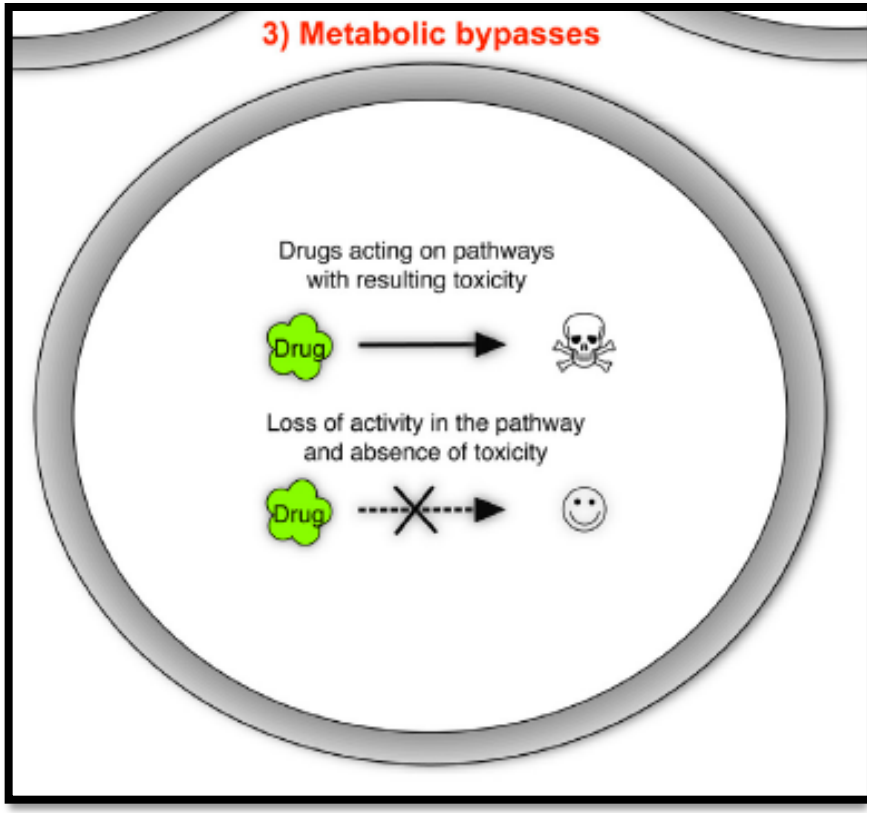


Sanglard, Frontier in Med, 2016

Fungi – Resistance

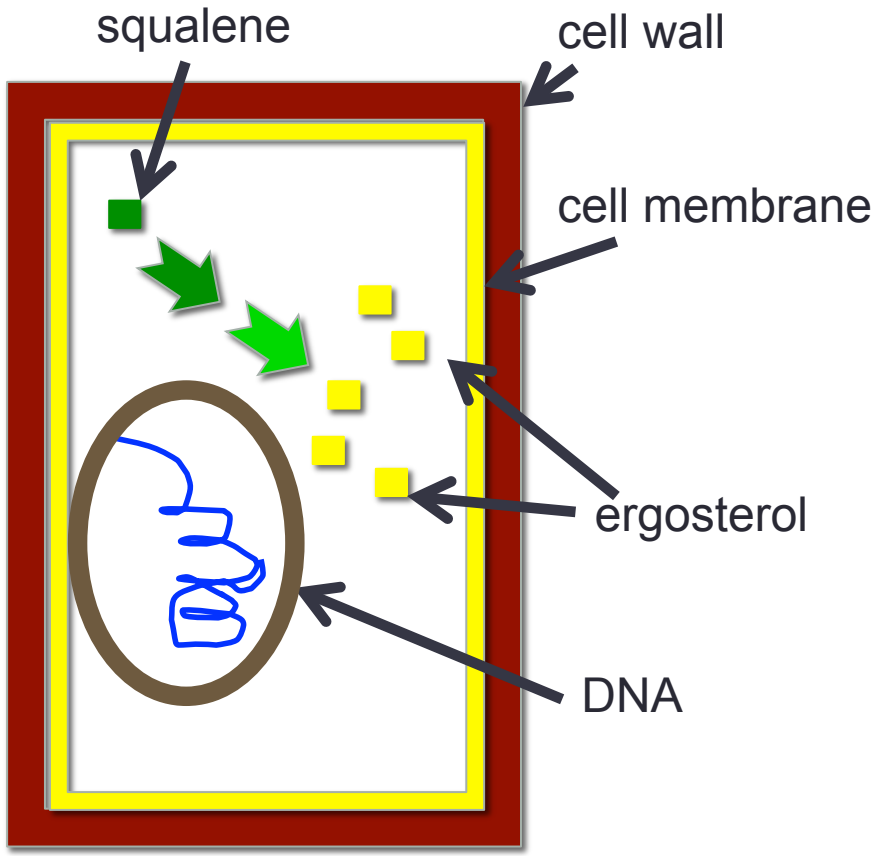
3. Alterations in metabolic pathways

- Exposure to antifungal → reduced development of byproduct of target pathway
- Incorporation of alternate byproduct in alternate pathway → production of functional fungal cell element blocked by antifungal



Antifungal Agents – MOA

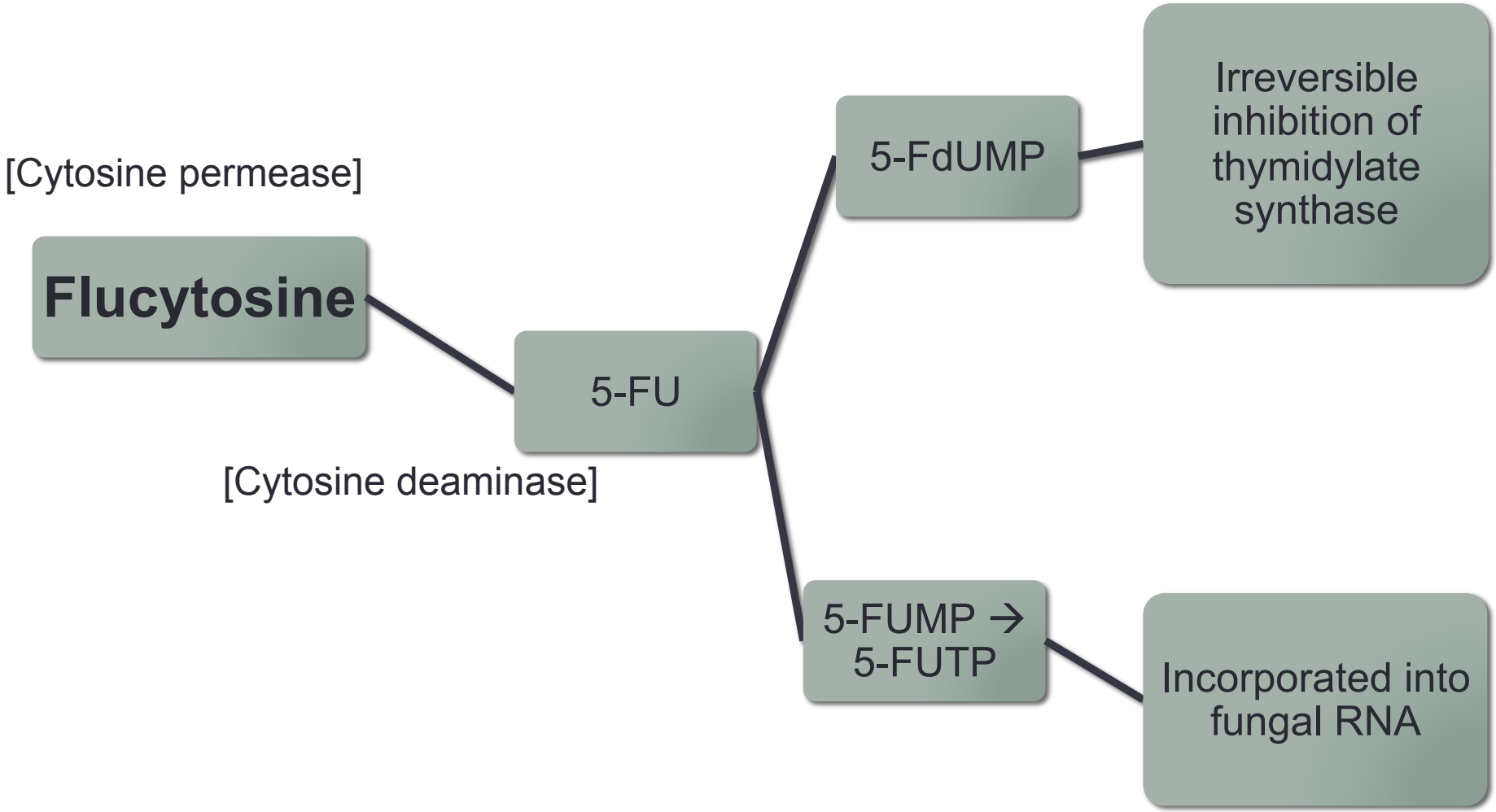
1. Decrease DNA/RNA synthesis
   - Pyrimidines
2. Alter cell *membrane* permeability
   - Polyenes
3. Alter cell *membrane* stability
   - Allylamines
   - Azoles
4. Alter cell *wall* stability
   - Echinocandins



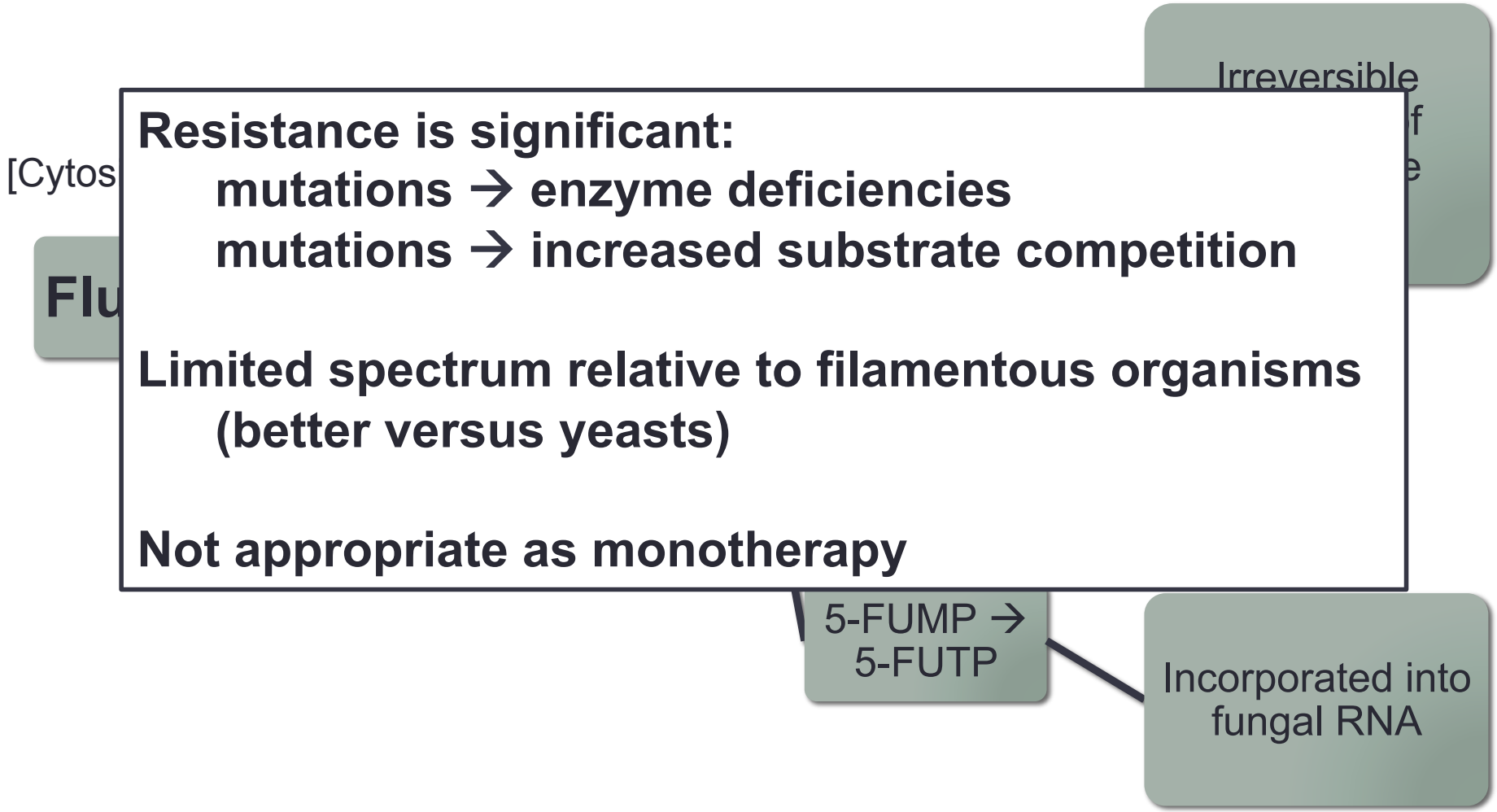
1. Inhibit DNA/RNA Synthesis

- Pyrimidines
  - 5-Fluorocytosine

Pyrimidine Antifungal



Pyrimidine Antifungal



Flucytosine Toxicity

- Human cells do not have cytosine deaminase, so presumptively decreased toxicity

- However…
  - Toxicity develops at [serum] 100 mg/L
  - Dose-dependent
  - Metabolites

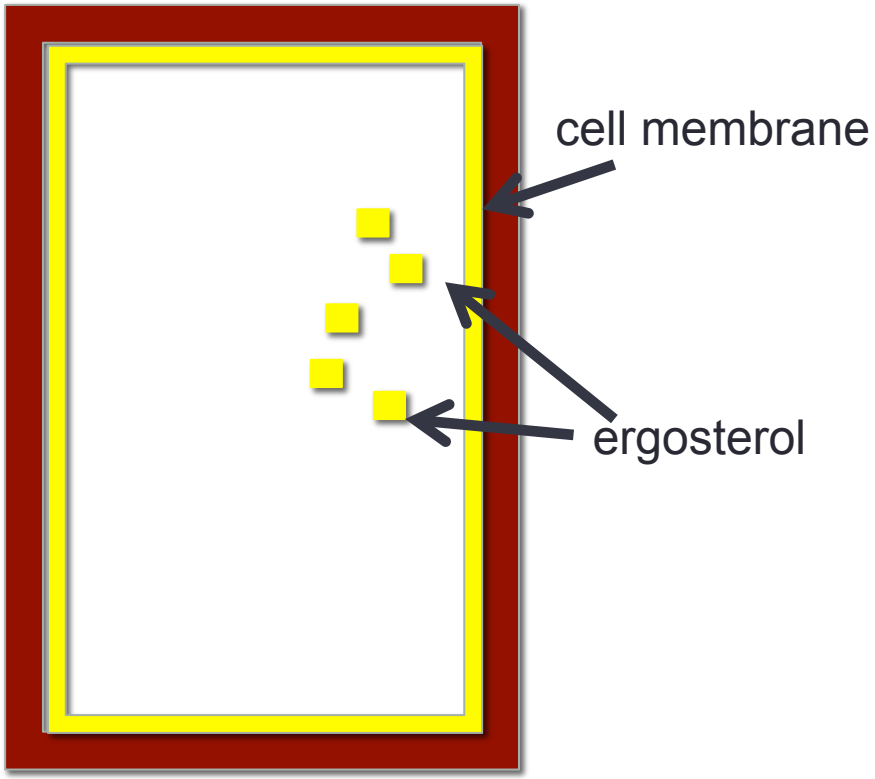
  - Bone marrow
  - Hepatotoxicity
  - GI

2. Alter cell membrane permeability

- Polyenes
  - Natamycin
  - Amphotericin B

Polyenes

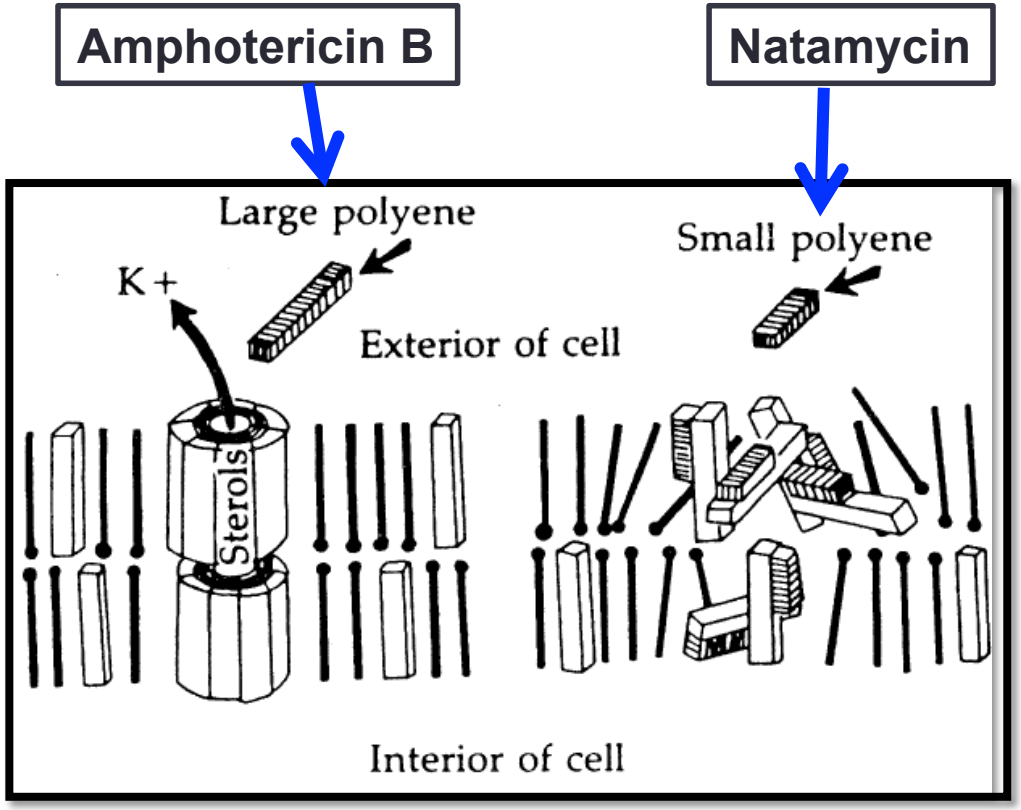
- Bind cell membrane ergosterol → polyene-sterol complex → increase permeability → leakage of intracellular constituents
- Fungistatic/fungicidal is concentration-dependent
- Resistance relatively rare
  - Alter total sterol content
  - Alter specific sterol present
  - Alter orientation of sterol



Natamycin

- Poorly water soluble
- 5% suspension
  - Good corneal adherence
  - Low toxicity
  - Variable transcorneal penetration
- Post-antifungal effect

- Spectrum:
  - Good versus yeast
  - Good versus dimorphic
  - Very good versus filamentous

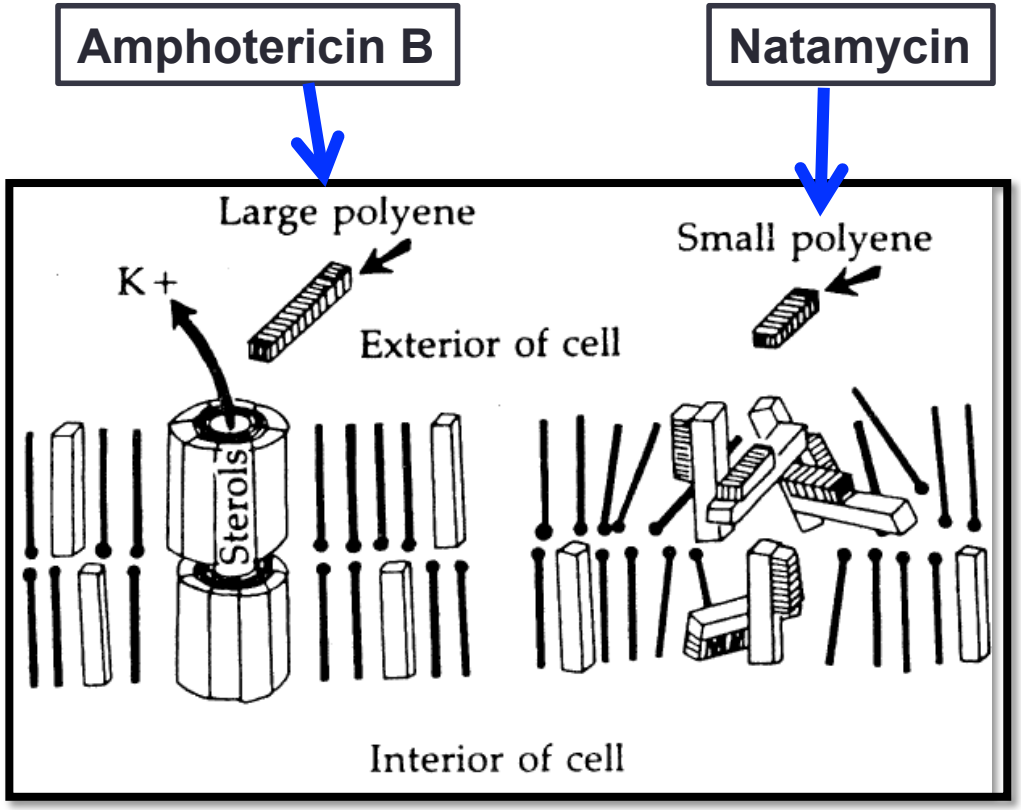


Amphotericin B

- Poorly water-soluble
- Highly protein-bound
  - Post-antifungal effect

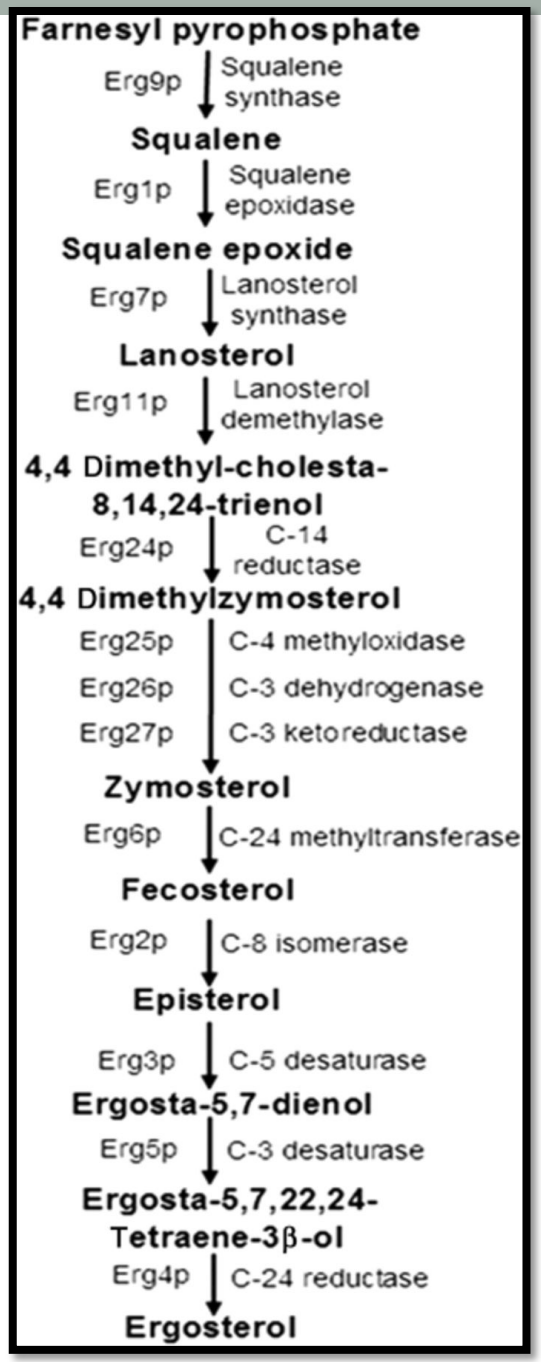
- Spectrum:
  - Very good versus yeast
  - Very good versus dimorphic
  - Variable versus filamentous

- Significant systemic toxicities
  - Deoxycholate solubilizer = toxic
  - Liposomal, lipid complex, colloidal dispersion → better solubility + less toxicity



3. Alter cell membrane stability

- Allylamines
  - Terbinafine
- Azoles
  - Ketoconazole
  - Fluconazole
  - Miconazole
  - Itraconazole
  - Voriconazole
  - Posaconazole
  - Ravuconazole



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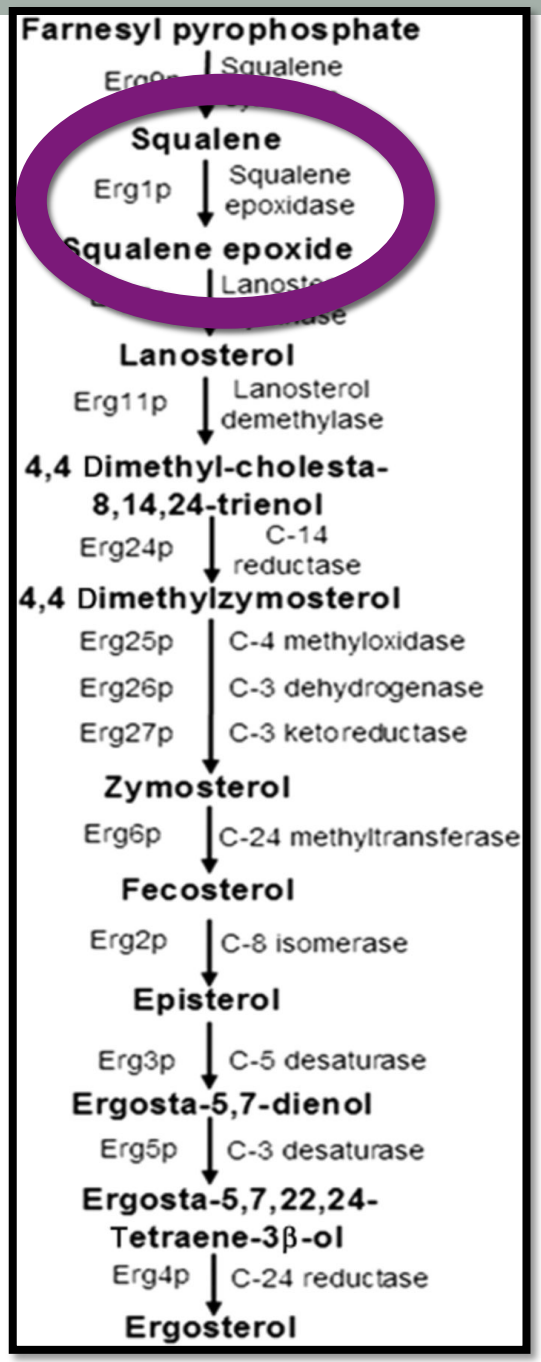

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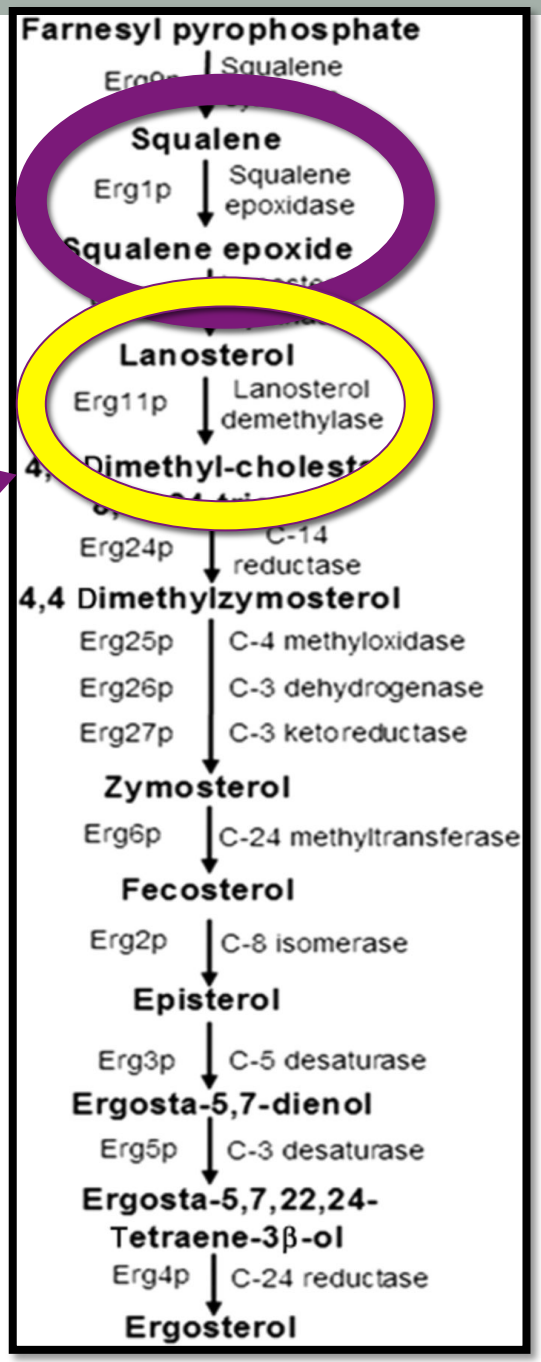
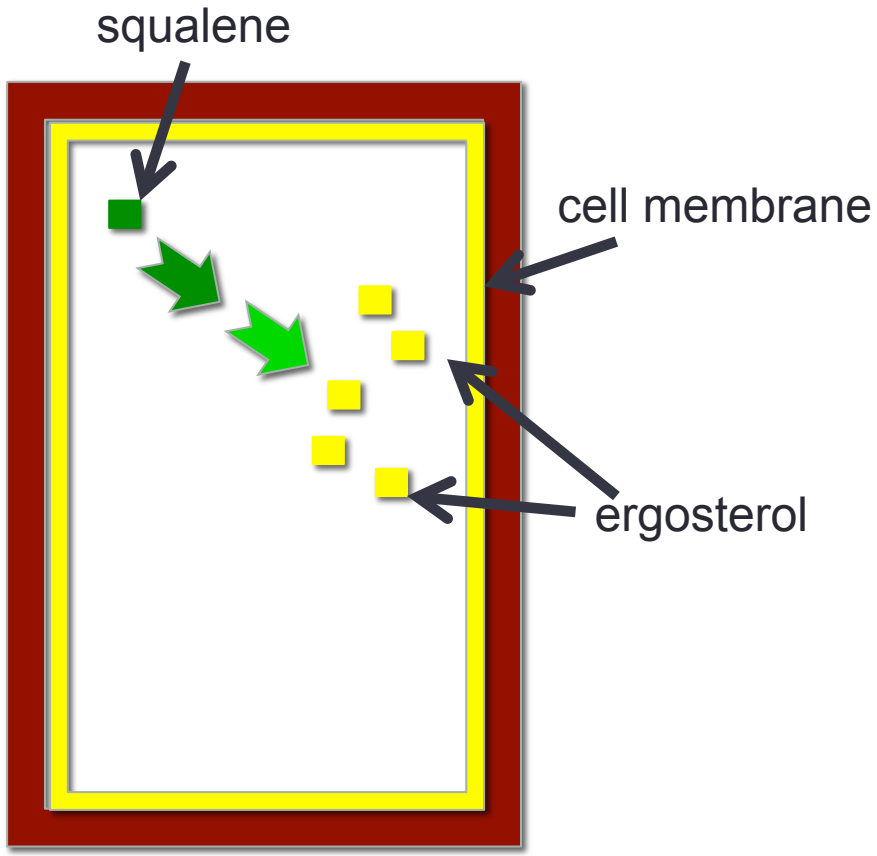


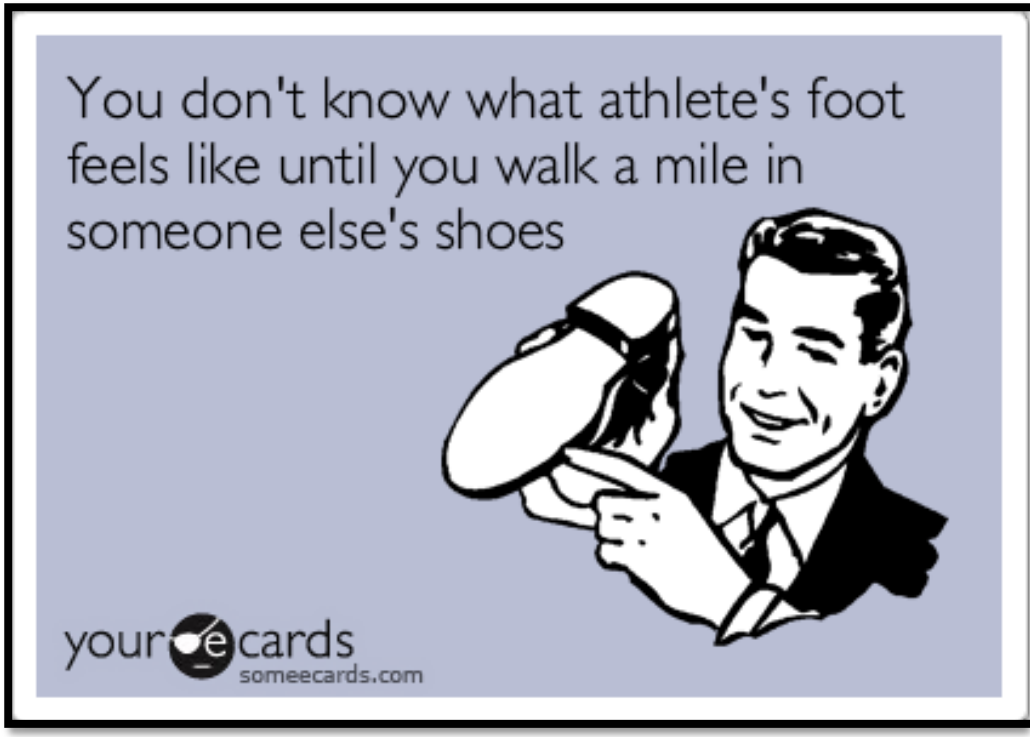
Allylamines

- Inhibit squalene epoxidase → squalene not converted to ergosterol
  - Squalene accumulates (toxic)
  - Increased cell permeability
- Comparable or greater efficacy than some azoles versus filamentous organisms
- Synergism with azoles and polyenes



Allylamines

- Primarily used to treat dermatophytosis (athlete's foot)
- Clinical efficacy comparable to natamycin in keratomycosis in people
- Topical administration resulted in measurable AH levels in rabbits



Liang QF, et al., Chin Med J 2009
Sun XG, et al., Ophthal Res 2007

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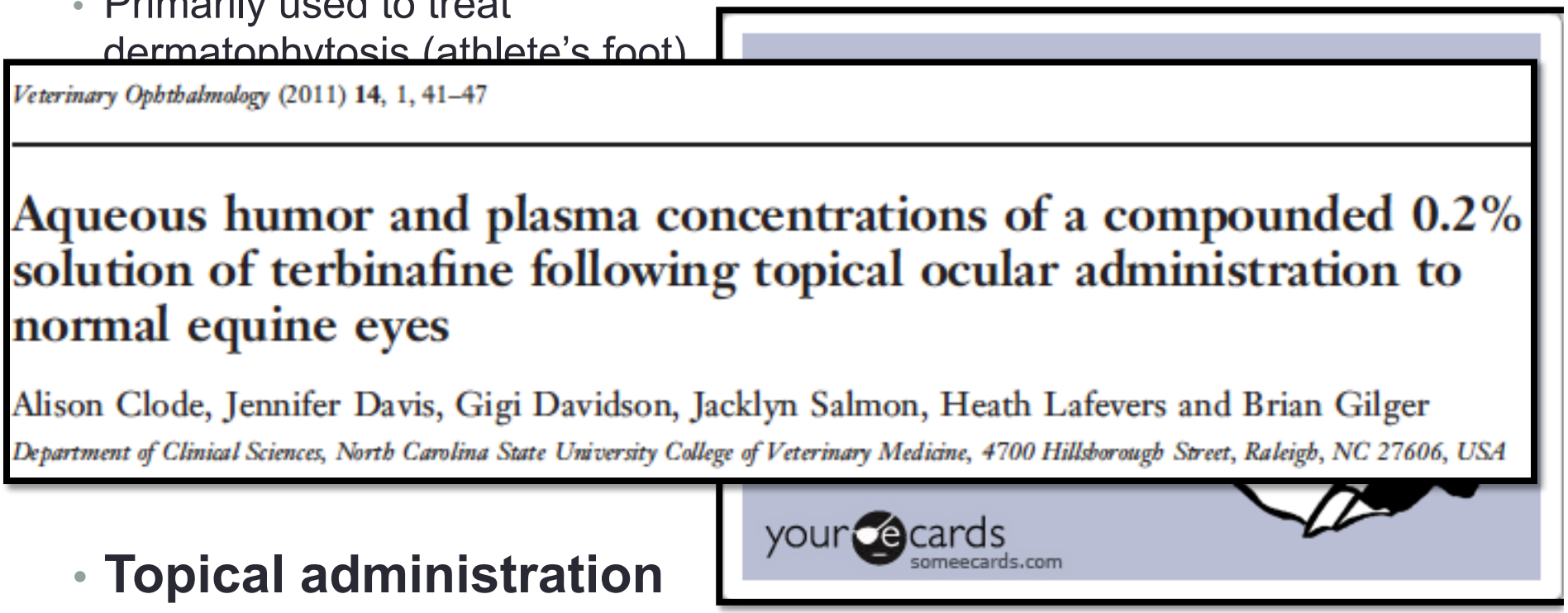


Veterinary Ophthalmology (2011) **14**, 1, 41–47

Aqueous humor and plasma concentrations of a compounded 0.2% solution of terbinafine following topical ocular administration to normal equine eyes

Alison Clode, Jennifer Davis, Gigi Davidson, Jacklyn Salmon, Heath Lafevers and Brian Gilger

Department of Clinical Sciences, North Carolina State University College of Veterinary Medicine, 4700 Hillsborough Street, Raleigh, NC 27606, USA

- **Topical administration did *not* reach measurable AH levels in horses**

Allylamines

Veterinary Ophthalmology (2015) 1–6 DOI:10.1111/vop.12318

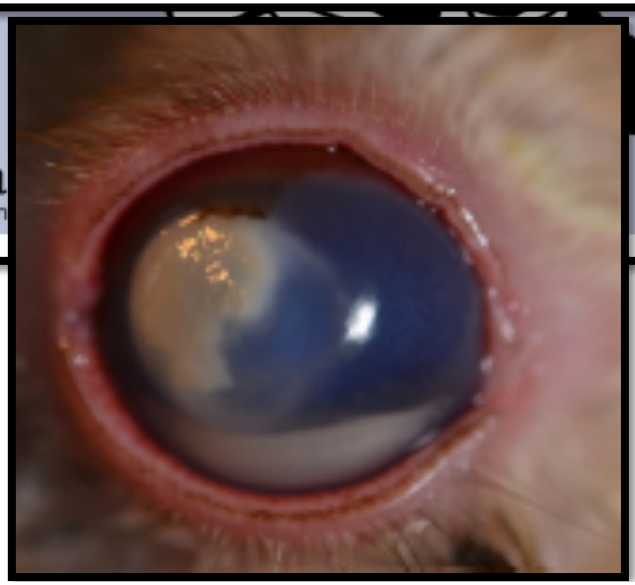
CASE REPORT

Keratomycosis in a pet rabbit (*Oryctolagus cuniculus*) treated with topical 1% terbinafine ointment

Aurélie Bourguet,* Alexandre Guyonnet,* Elise Donzel,* Jacques Guillot,† Charly Pignon‡ and Sabine Chahory*

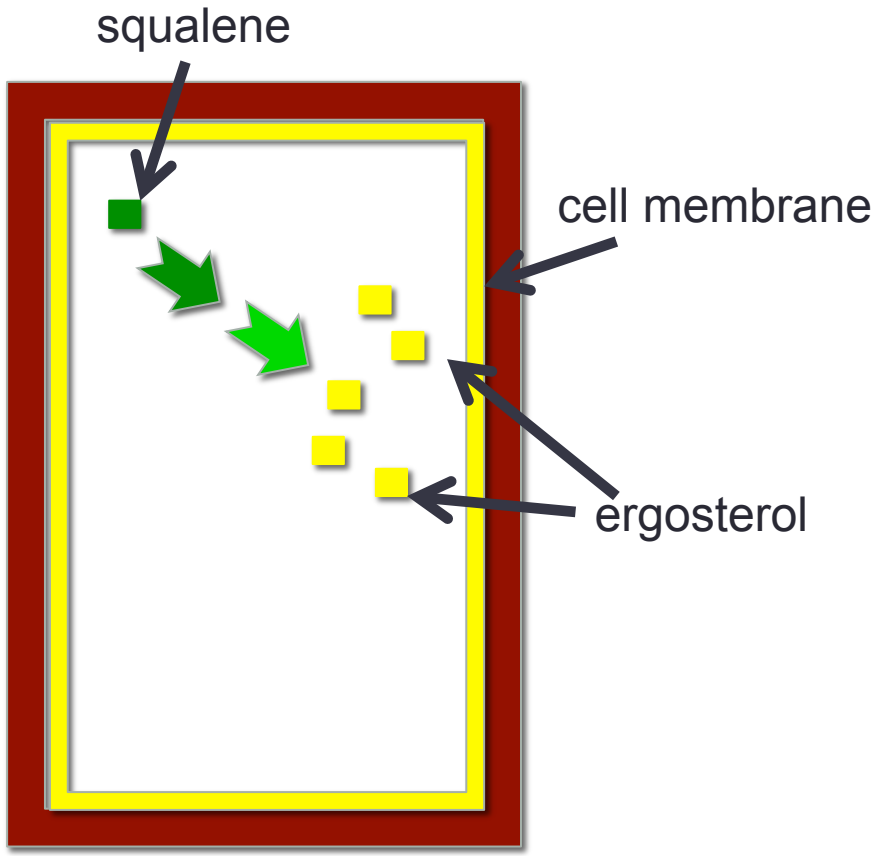
- Topical administration resulted in measurable AH levels in rabbits

- **Topical administration *was* efficacious in a rabbit with *Aspergillus* keratomycosis**



Azoles

- Inhibit 14α-sterol demethylase → no conversion of squalene to ergosterol
  - Increased cell permeability
  - Intracellular accumulation of toxic metabolites
- Decrease function of immune cells
- Decrease cytochrome P450 metabolism
  - Significant potential for drug interactions



Azoles in Ophthalmology

	Spectrum	Route(s)	Other
Ketoconazole	**Good**: *Candida* **Poor**: filamentous	Oral Topical	Fungistatic Ineffective for significant infections Significant systemic side effects
Miconazole	**Good**: yeasts **Good**: filamentous	Topical Subconjunctival	Good penetration
Fluconazole	**Good**: yeasts **Poor**: filamentous	Oral Topical	Good penetration
Itraconazole	**Good**: filamentous	Oral Topical	Poor penetration
Voriconazole	**Very good**: filamentous	Oral Topical Intravitreal	Good penetration

Voriconazole in Horses

Route	Dose	Findings
Oral	4 mg/kg single dose *(Clode et al)* 3 mg/kg PO BID x 10 days *(Colitz et al)* 4 mg/kg PO q24h x 14 days *(Passler et al)*	Measurable AH levels Measurable PTF levels Measurable AH levels
Topical	0.5%, 1%, 3% q4h x 7 doses *(Clode et al)*	Measurable AH levels 3% → topical irritation
Intrastromal and subconjunctival	22.5 mg intrastromal *(Smith et al)* 0.5 mg intrastromal + 4 mg subconj *(Tsujita et al)* 1.5 mg intrastromal + 5 mg subconj *(Tsujita et al)*	Reported clinical resolution of stromal abscessation

Clode AB et al., AJVR 2006
Colitz CMH et al., AJVR 2007
Passler NH et al., JVPT 2010
Smith KM et al., VO 2014
Tsujita H et al., VO 2013

Voriconazole in Horses

Table 3. Minimal inhibitory concentrations (MIC) values in μg/mL for 5 *Aspergillus fumigatus* colonies

	Voriconazole	Fluconazole	Itraconazole	Amphotericin B	Miconazole
1	0.75	>256	1.5	3.0	10
2	0.75	>256	2.0	0.75	10
3	0.75	>256	2.0	0.75	10
4	0.38	>256	0.75	2.0	10
5	0.19	>256	0.75	1.0	10
Mean MIC	0.56	256	1.4	1.5	10

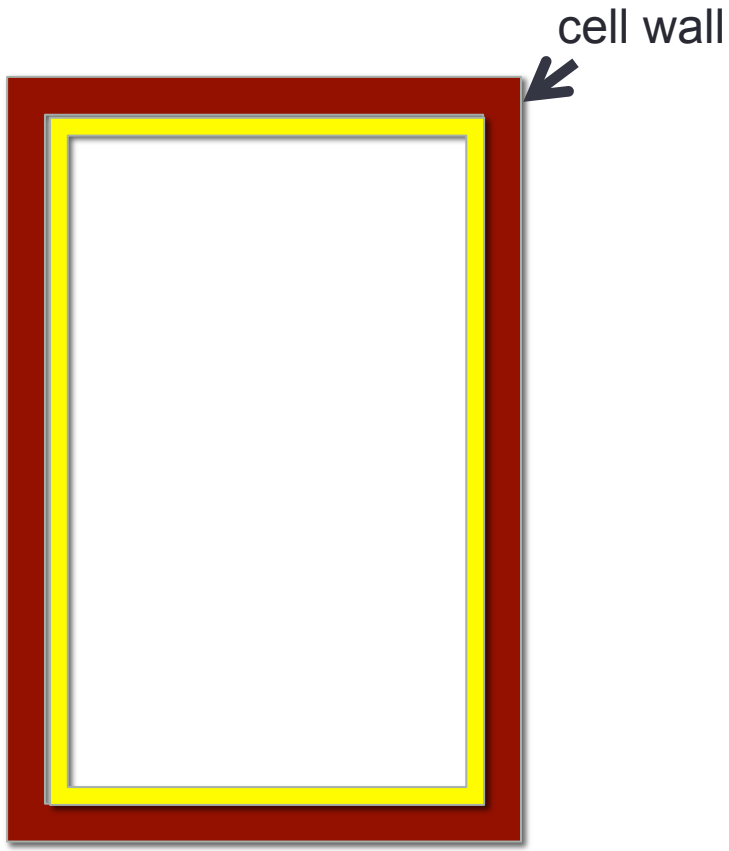
Pearce J et al., Vet Ophthalmol 2009
Voelter-Ratson K et al., Vet Ophthalmol 2014

4. Alter cell wall stability

- Echinocandins

Echinocandins

- β-1,3-glucan synthase → produce β-1,3-glucan → cell wall
- Bind β-1,3-glucan synthase enzyme complex → inhibit synthesis of β-1,3-glucan polymers → inhibit cell wall synthesis
- Enzyme specific to fungal cells → limited mammalian toxicity



Echinocandins

- Fungicidal versus *Candida*
- Fungistatic versus *Aspergillus*
- Poor activity versus *Fusarium, Cryptococcus, Mucormycetes*

- Resistance develops due to mutations in catalytic subunits of glucan synthase

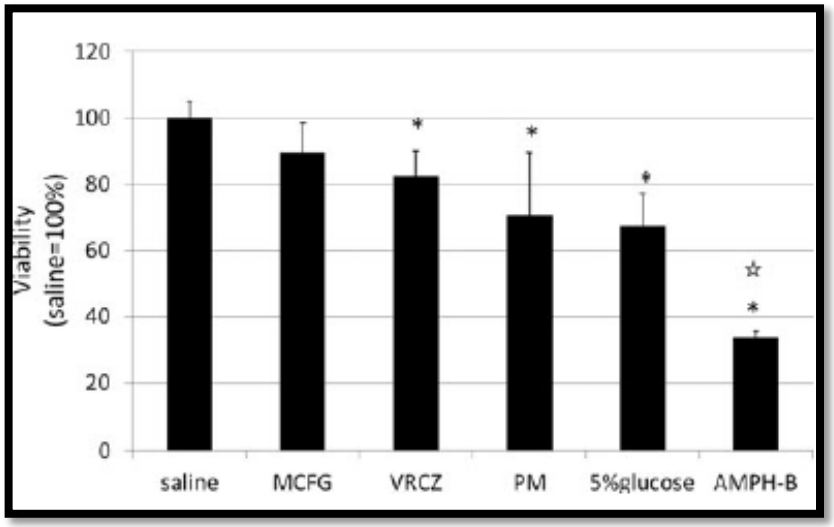
- Poor oral bioavailability → intravenous administration
- Limited evaluation shows reasonable intraocular penetration after IV administration

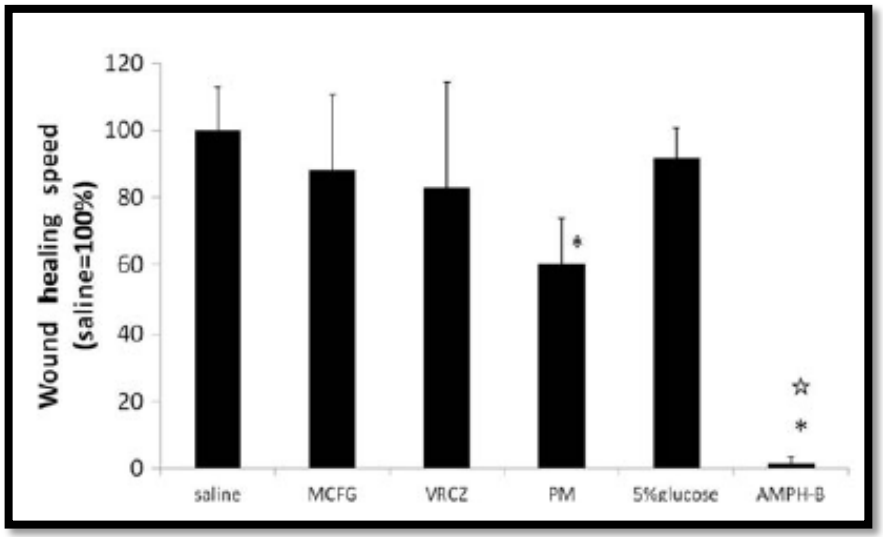
Mochizuki et al., JOPT 2011
Suzuki et al., JIC 2008

Echinocandins in Ophthalmology

- *In vitro* human corneal epithelial cell culture
- Cell viability, barrier function, wound closure

- Overall decreased toxic effects of 0.1% micafungin and 1% voriconazole versus 0.1% amphotericin B and 5% pimaricin





JOURNAL OF OCULAR PHARMACOLOGY AND THERAPEUTICS
Volume 30, Number 10, 2014

DOI: 10.1089/jop.2014.0044

Toxicity of Topical Antifungal Agents to Stratified Human Cultivated Corneal Epithelial Sheets

Mikiko Kimakura, Tomohiko Usui, Seiichi Yokoo, Suguru Nakagawa, Satoru Yamagami, and Shiro Amano

Summary…

- Mechanisms of action =
  - Interrupt DNA/RNA synthesis
  - Alter cell membrane permeability
  - Alter cell membrane stability
  - Alter cell wall stability
- Although antifungal drugs target constituents of the fungal cell not found in mammalian cells, toxicities do exist
- Mechanisms of resistance are similar to those of bacteria