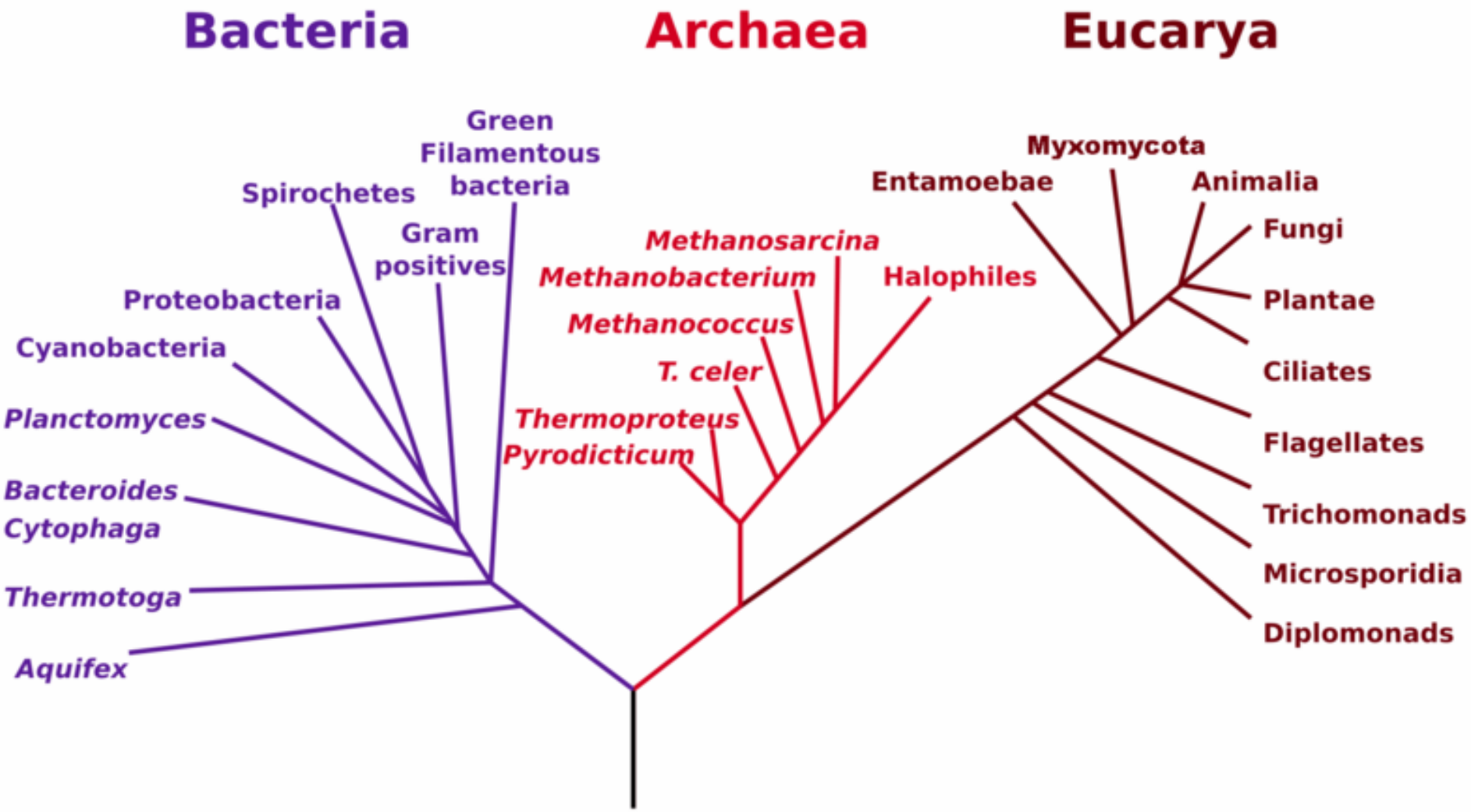
Introduction to mycology

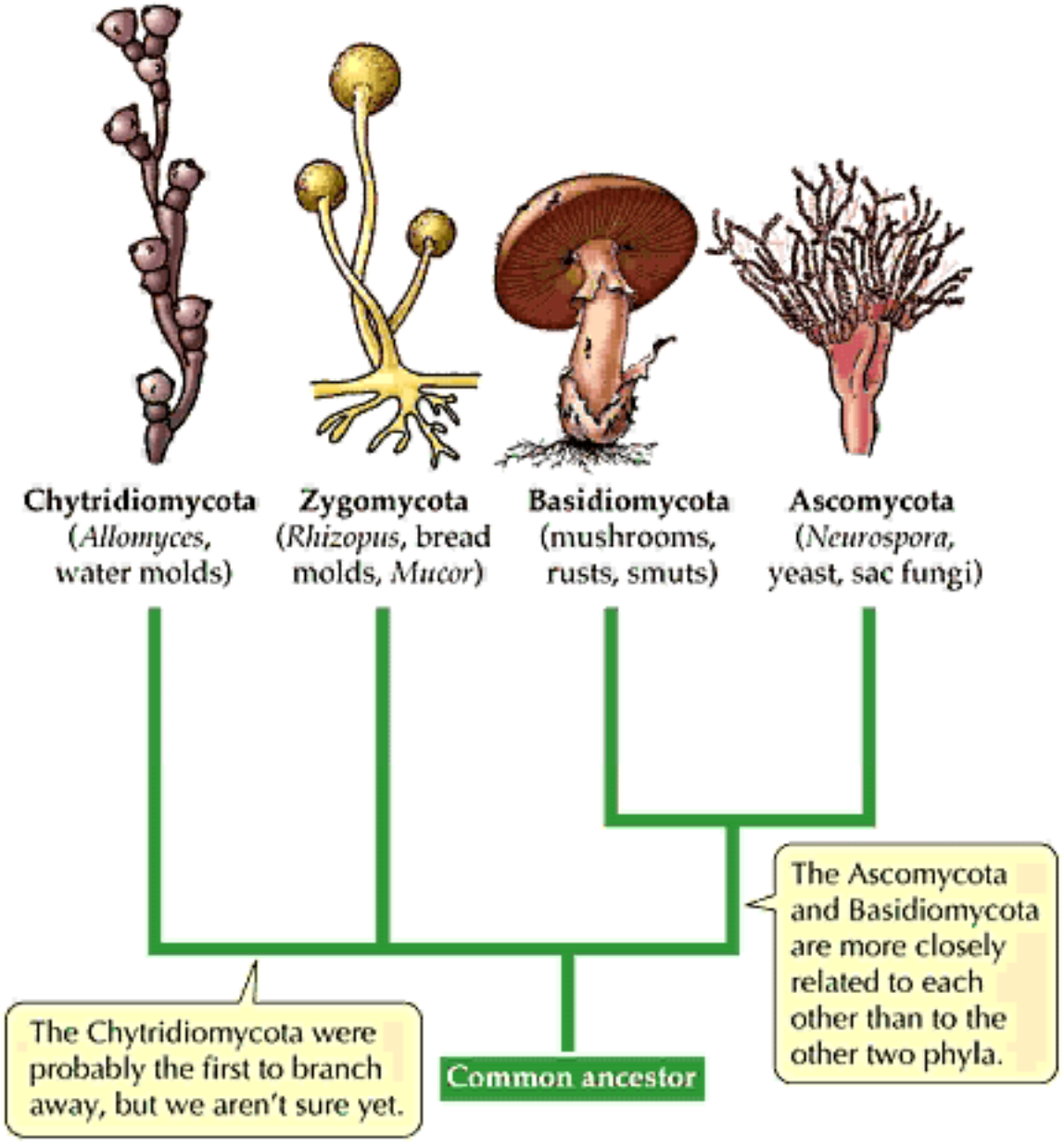
Dr Darius Armstrong-James BM MRCP MSc PhD DipHIVMed
MRC Clinician Scientist
Clinical Senior Lecturer in Fungal Immunobiology
Consultant Physician and Medical Mycologist

Talk overview

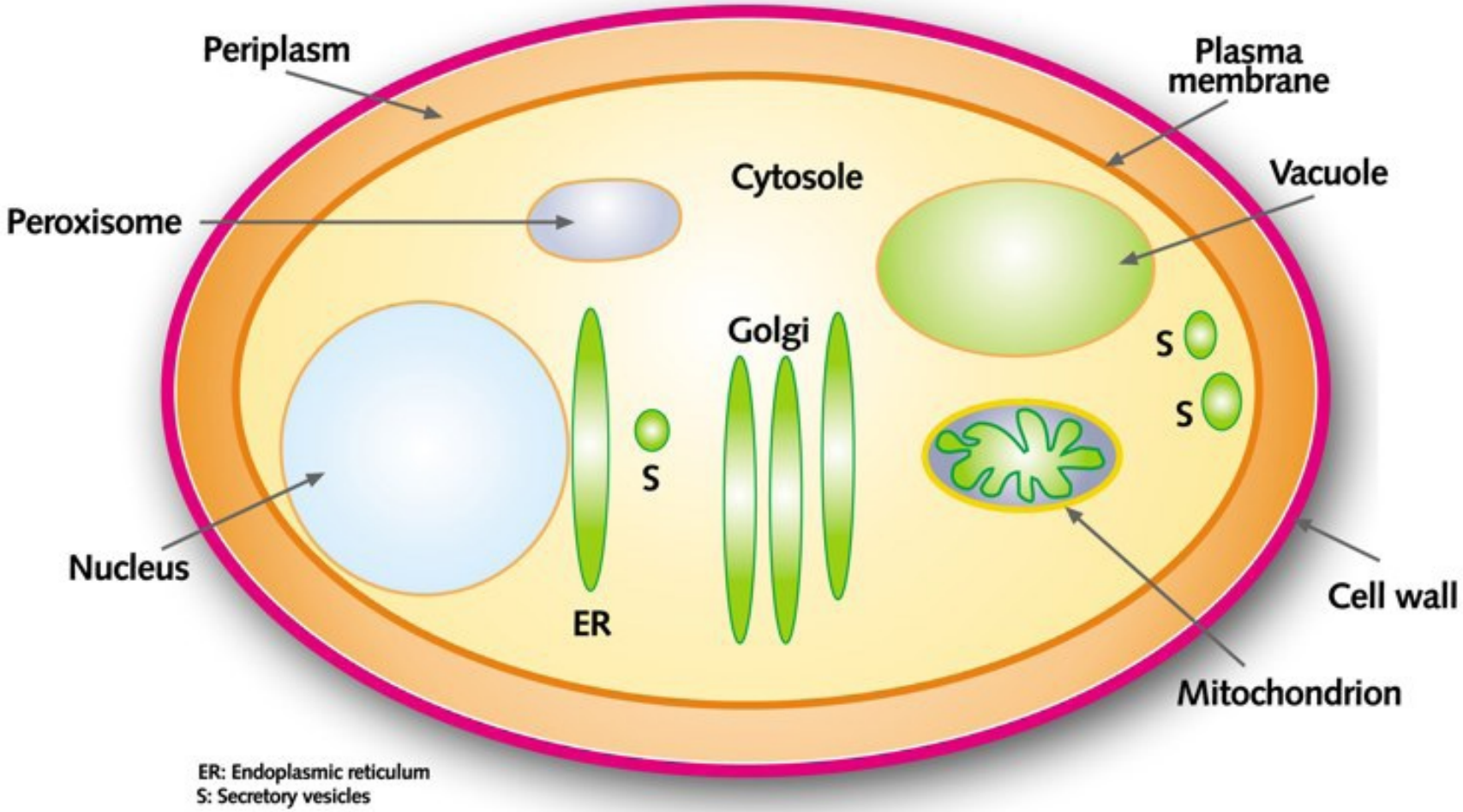
- Fungal overview
- Candidasis
- Aspergillosis
- Cryptococcosis
- Antifungals

Phylogenetic Tree of Life




Chytridiomycota (*Allomyces*, water molds)
Zygomycota (*Rhizopus*, bread molds, *Mucor*)
Basidiomycota (mushrooms, rusts, smuts)
Ascomycota (*Neurospora*, yeast, sac fungi)
The Chytridiomycota were probably the first to branch away, but we aren't sure yet.
Common ancestor
The Ascomycota and Basidiomycota are more closely related to each other than to the other two phyla.

The Yeast Cell

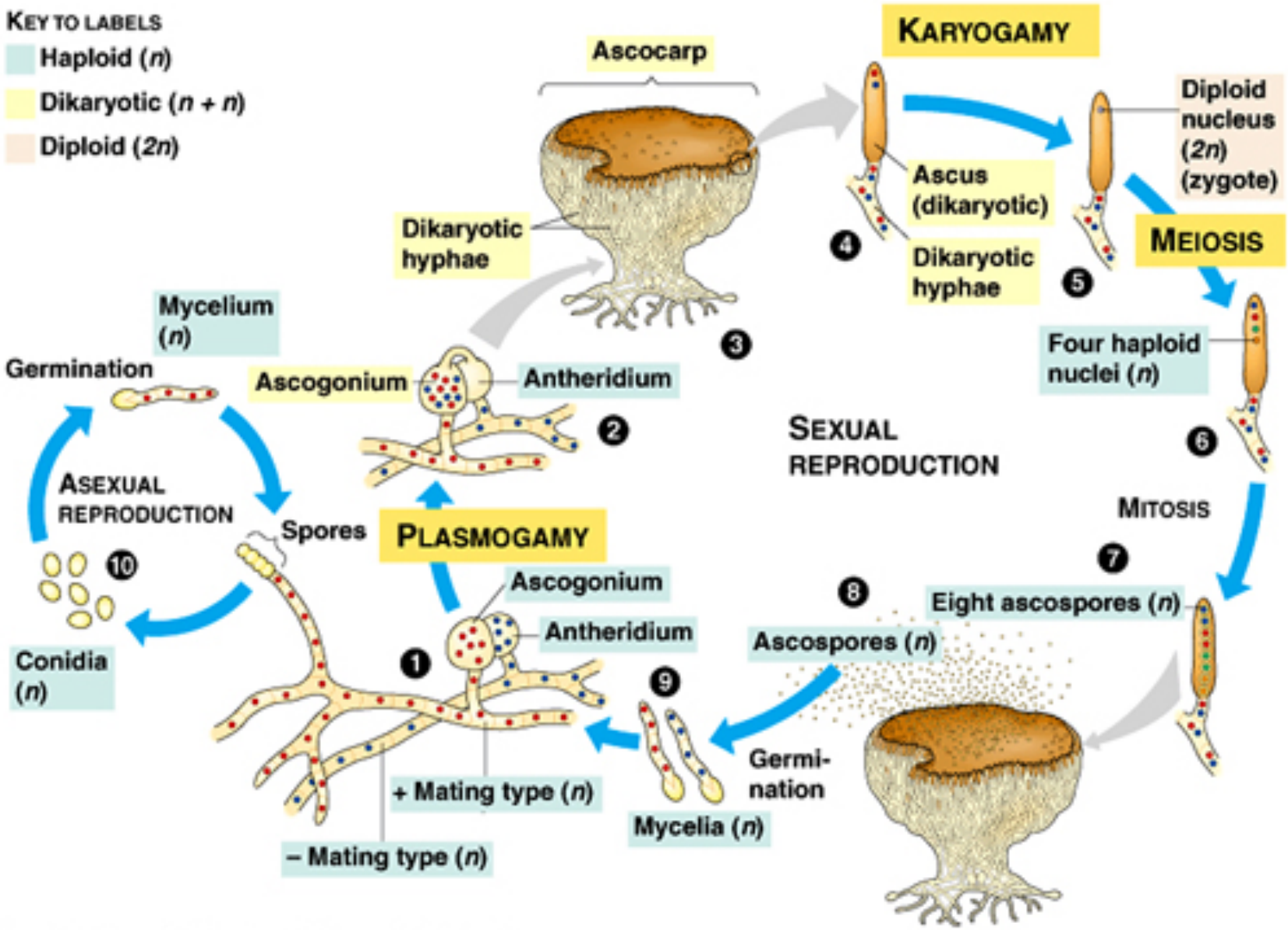


Candidiasis

A primary or secondary mycotic infection caused by members of the genus *Candida*. The clinical manifestations may be acute, subacute or chronic to episodic. Involvement may be localized to the mouth, throat, skin, scalp, vagina, fingers, nails, bronchi, lungs, or the gastrointestinal tract, or become systemic as in septicaemia, endocarditis and meningitis.

Distribution: World-wide.

Aetiological Agents: *Candida albicans, C. glabrata, C. tropicalis, C. krusei. C. parapsilosis, C. guilliermondii* and *C. pseudotropicalis.* All are ubiquitous and occur naturally on humans.



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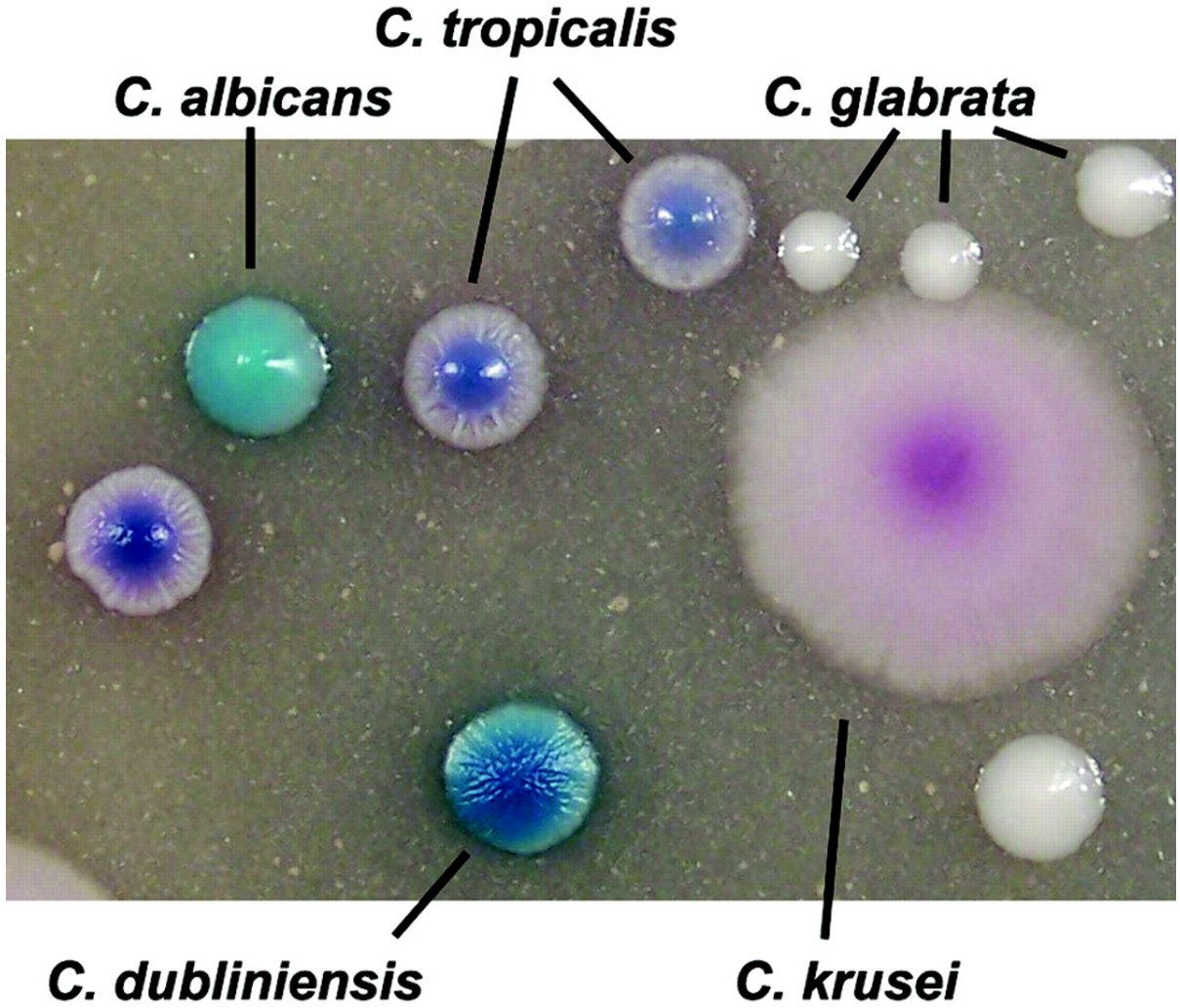
Colonies of different species of Candida after growing for 48 h at 37°C in CHROMagar Candida medium supplemented with Pal's agar.



Sahand I H et al. J. Clin. Microbiol. 2005;43:5768-5770

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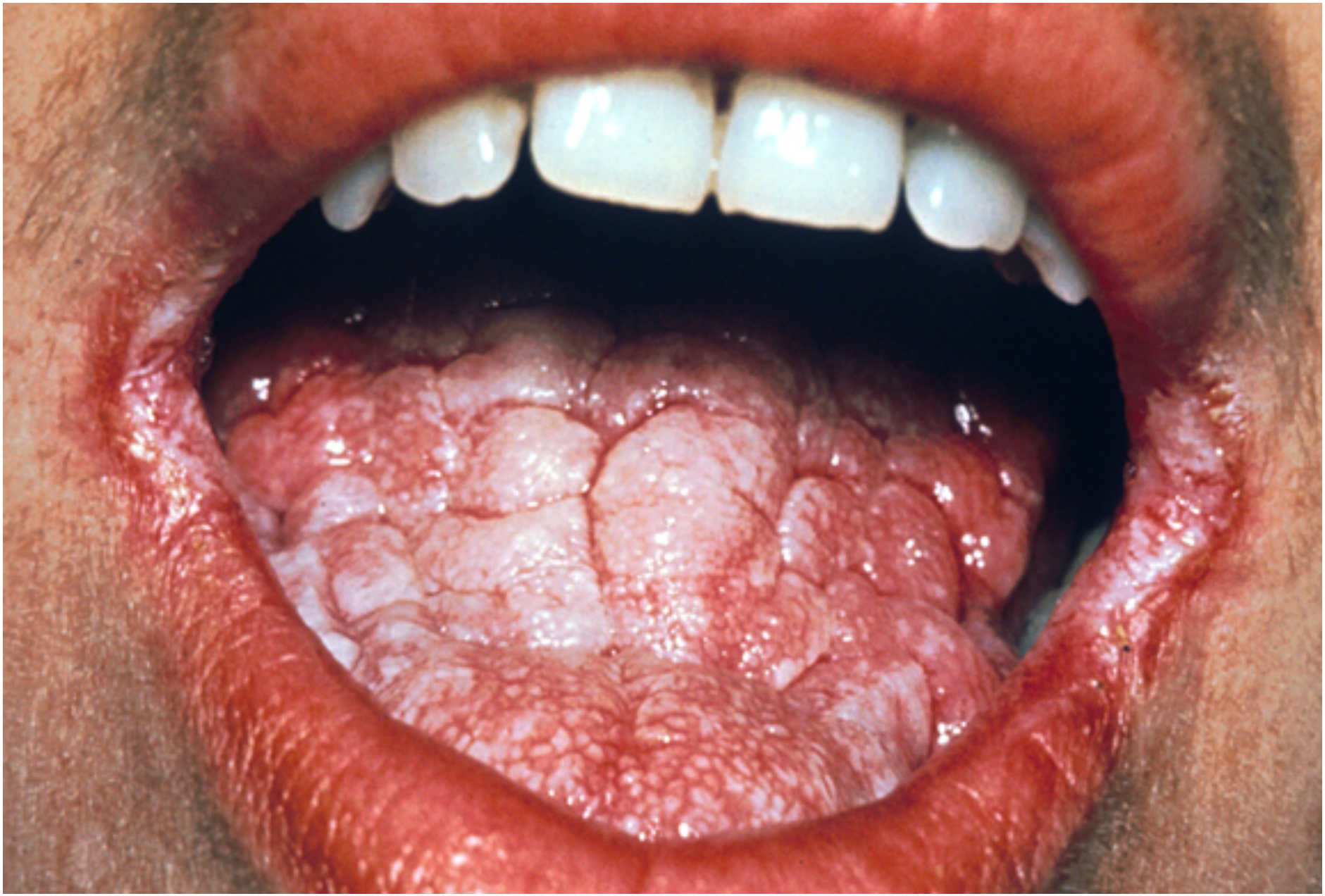
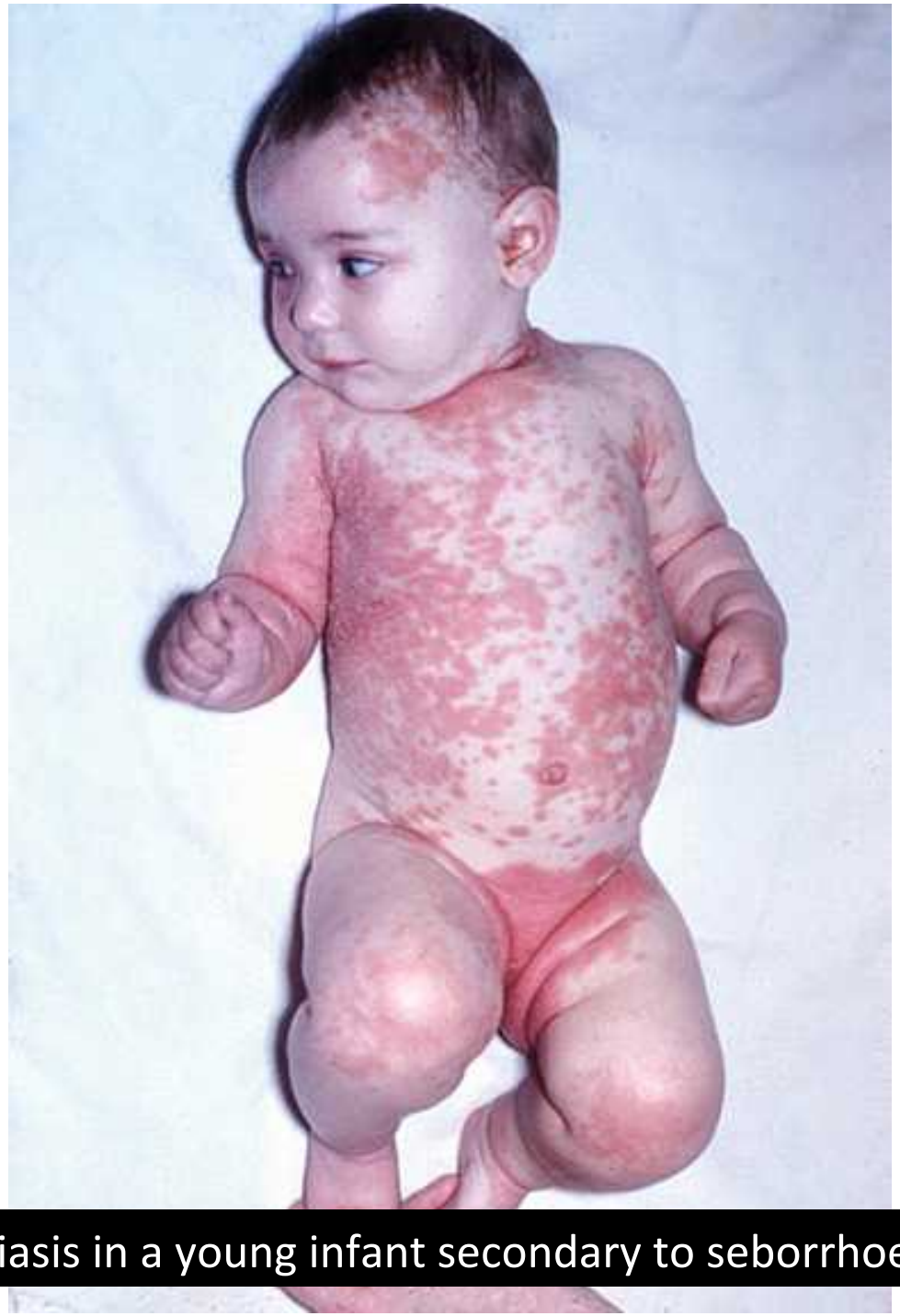
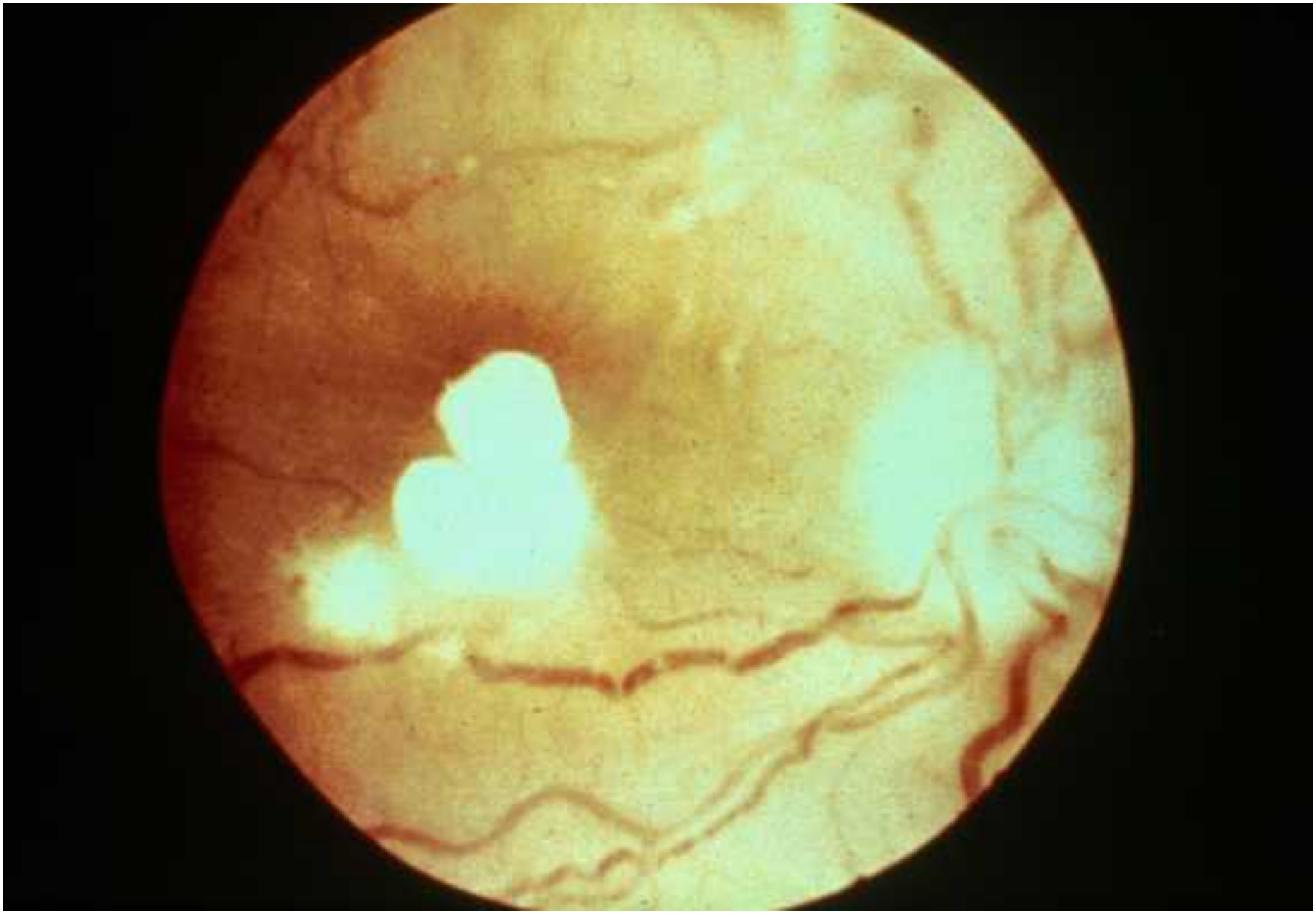


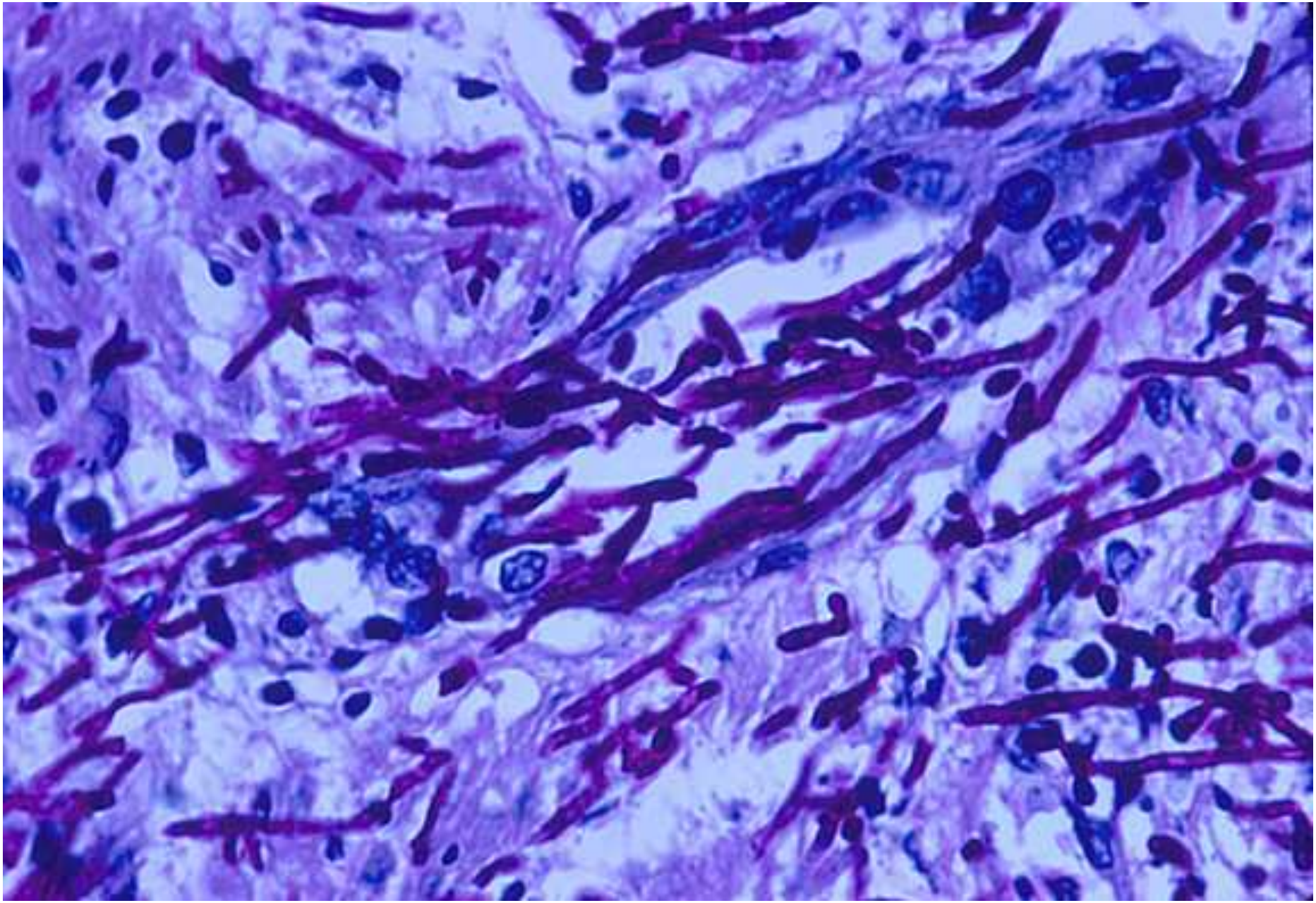
Chronic oral candidiasis



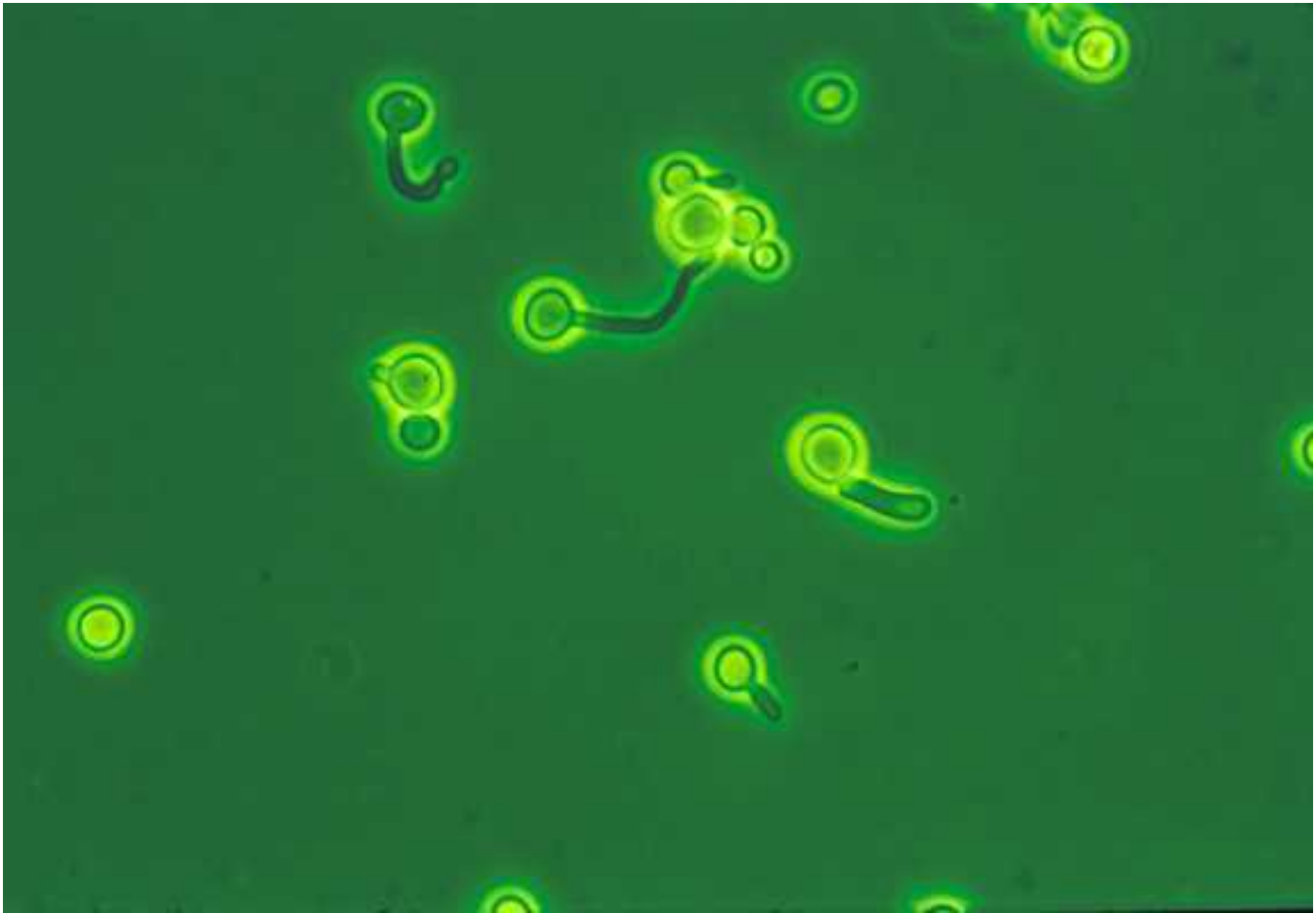
Generalized candidiasis in a young infant secondary to seborrhoeic dermatitis



Candida endophthalmitis



Invasive candidiasis-PAS-stained tissue section



Screening test for the identification of *C. albicans*.

Diagnosis of Candidasis

- Blood cultures for candidaemia
- Imaging for hepatosplenic candidaisis
- B D Glucan assay
- EORTC Criteria
  - Host
  - Clinical
  - Mycological

Management of Candidasis

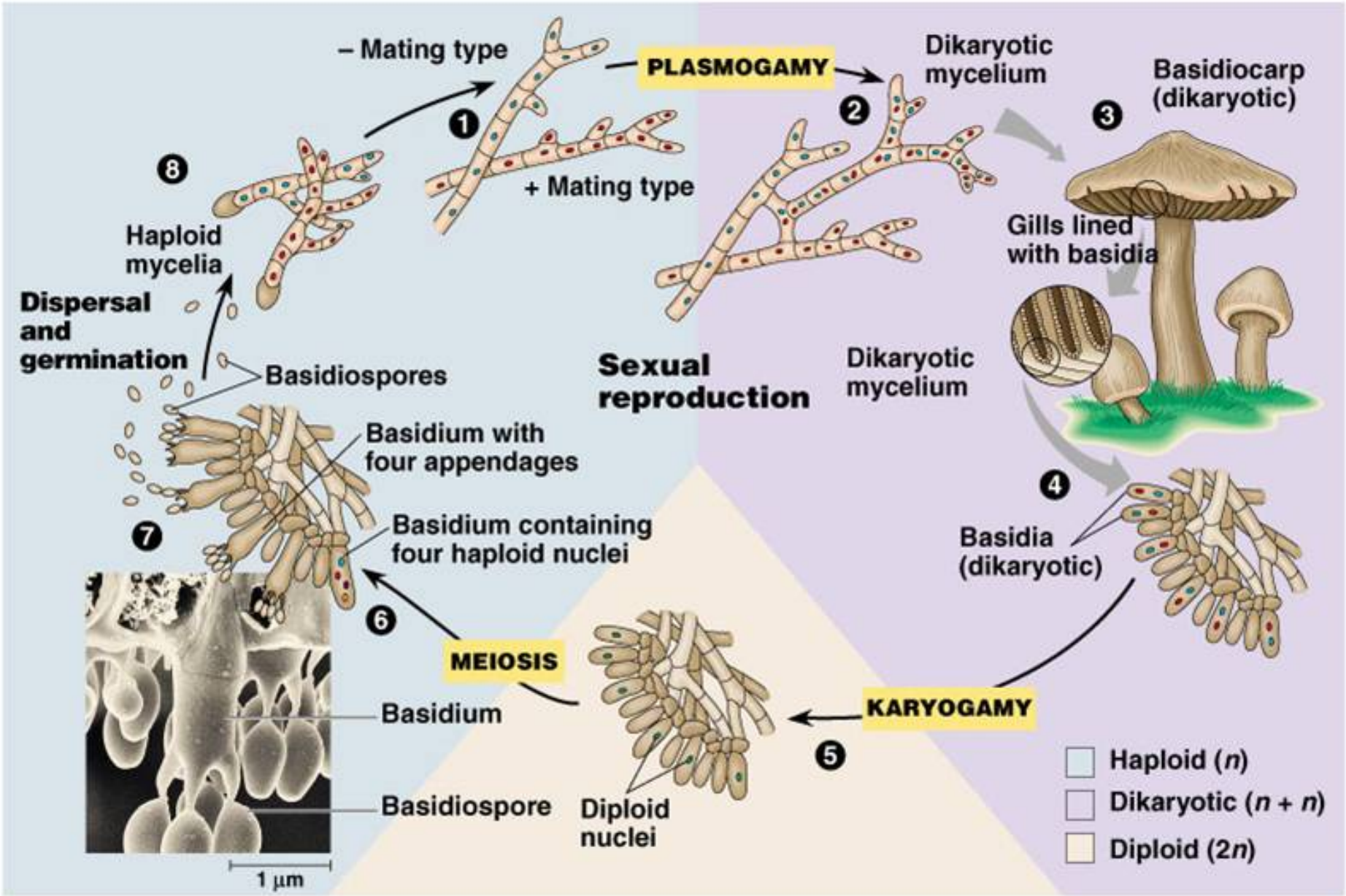
- At least 2 weeks of antifungals
- Fluconazole for Candida albicans
- Echinicandin for non-Candida albicans
- Ambisome, Fluconazole or Voriconazole for organ-based disease

Cryptococcosis

A chronic, subacute to acute pulmonary, systemic or meningitic disease, initiated by the inhalation of the fungus. Primary pulmonary infections have no diagnostic symptoms and are usually subclinical. On dissemination, the fungus usually shows a predilection for the central nervous system, however skin, bones and other visceral organs may also become involved.

Distribution: World-wide.

Aetiological Agent: *Cryptococcus neoformans*.



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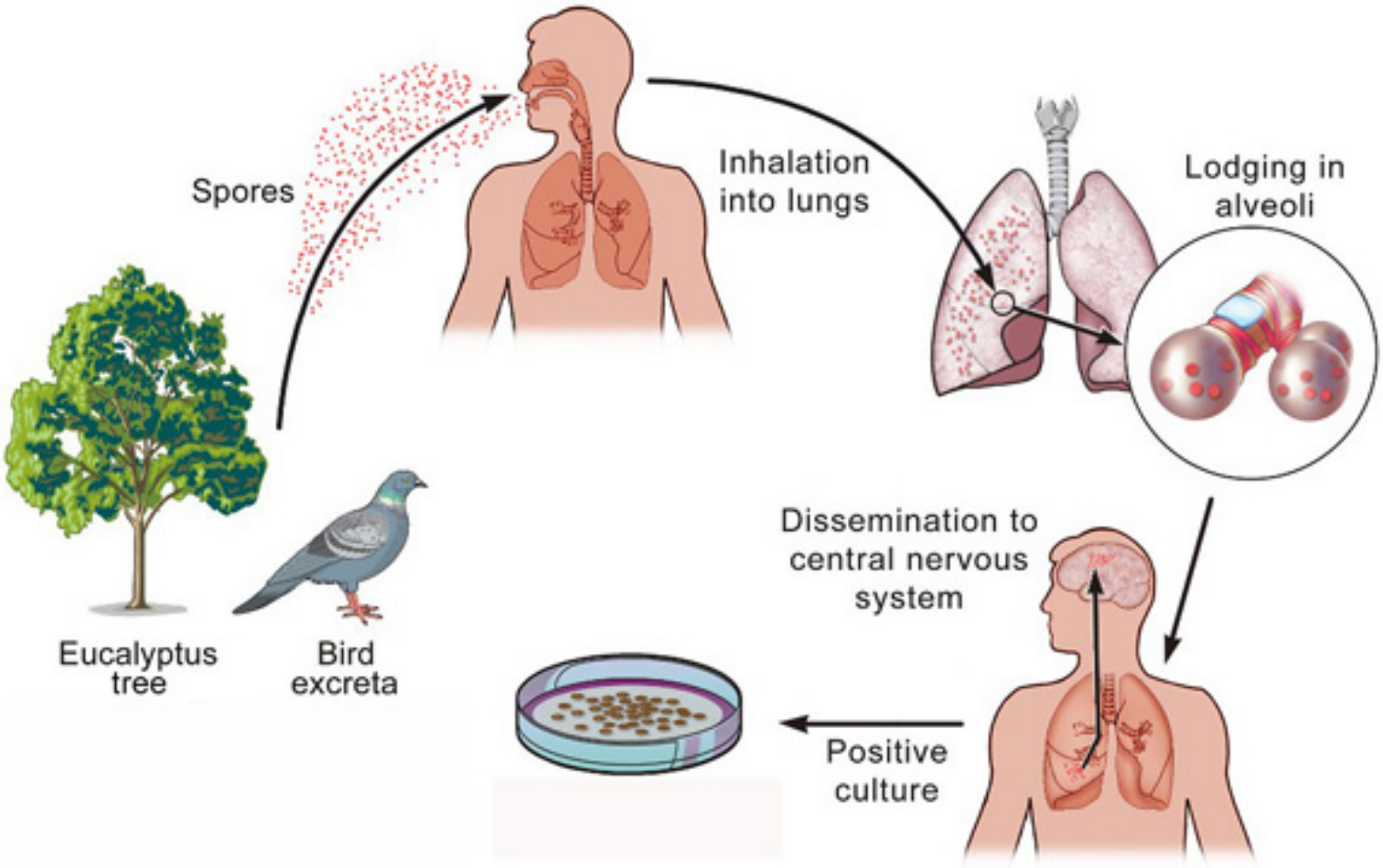
Susceptibility to cryptococcosis

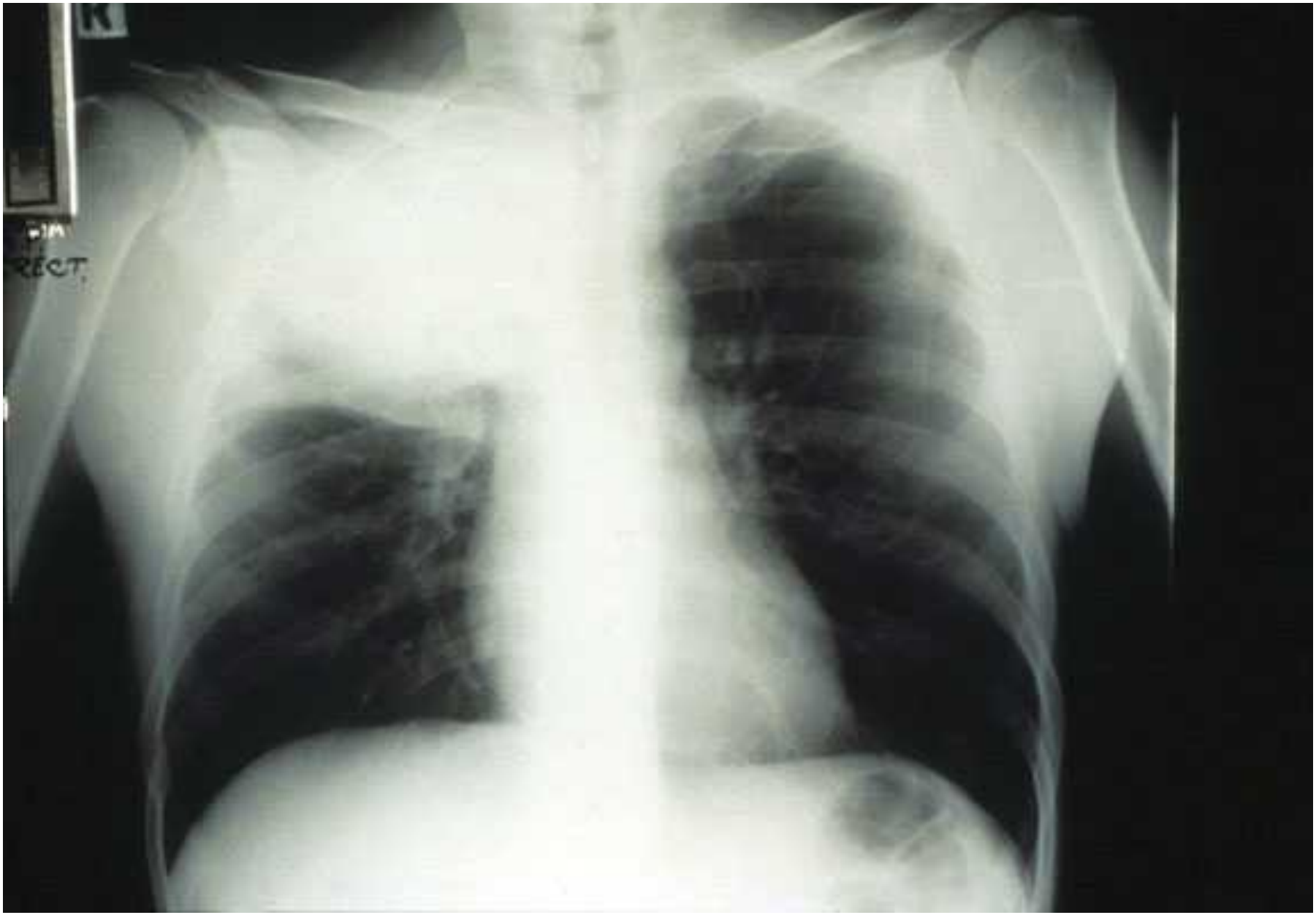
- Greatly increased in patients with impaired T-cell immunity
- Particularly AIDS patients, who have reduced CD4 helper T-cell numbers (typically less than 200/ml)
- Second most common cause of death in AIDS
- Patients taking T-cell immunosuppressants for solid organ transplant also have a 6% lifetime risk

Crytococcus neoformans var. gattii

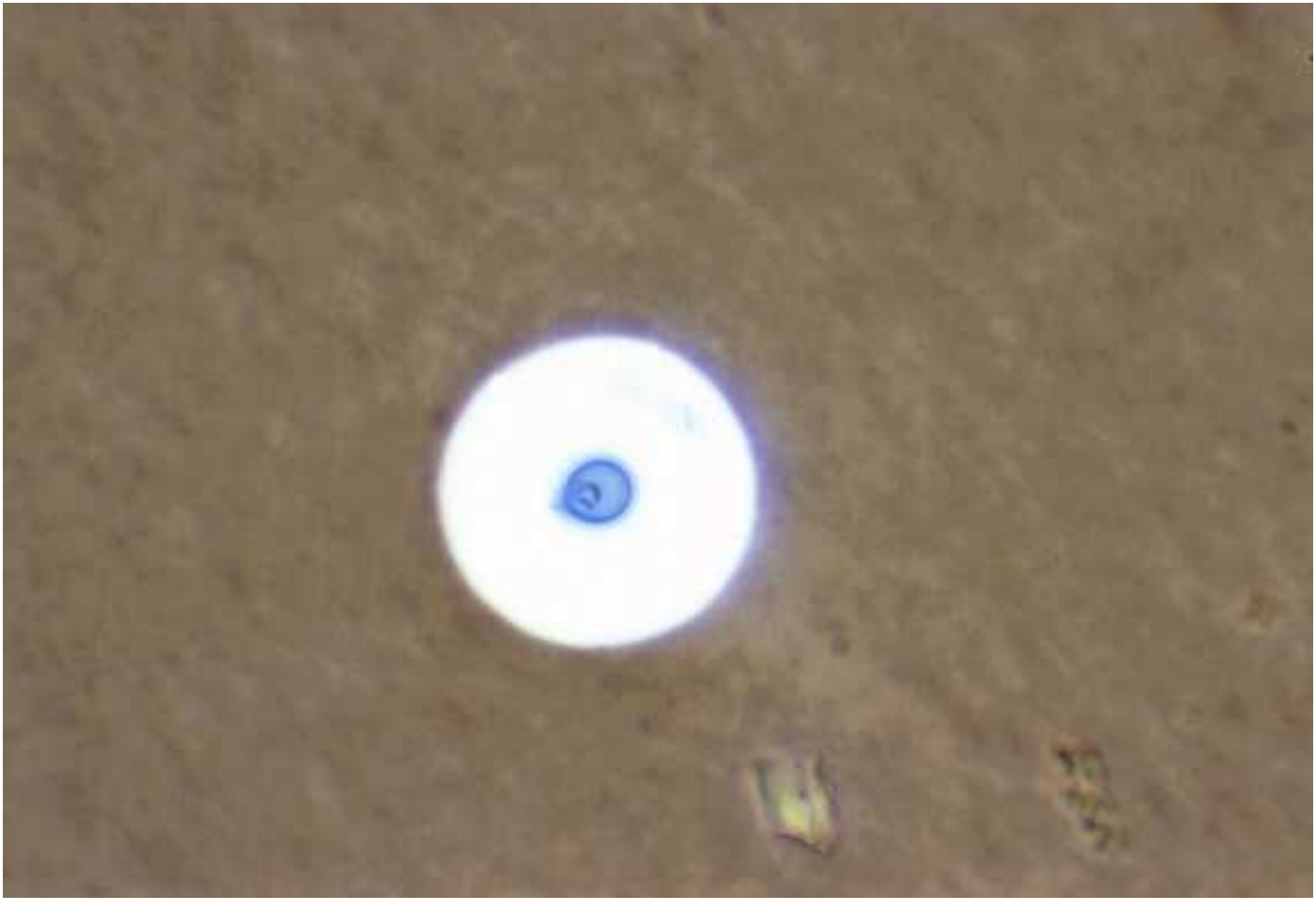
- Causes a meningitis in apparently immunocompetent individuals in tropical latitudes, esp. SE Asia and Australia
- Recent outbreak in Vancouver Island
- High incidence of space-occupying lesions in brain and lung
- Increased resistance to amphotericin B clinically

Life cycle of cryptococcosis

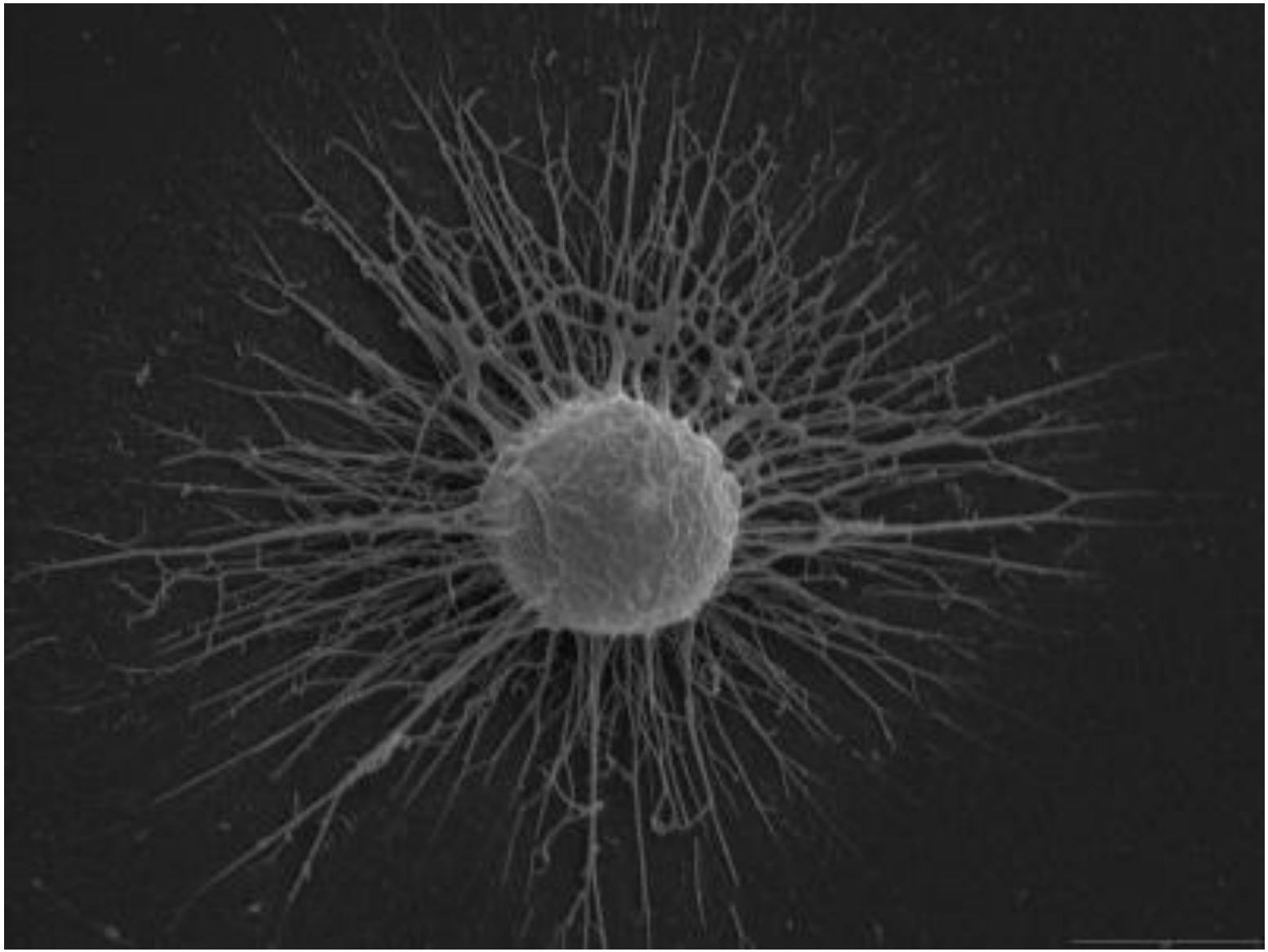


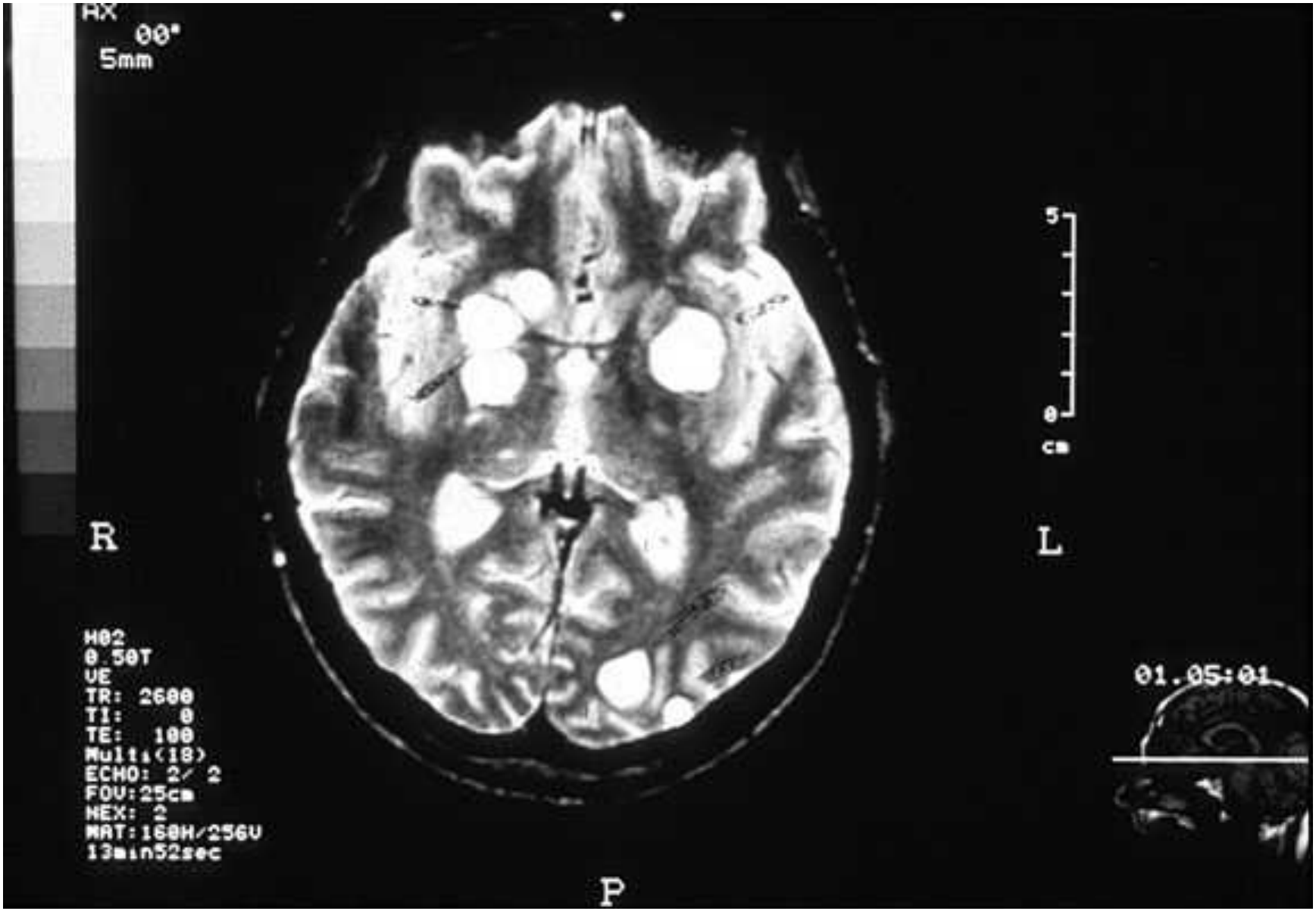


X-ray showing pulmonary cryptococcal infection



India ink preparation of cerebrospinal fluid from a patient with cryptococcal meningitis





MRI scans showing multiple cryptococcomas in the brain.

Eucalyptus camaldulensis

Cryptococcus gattii growing on water agar from Eucalyptus leaves

Diagnosis and management of cryptococcosis

- Diagnosis almost entirely around detection of Cryptococcal antigen in blood or CSF
- Typical clinical features
- Immunosuppressed host
- Often culturable from blood, body fluids

Cryptoccocosis Management

- 3/52 Amphotericin B +/- flucytosine
- Repeat LP for pressure management
- Secondary suppression with fluconazole
- Some evidence that high dose fluconazole effective
- Interferon-gamma may have utility

Aspergillosis

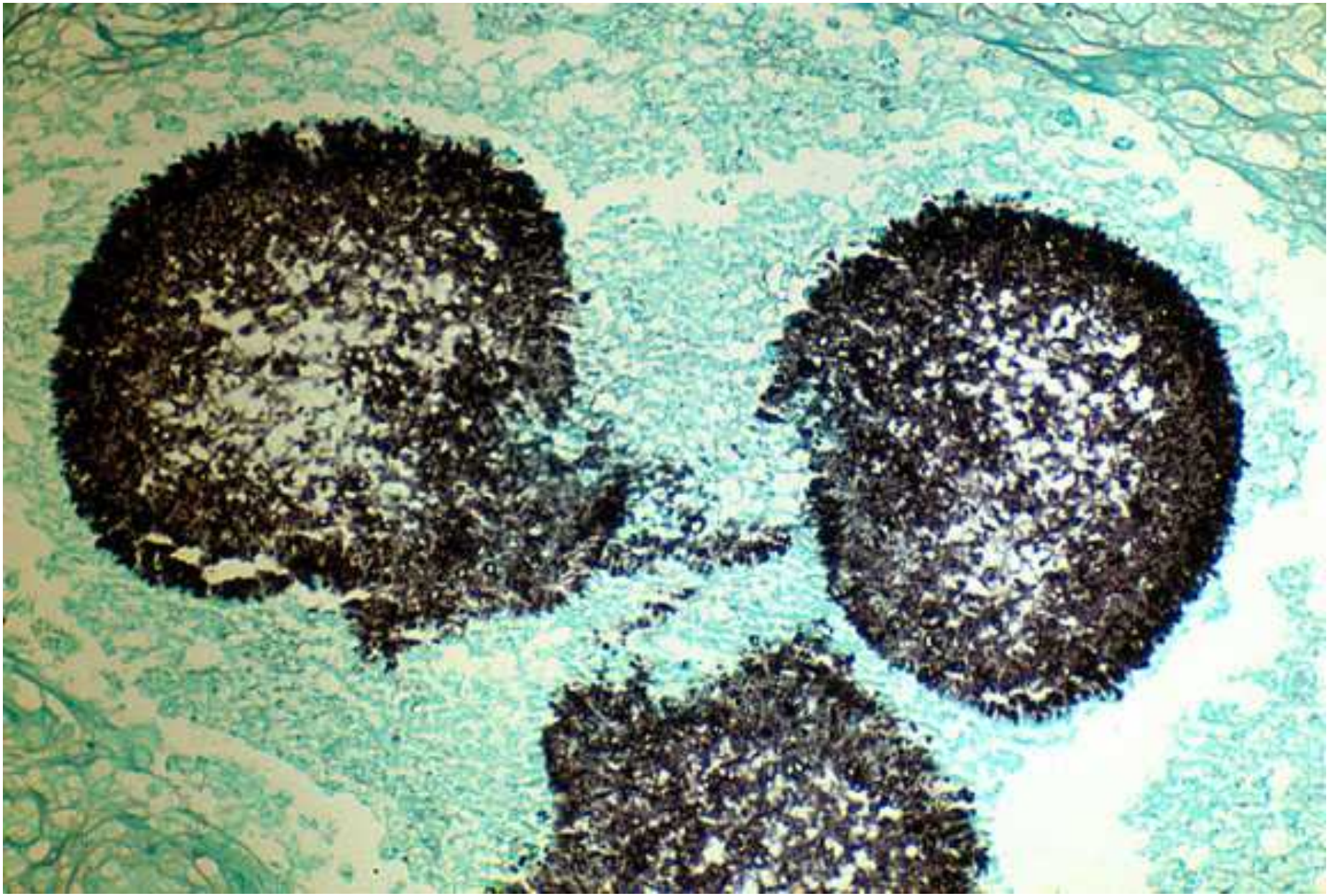
Aspergillosis is a spectrum of diseases of humans and animals caused by members of the genus *Aspergillus*. These include (1) mycotoxicosis due to ingestion of contaminated foods; (2) allergy and sequelae to the presence of conidia or transient growth of the organism in body orifices; (3) colonization without extension in preformed cavities and debilitated tissues; (4) invasive, inflammatory, granulomatous, necrotizing disease of lungs, and other organs; and rarely (5) systemic and fatal disseminated disease. The type of disease and severity depends upon the physiologic state of the host and the species of *Aspergillus* involved.

Distribution: World-wide.

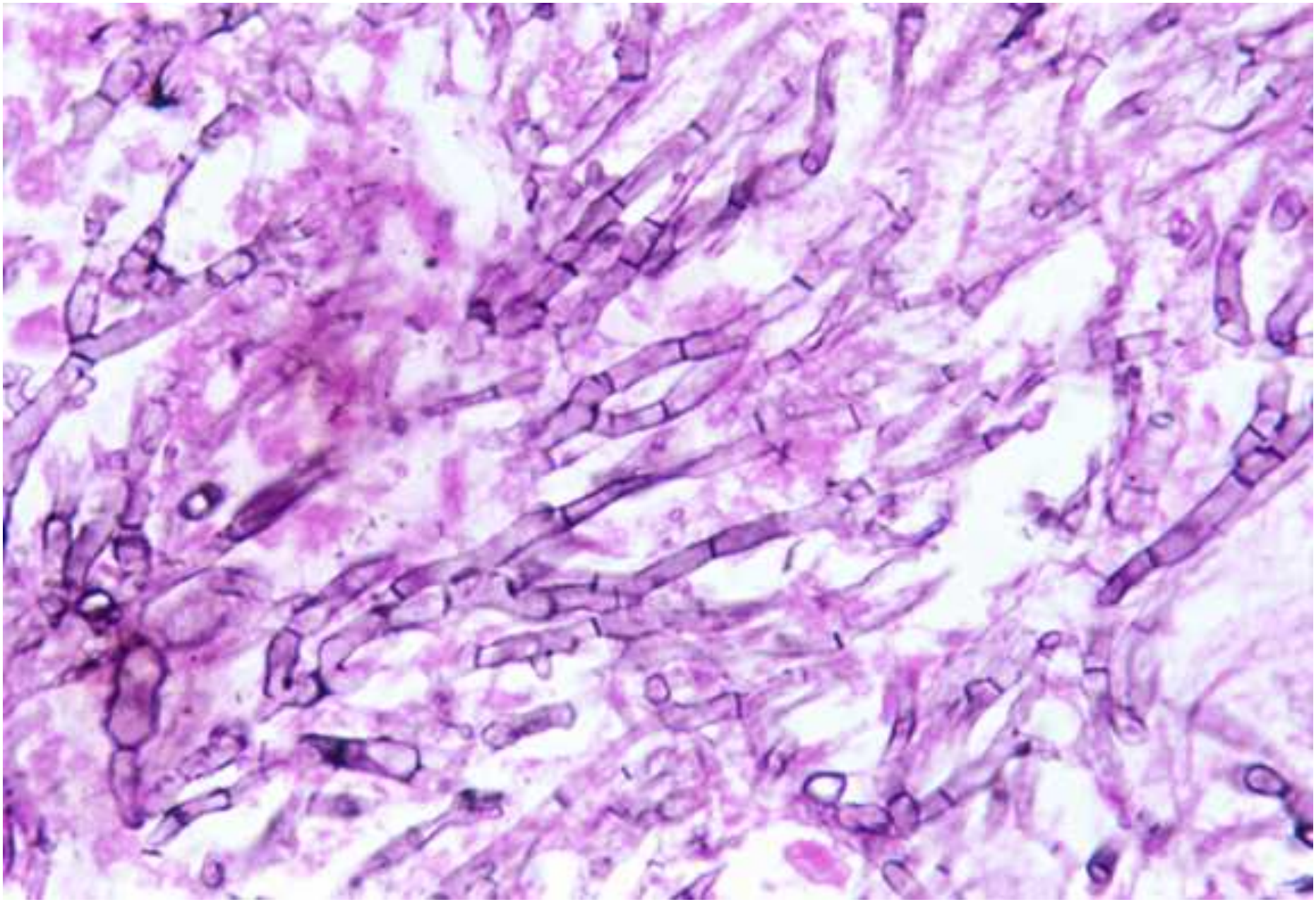
Aetiological Agents: *Aspergillus fumigatus, A. flavus, A. niger, A. nidulans* and *A. terreus*.

Aspergillus – scanning electron microscopy

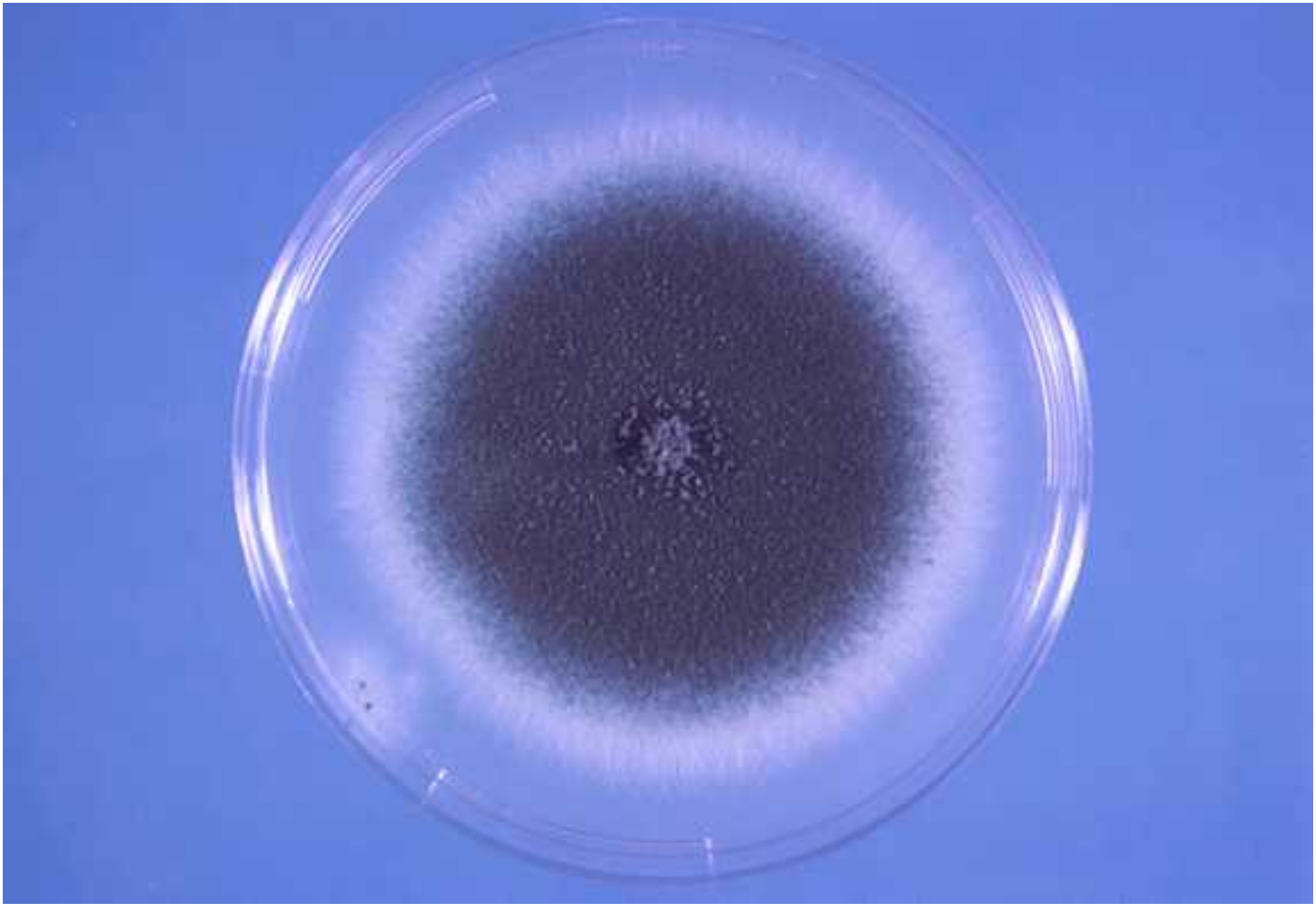
Aspergilloma - Leukaemia patient



Fungus ball-Previous TB



Invasive aspergillosis - lung

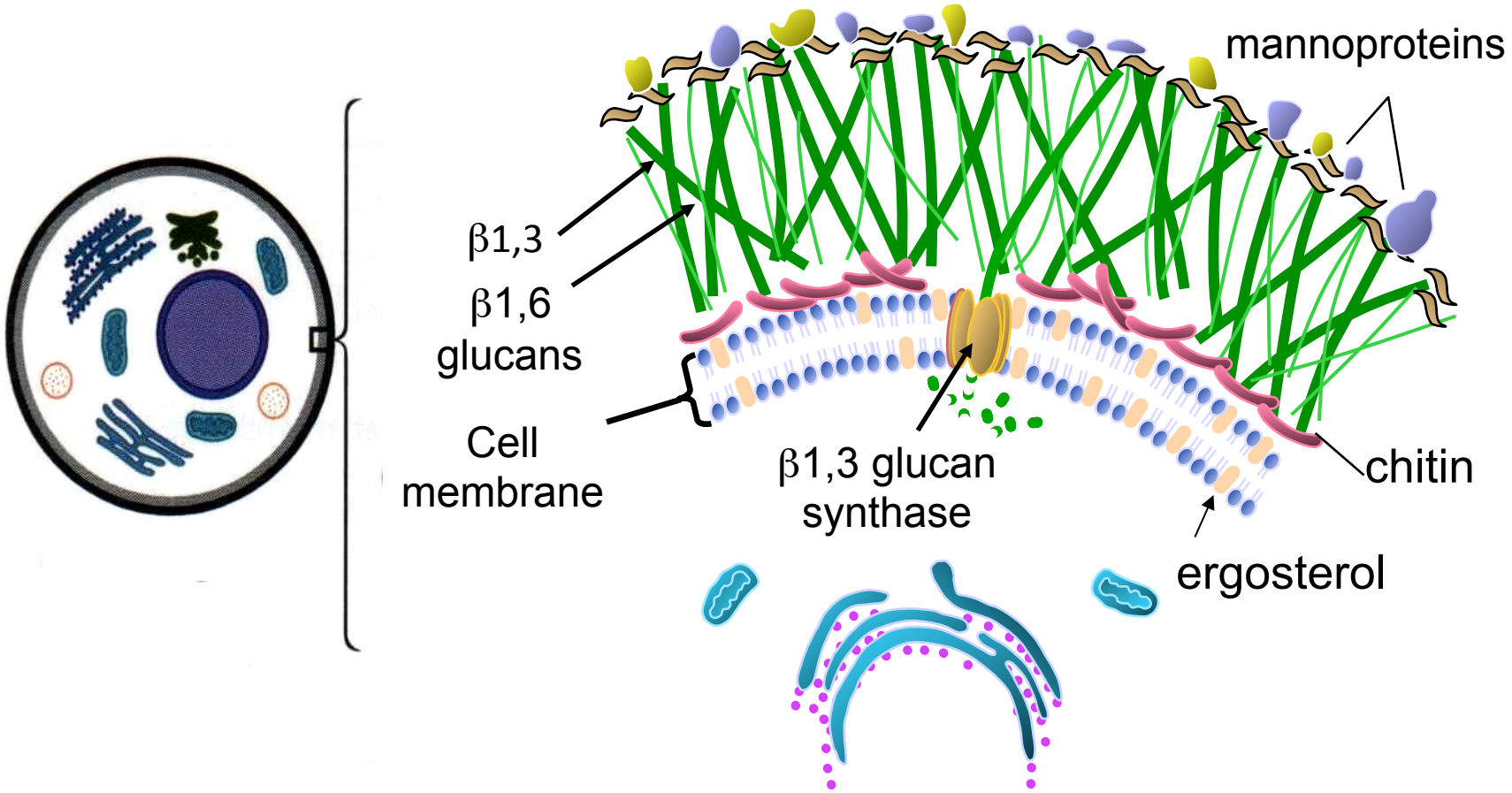


Aspergillosis management

- Voriconazole
- Ambisome
- Caspofungin/Itraconazole less good
- At least 6 weeks of therapy
- Duration based on host/radiological/ mycological factors

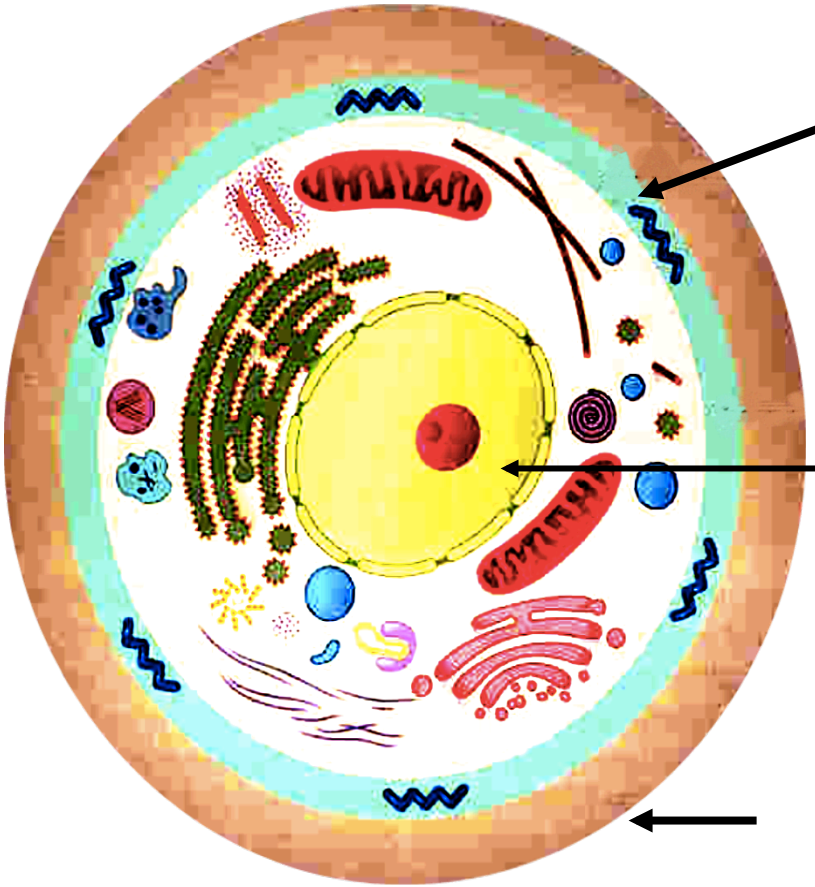
Antifungal agents: activity, resistance and monitoring

The Fungal Cell Wall



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

What are the targets for antifungal therapy?



Cell membrane

Fungi use principally ergosterol instead of cholesterol

DNA Synthesis

Some compounds may be selectively activated by fungi, arresting DNA synthesis.

Cell Wall

Unlike mammalian cells, fungi have a cell wall

Atlas of fungal Infections, Richard Diamond Ed. 1999
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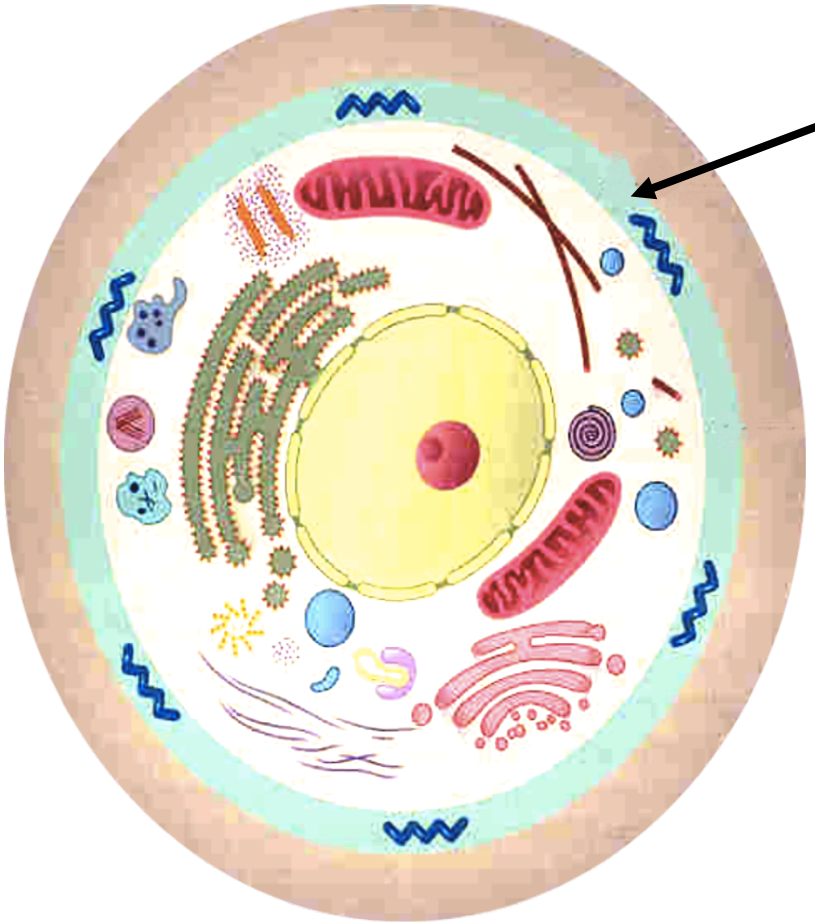
Cell Membrane Active Antifungals

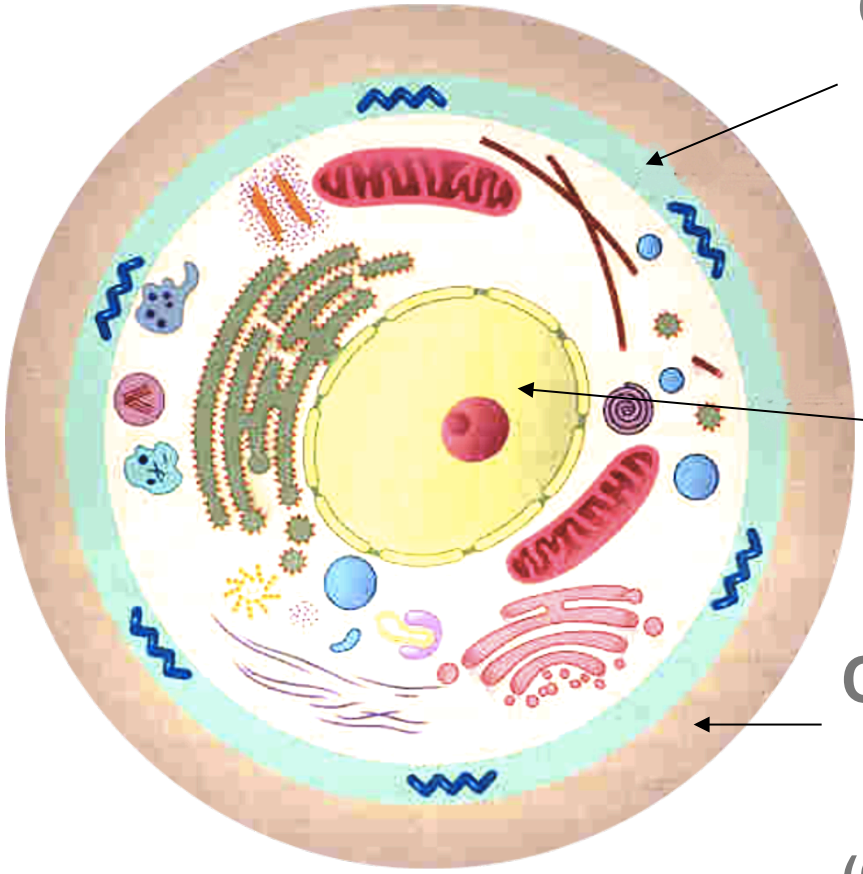


Cell membrane

- **Polyene antibiotics**
  - Amphotericin B, lipid formulations
  - Nystatin (topical)

- **Azole antifungals**
  - **Ketoconazole**
  - **Itraconazole**
  - **Fluconazole**
  - **Voriconazole**
  - **Miconazole, clotrimazole (and other topicals)**

Cell Wall Active Antifungals



Cell membrane

- Polyene antibiotics
- Azole antifungals

DNA/RNA synthesis

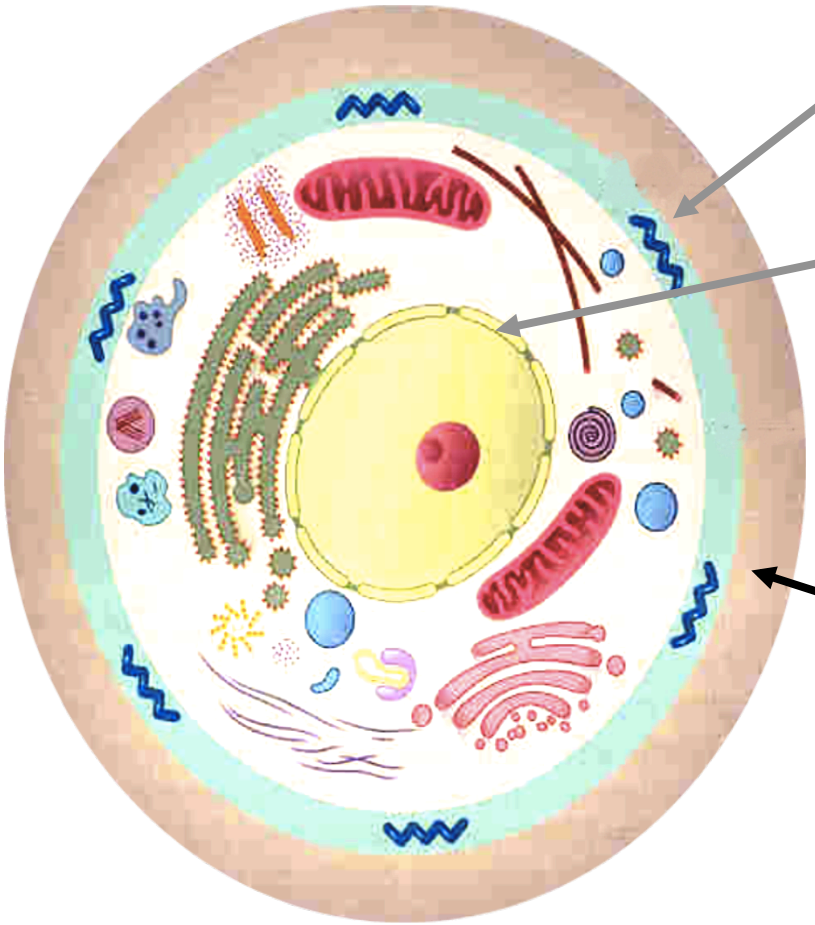
- **Pyrimidine analogues**
  - **Flucytosine**

Cell wall

- Echinocandins
  - -Caspofungin acetate

(Cancidas)

Cell Wall Active Antifungals



Cell membrane
- Polyene antibiotics
- Azole antifungals

DNA/RNA synthesis
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Cell wall
- **Echinocandins**
  - Caspofungin acetate

(Cancidas)

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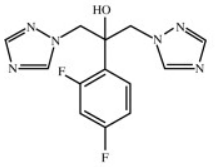
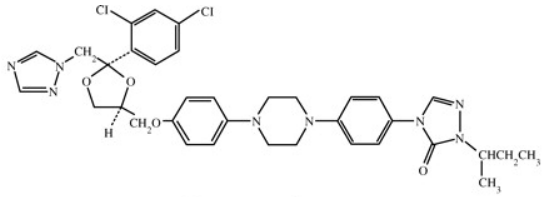
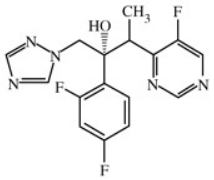
Chemical Structures of the Licensed and New Azoles and Echinocandins



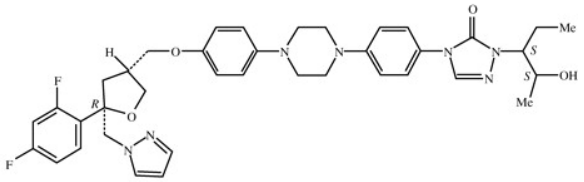
Fluconazole



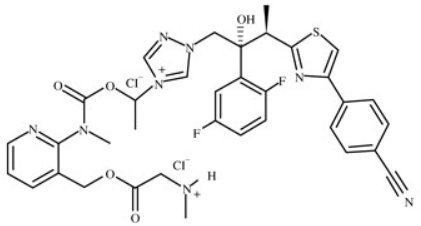
Itraconazole



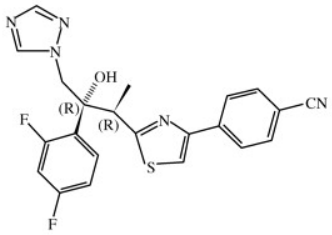
Voriconazole



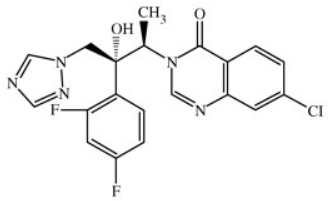
Posaconazole



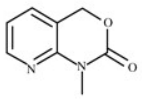
Isavuconazonium (pro-drug BAL-8557)



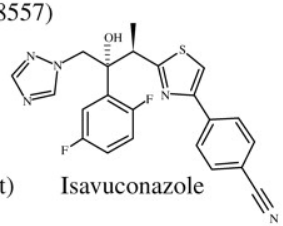
Ravuconazole



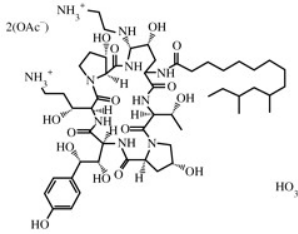
Albaconazole



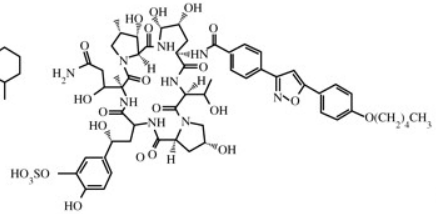
BAL-8728 (pro-drug cleavage product)



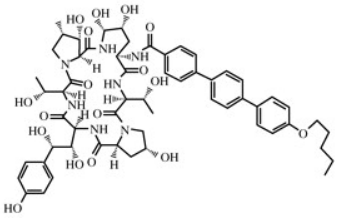
Isavuconazole



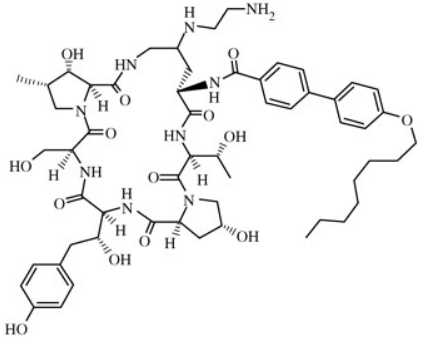
Caspofungin



Micafungin

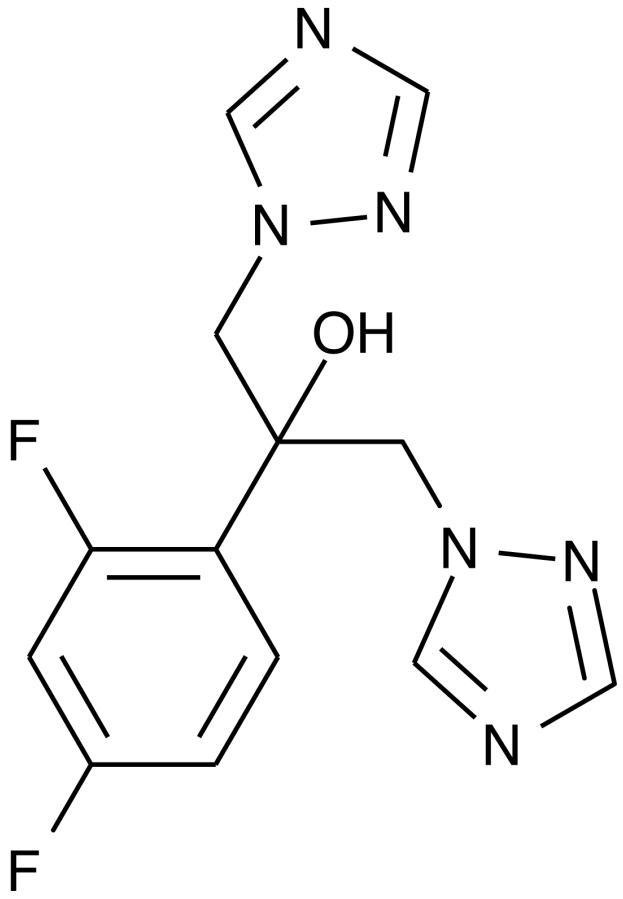


Anidulafungin

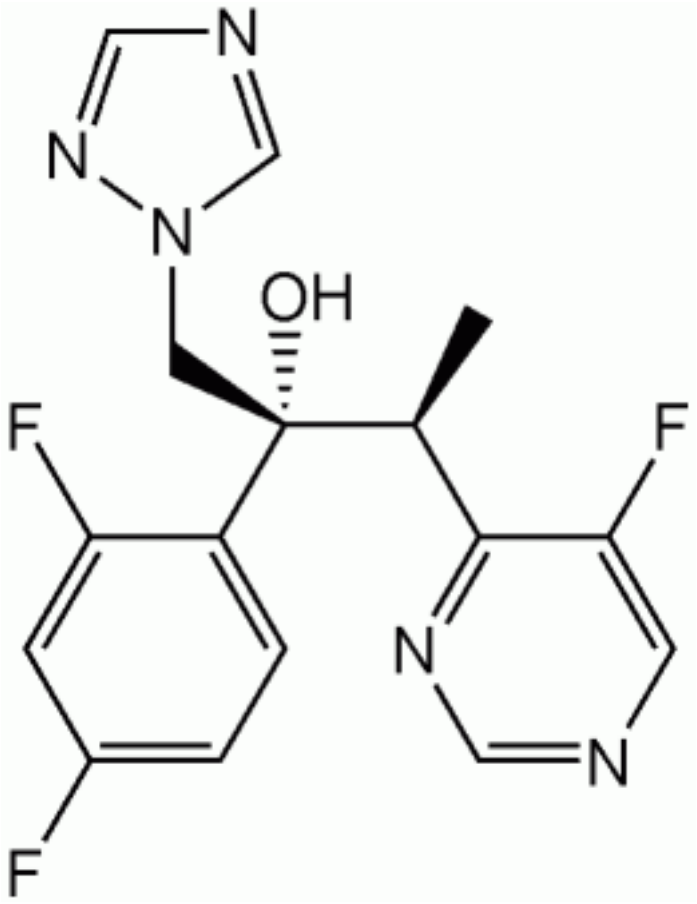


Aminocandin

Structure of the Water-Soluble Triazoles

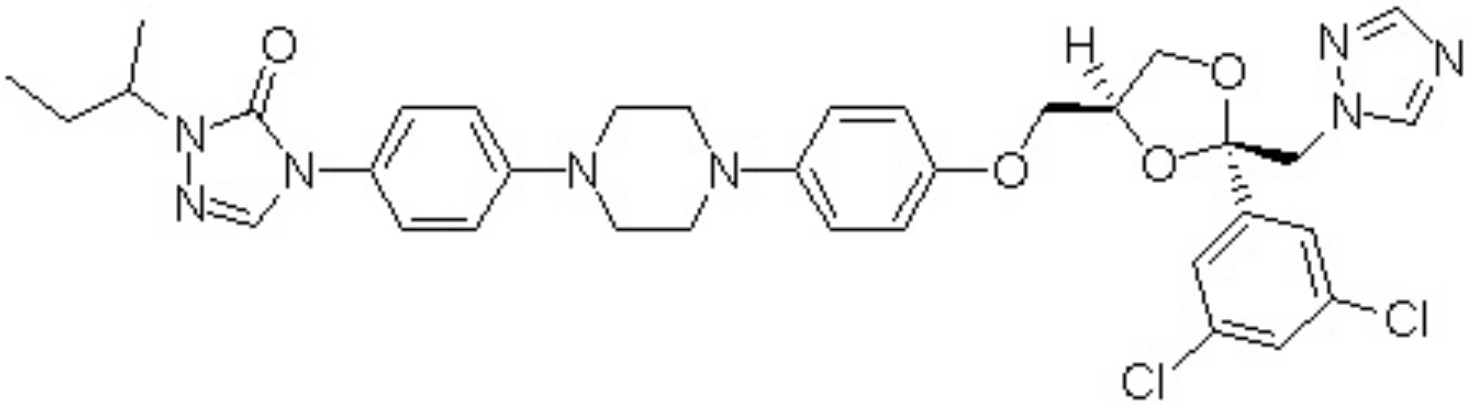


Fluconazole

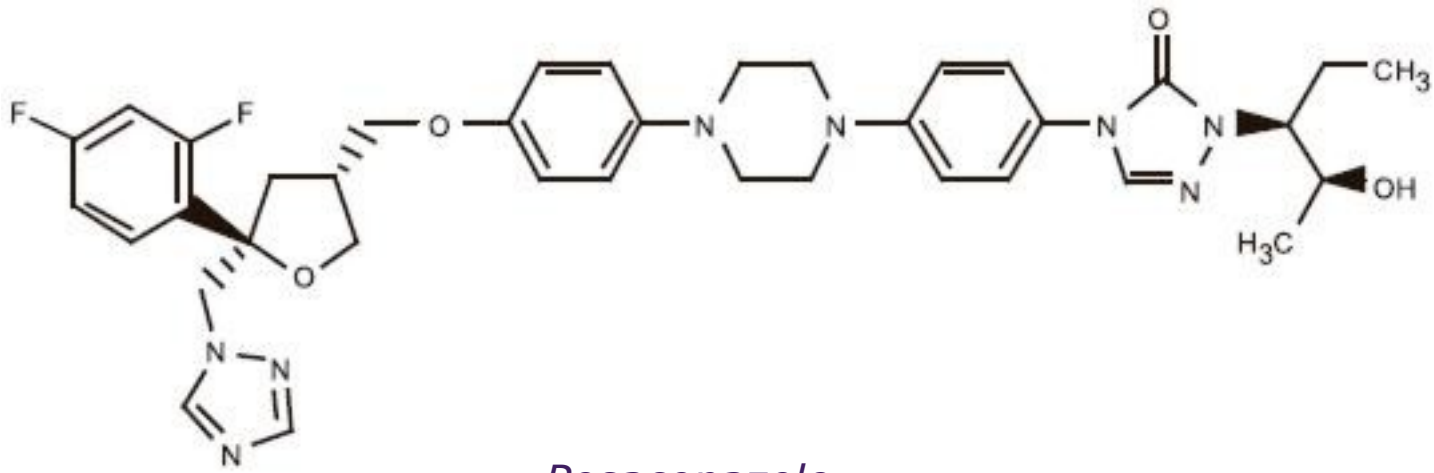


Voriconazole

Structure of the Lipophilic Triazoles



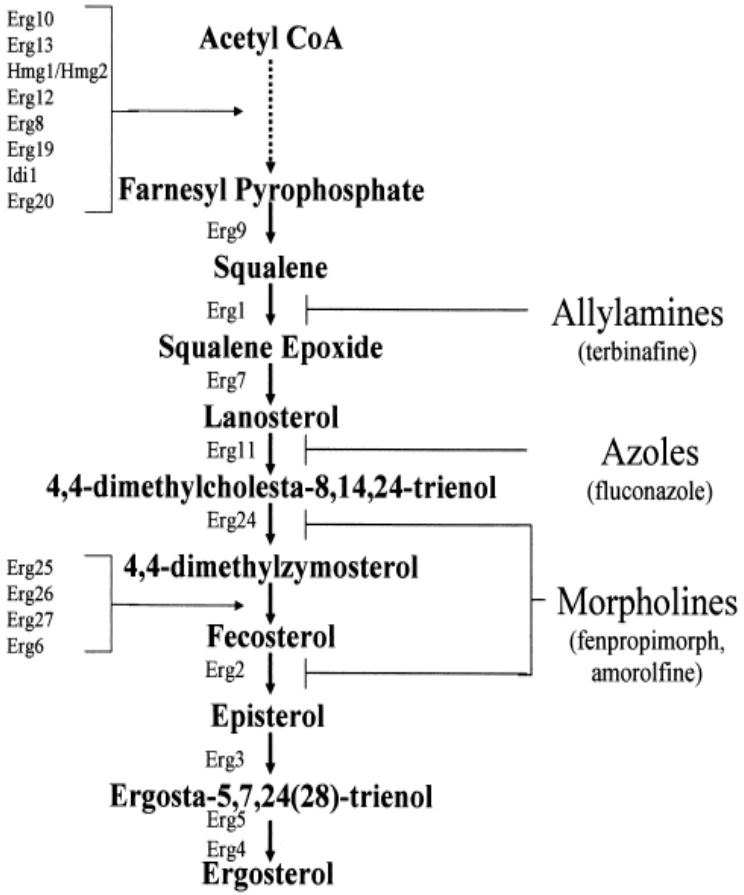
Itraconazole



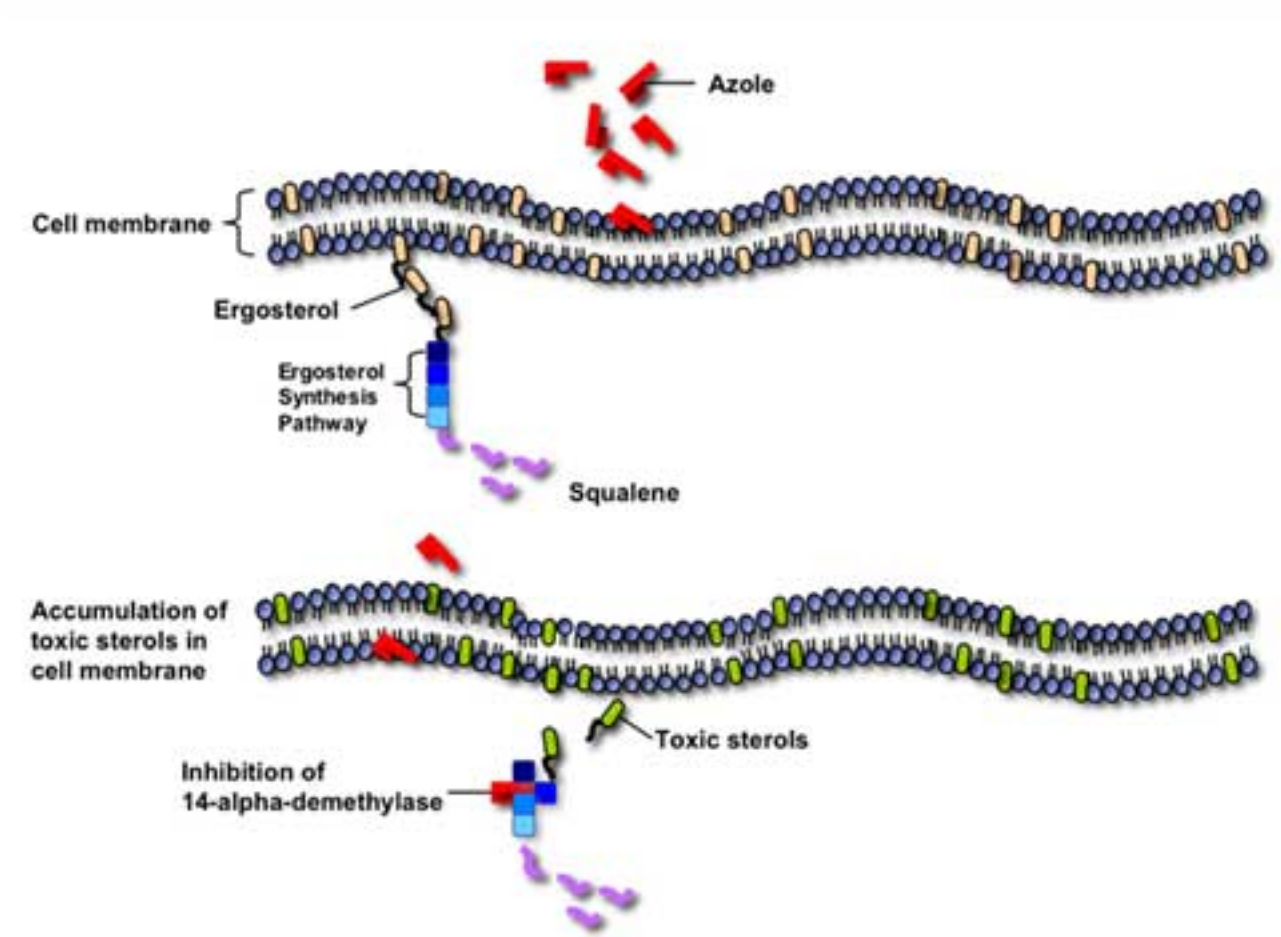
Posaconazole

Azoles - Mechanism

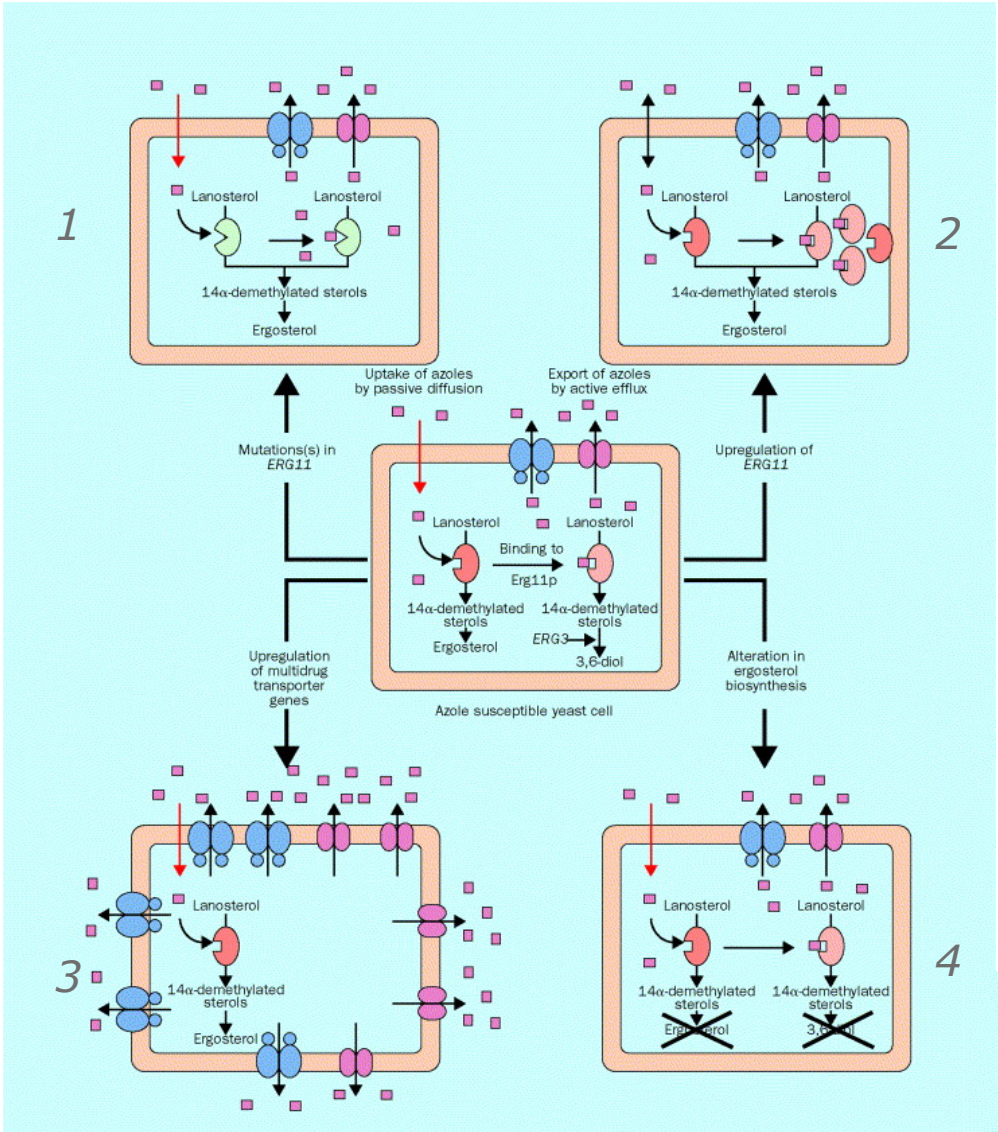
- In fungi, the cytochrome P450-enzyme lanosterol 14-α demethylase is responsible for the conversion of lanosterol to ergosterol
- Azoles bind to lanosterol 14α-demethylase inhibiting the production of ergosterol
  - Some cross-reactivity is seen with mammalian cytochrome p450 enzymes
    - Drug Interactions
    - Impairment of steroidneogenesis (ketoconazole, itraconazole)



Azoles: Mechanism of action

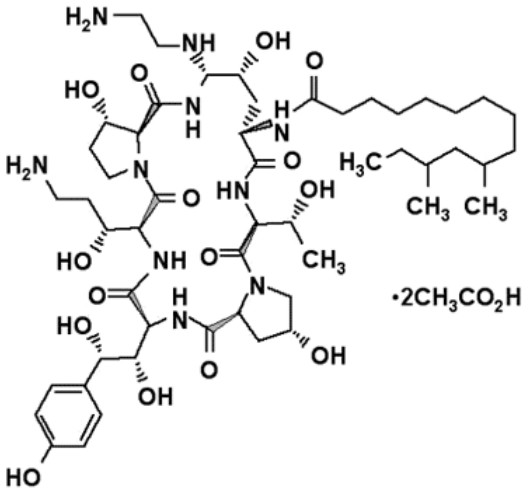


Mechanisms of Triazole Drug Resistance

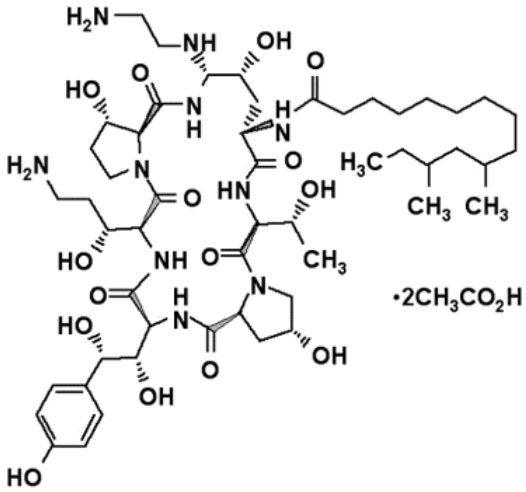


1. Erg11 mutations
2. Erg11 up-regulation
3. Multidrug transporter up-regulation
4. Alteration in ergosterol synthesis

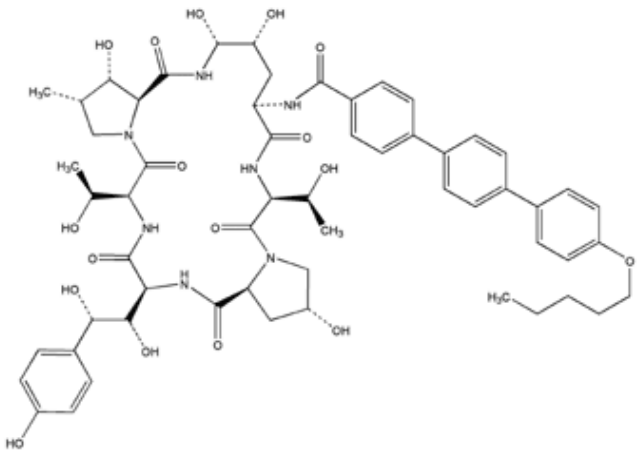
The Echinocandin Antifungals



Caspofungin

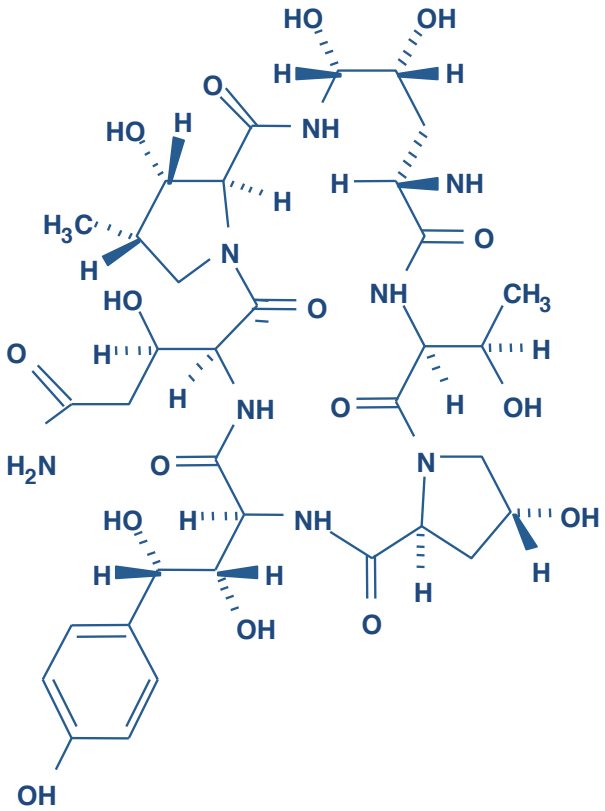


Micafungin



Anidulafungin

Echinocandins - Pharmacology

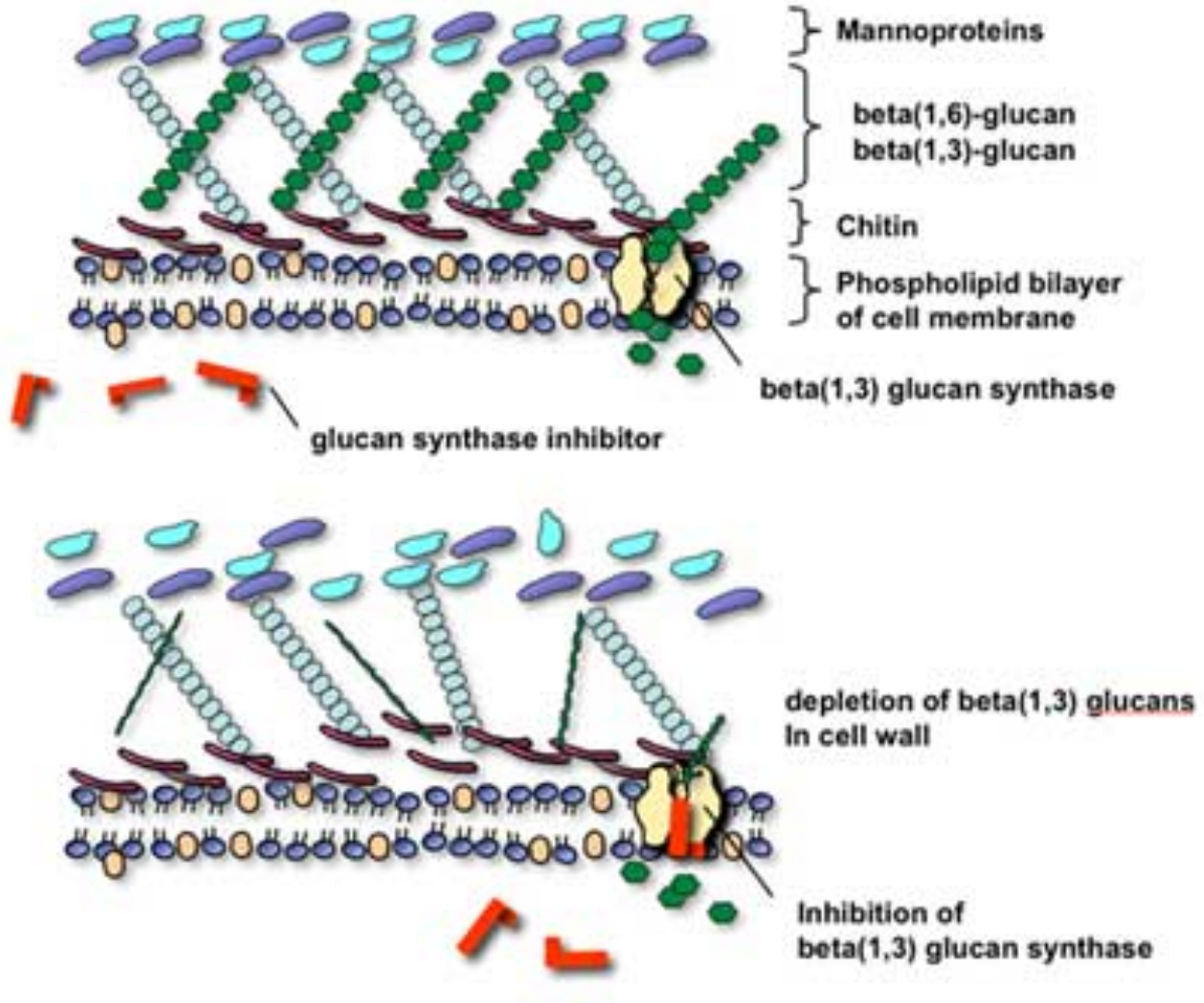


- Cyclic lipopeptide antibiotics that interfere with fungal cell wall synthesis by inhibition of ß-(1,3) D-glucan synthase
- Loss of cell wall glucan results in osmotic fragility

Spectrum:

- Candida *species including non-*albicans *isolates resistant to fluconazole*
- Aspergillus *spp. but not activity against other moulds (*Fusarium*,* Zygomycosis*)*
- No *coverage of* Cryptococcus neoformans

Echinocandins: Mechanism of Action



Echinocandins - spectrum

Highly active

Candida albicans,
Candida glabrata,
Candida tropicalis,
Candida krusei
Candida kefyr
Pneumocystis carinii

Low MIC ,with fungicidal activity and good in-vivo activity.

Very active

Candida parapsilosis
Candida gulliermondii
Aspergillus fumigatus
Aspergillus flavus
Aspergillus terreus
Candida lusitaniae

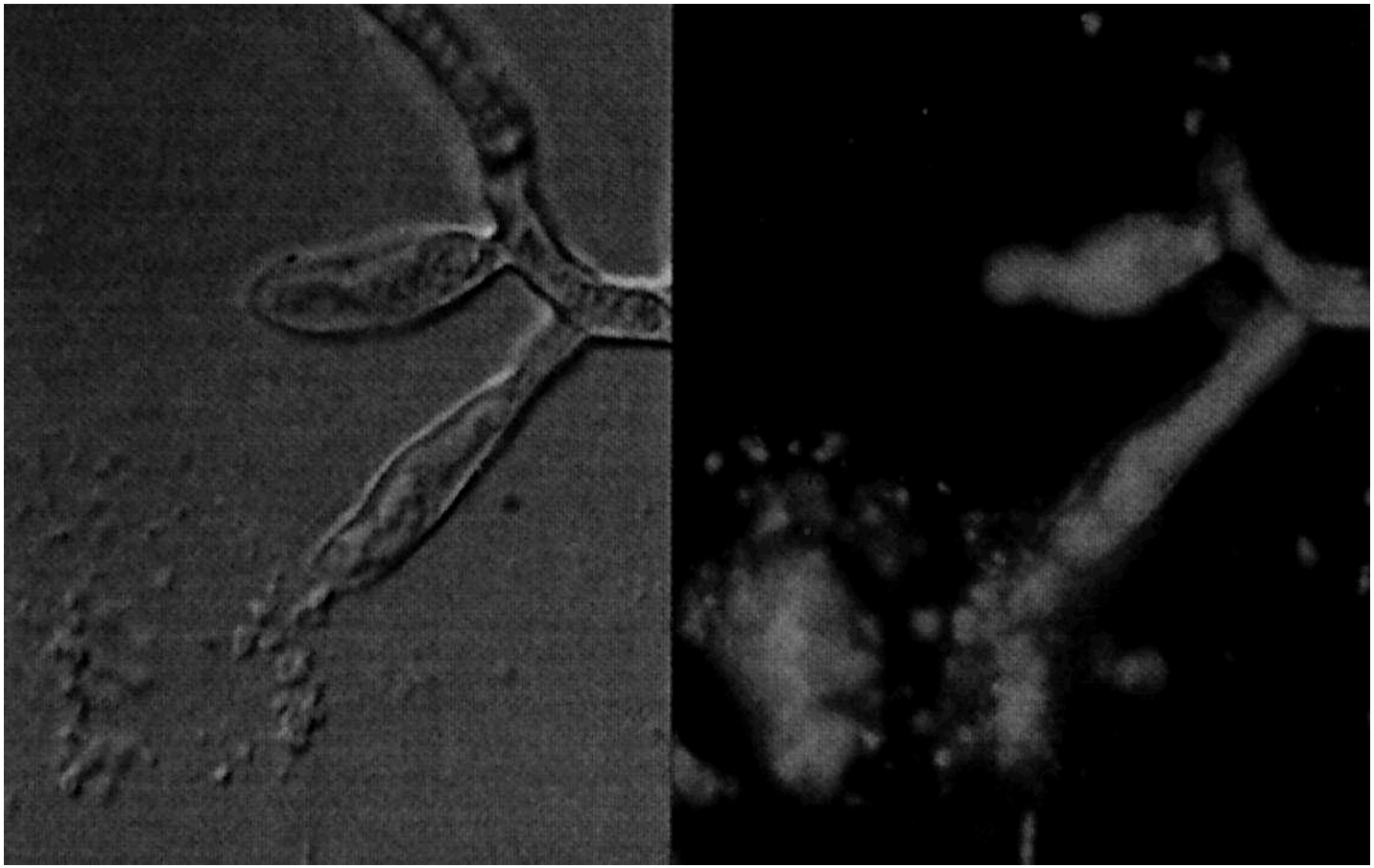
Low MIC, but without fungicidal activity in most instances.

Some activity

Coccidioides immitis
Blastomyces dermatididis
Scedosporium species
Paecilomyces variotii
Histoplasma capsulatum

Detectable activity, which might have therapeutic potential for man (in some cases in combination with other drugs).

Echinocandins act at the apical tips of *Aspergillus* hyphae



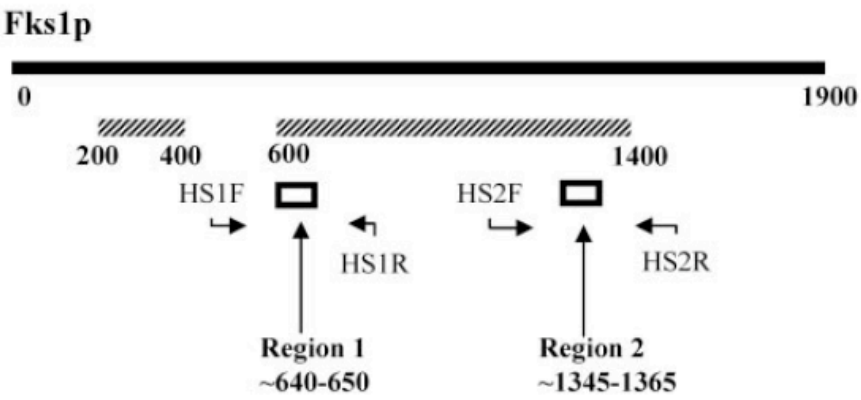
Bowman et al. *Antimicrob Agent Chemother* 2002;46:3001-12

Acquired Echinocandin Resistance

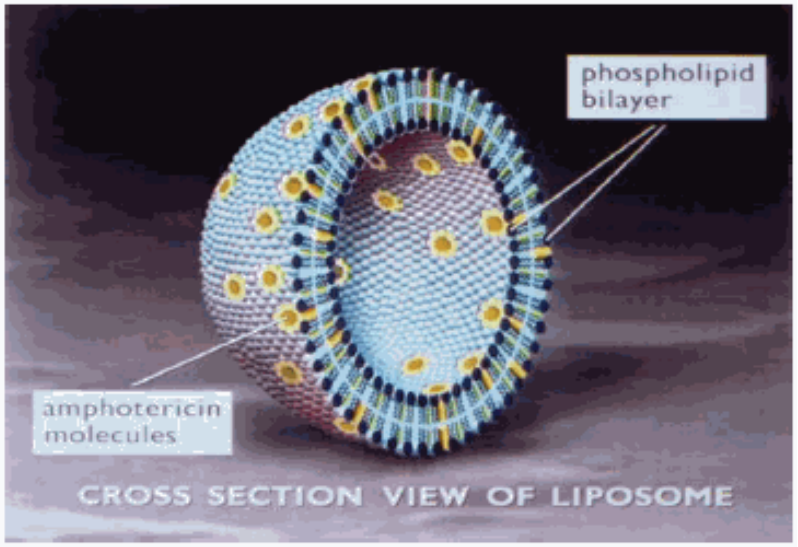
- In *Candida* species, emergent resistance has been detected.
- Found to be due to point mutations in the FKS1 gene
- FKS1 is a catalytic subunit of 1,3-D glucan synthase

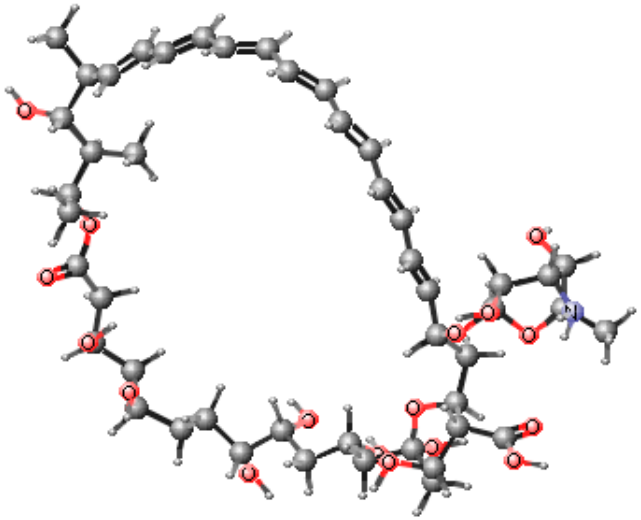
TABLE 3. Properties of *C. albicans* laboratory strains with reduced caspofungin susceptibility

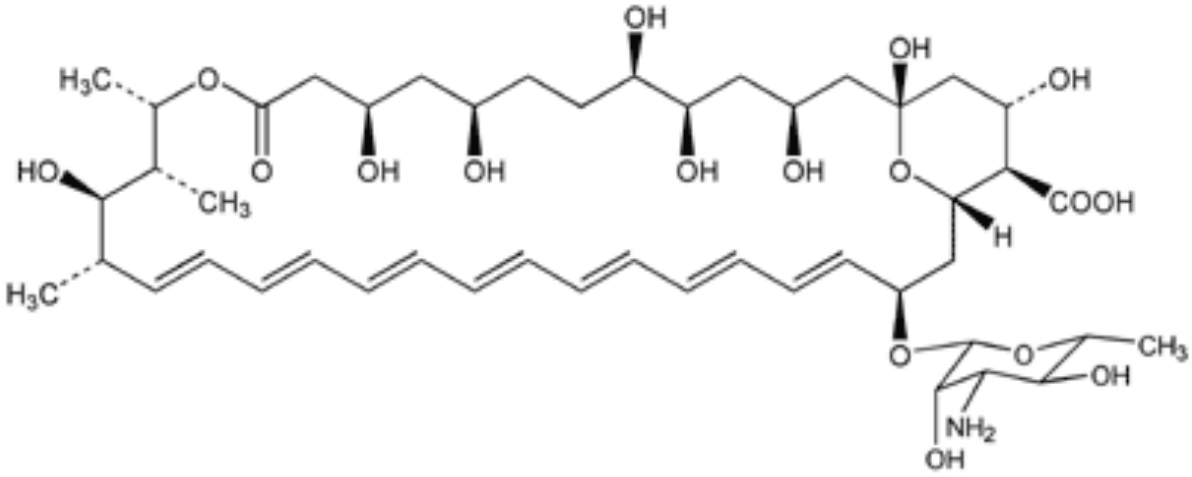
Strain	Fks1p mutation	MIC[a] (μg/ml) in:		Glucan synthesis IC_{50} (ng/ml)	Mouse ED_{90} (mg/kg)
		RPMI	AM3		
CAI4	S645/S645	0.12	<0.06	0.91	0.002
CAI4-R1	S645/P645	>32	>2	0.5 and 100	0.14
NR2	S645/P645	>32	>2	ND[b]	0.09
NR4	P645/S645	>32	1	0.5 and 100	0.07
NR3	Y645/Y645	>32	>2	2500	3.20
T25	Δ[c]/P645	>32	>2	133	3.39



The Polyene Antifungals







The Polyenes: Mechanism of Action

Polyene Antifungals: Intrinsic Resistance

- Frequently reported in:
  - *C. lusitaneae* and
  - *Trichosporon beiglii.*
- *Aspergillus terreus* generally resistant.
- *Scedosporium apiospermum* / *prolificans*.
- *Fusarium* spp.

Polyene Antifungals: Acquired Resistance

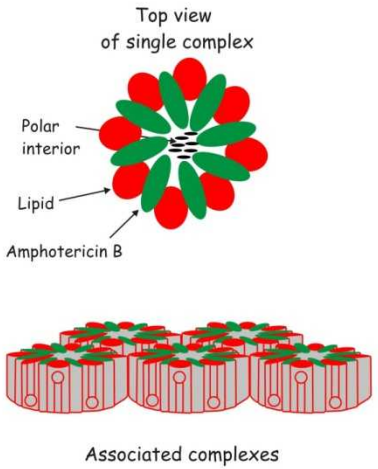
- Mechanisms not well described
- May be associated with defects in the ERG3 gene (ergosterol synthesis)
- Leads to accumulation of other sterols in the fungal membrane
- Resistant strains have low ergosterol content
- May also be mediated by increases catalase activity and reduction of oxidative stress.

Amphotericin B

- Polyene antibiotic
- Fermentation product of *Streptomyces nodusus*
- Binds sterols in fungal cell membrane
- Creates transmembrane channel and electrolyte leakage.
- Active against most fungi except *Aspergillus terreus*, *Scedosporium* spp.

Lipid Amphotericin B Formulations

Abelcet ® ABLC

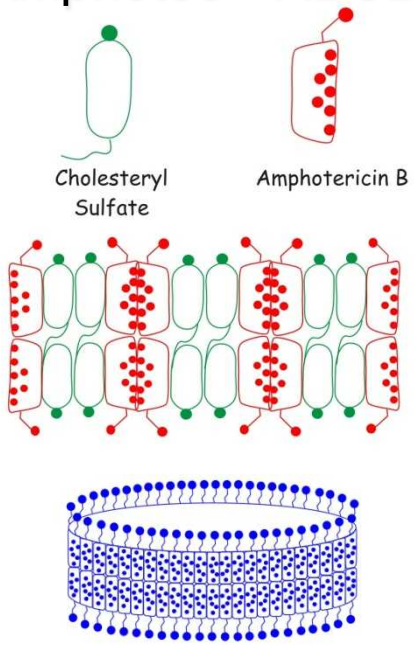


Ribbon-like particles

Carrier lipids: DMPC, DMPG

Particle size (µm): 1.6-11

Amphotec ® ABCD

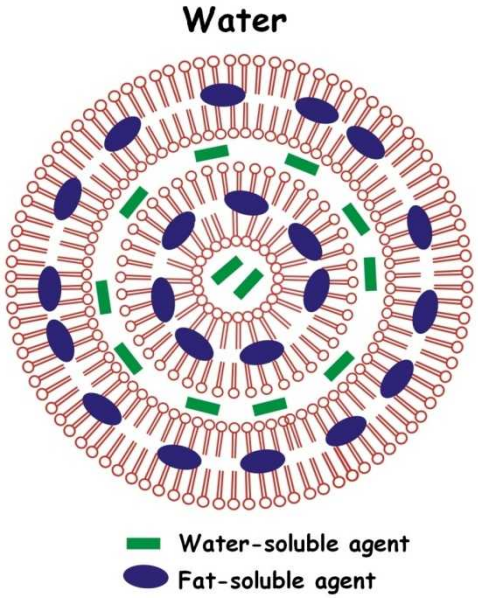


Disk-like particles

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 HSPC-Hydrogenated soy phosphatidylcholine
DMPG- Dimyristoyl phospitidylcglycerol DSPG-Distearoyl phosphitidylcholine

Amphotericin B

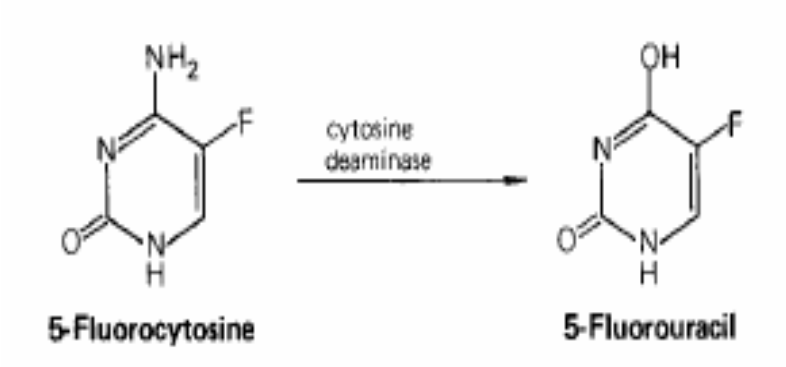
- Classic amphotericin B deoxycholate (Fungizone™) formulation: serious toxic side effects.
- Less toxic preparations:

1) Liposomal amphotericin B
2) Amphotericin B colloidal dispersion
3) Amphotericin B lipid complex

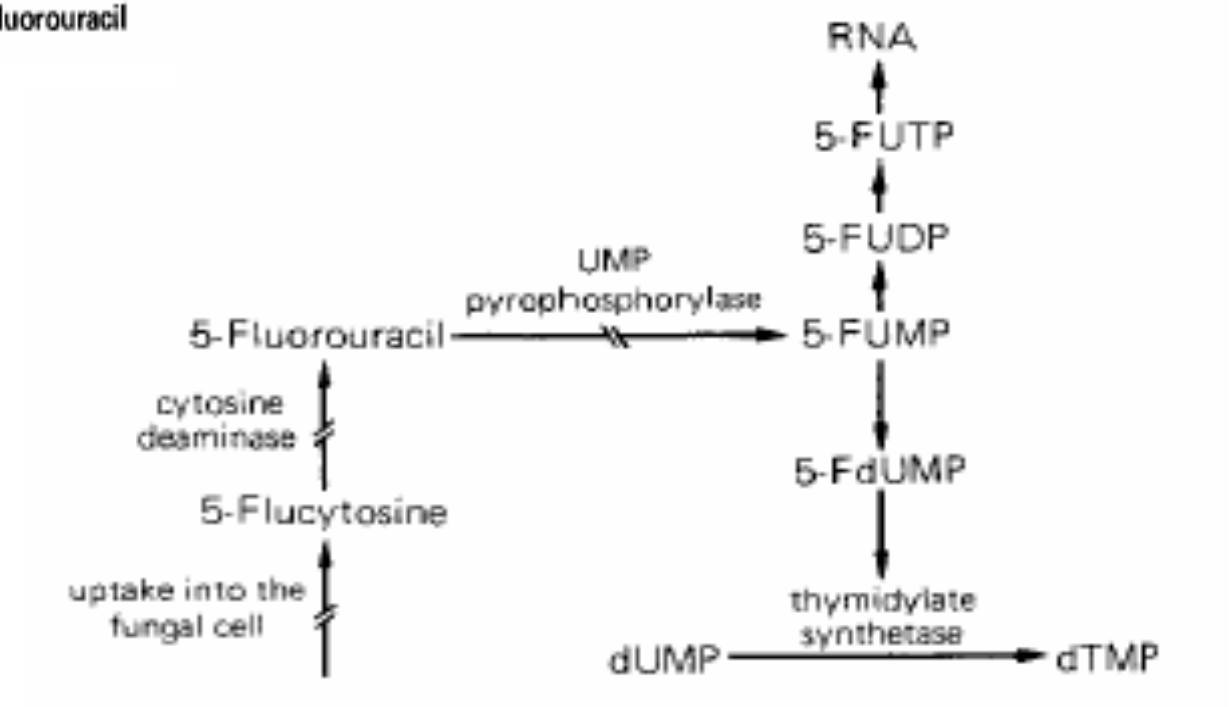
Amphotericin B - Nephrotoxicity

- Most significant delayed toxicity
- Renovascular and tubular mechanisms
  - Vascular-decrease in renal blood flow leading to drop in GFR, azotemia
  - Tubular-distal tubular ischemia, wasting of potassium, sodium, and magnesium
- Enhanced in patients who are volume depleted or who are on concomitant nephrotoxic agents

Flucytosine



Fluorinated pyrimidine related to flurouracil.



Flucytosine

- Restricted spectrum of activity.

- Acquired Resistance.
    > result of monotherapy
    > rapid onset

Due to:

1) Decreased uptake (permease activity)
2) Altered 5-FC metabolism (cytosine deaminase or UMP pyrophosphorylase activity)

Flucytosine – Clinical uses

Monotherapy : now limited

- Candidiasis
- Cryptococcosis
- ?Aspergillosis

} **In combination with amphotericin B or fluconazole.**

Thank you

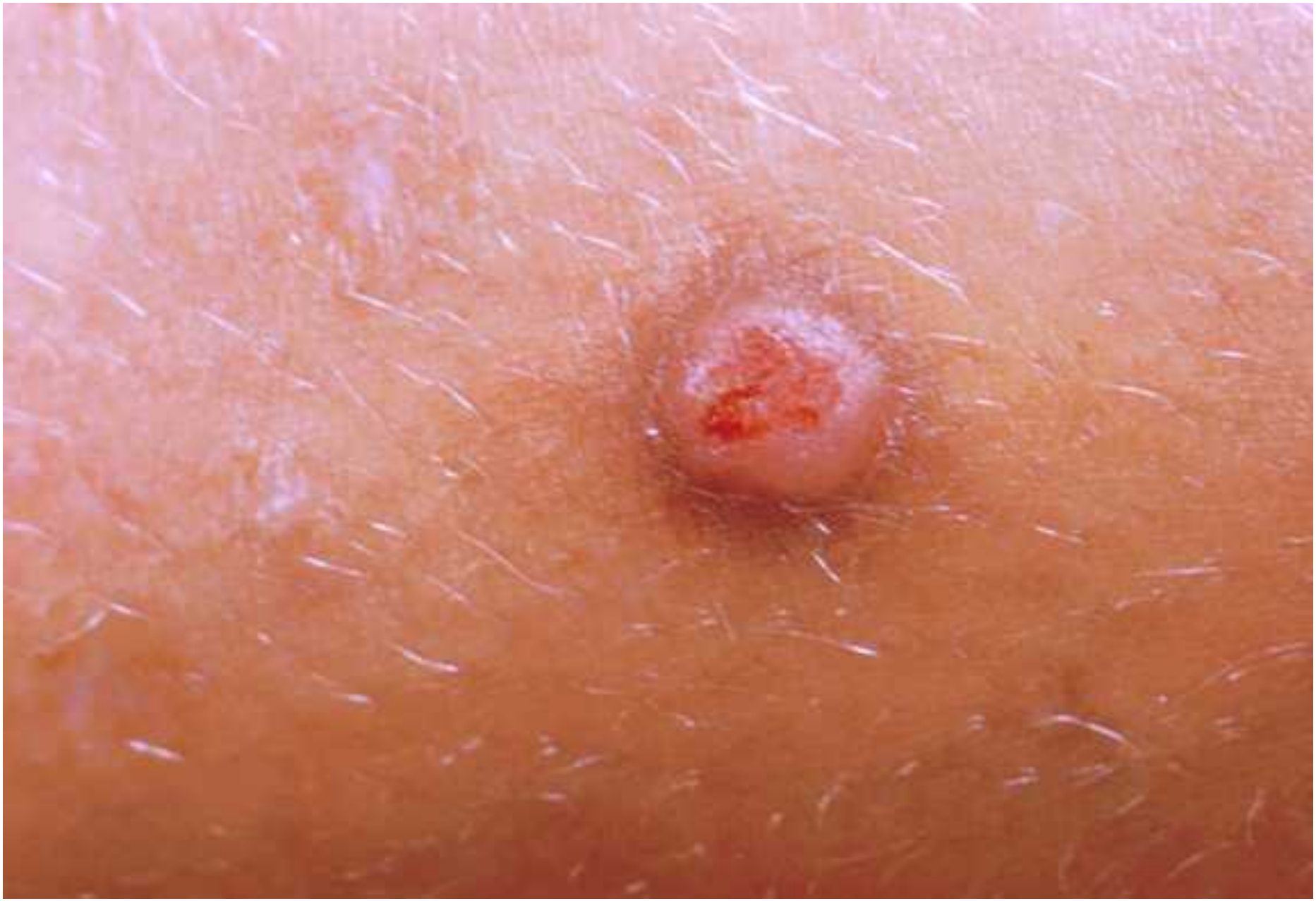
Flucytosine - pharmacokinetics

Oral absorption	complete
Plasma half-life	3-6 hrs
Volume of distribution	0.7-1l/kg (low)
Plasma protein binding	~12%

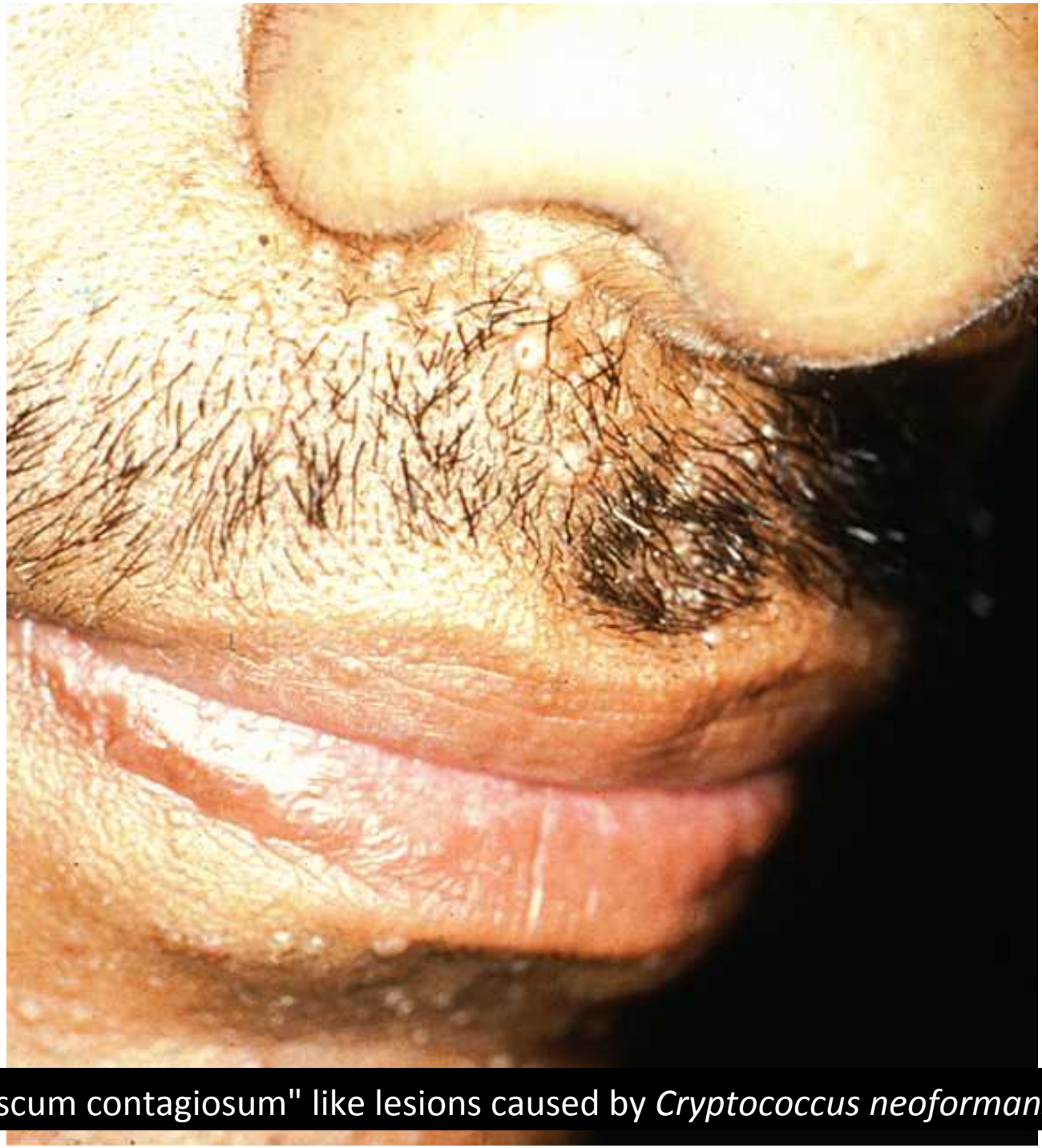
Flucytosine - side effects

- Infrequent – include D&V, alterations in liver function tests and blood disorders.

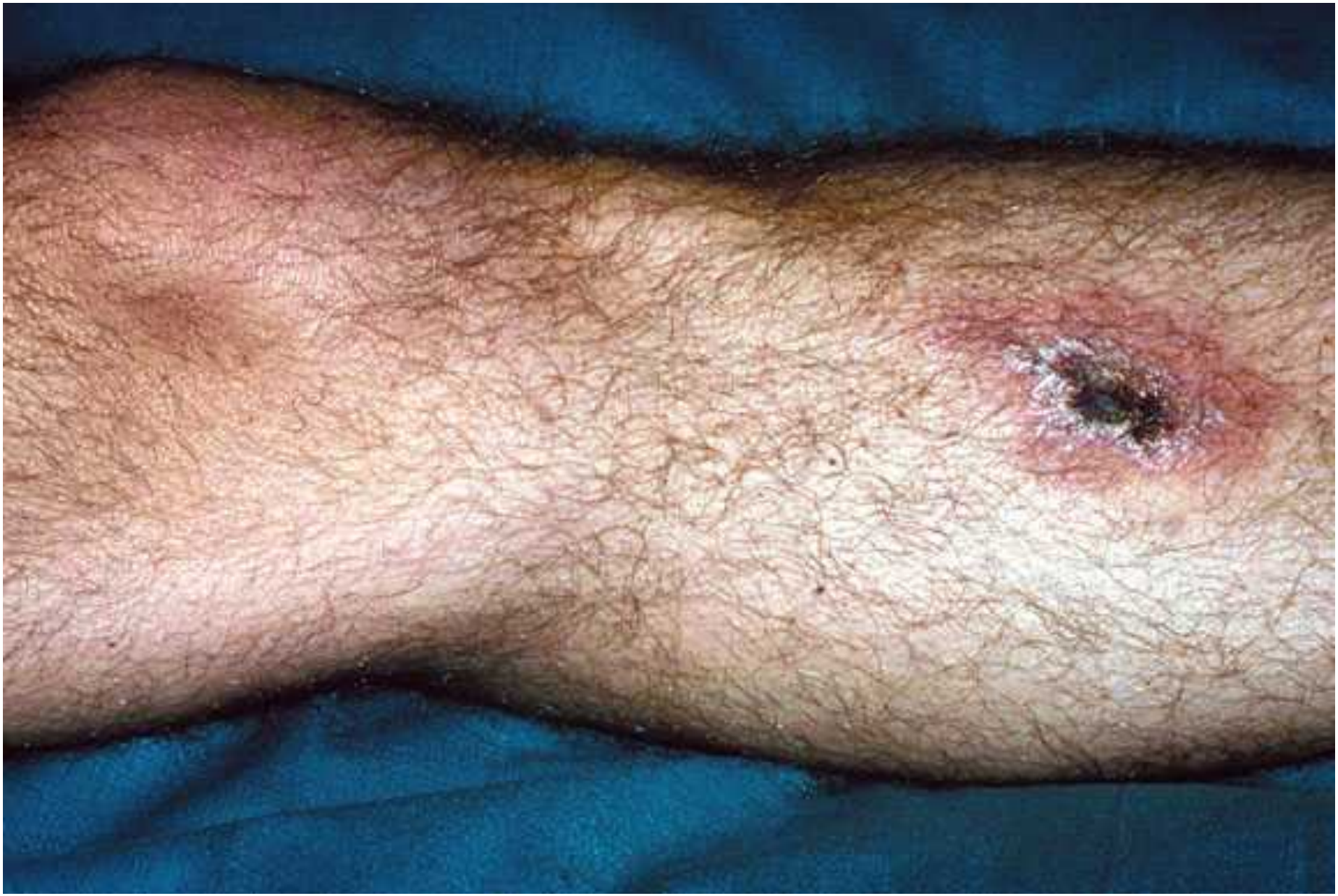
- Blood concs need monitoring when used in conjunction with Amphotericin B.



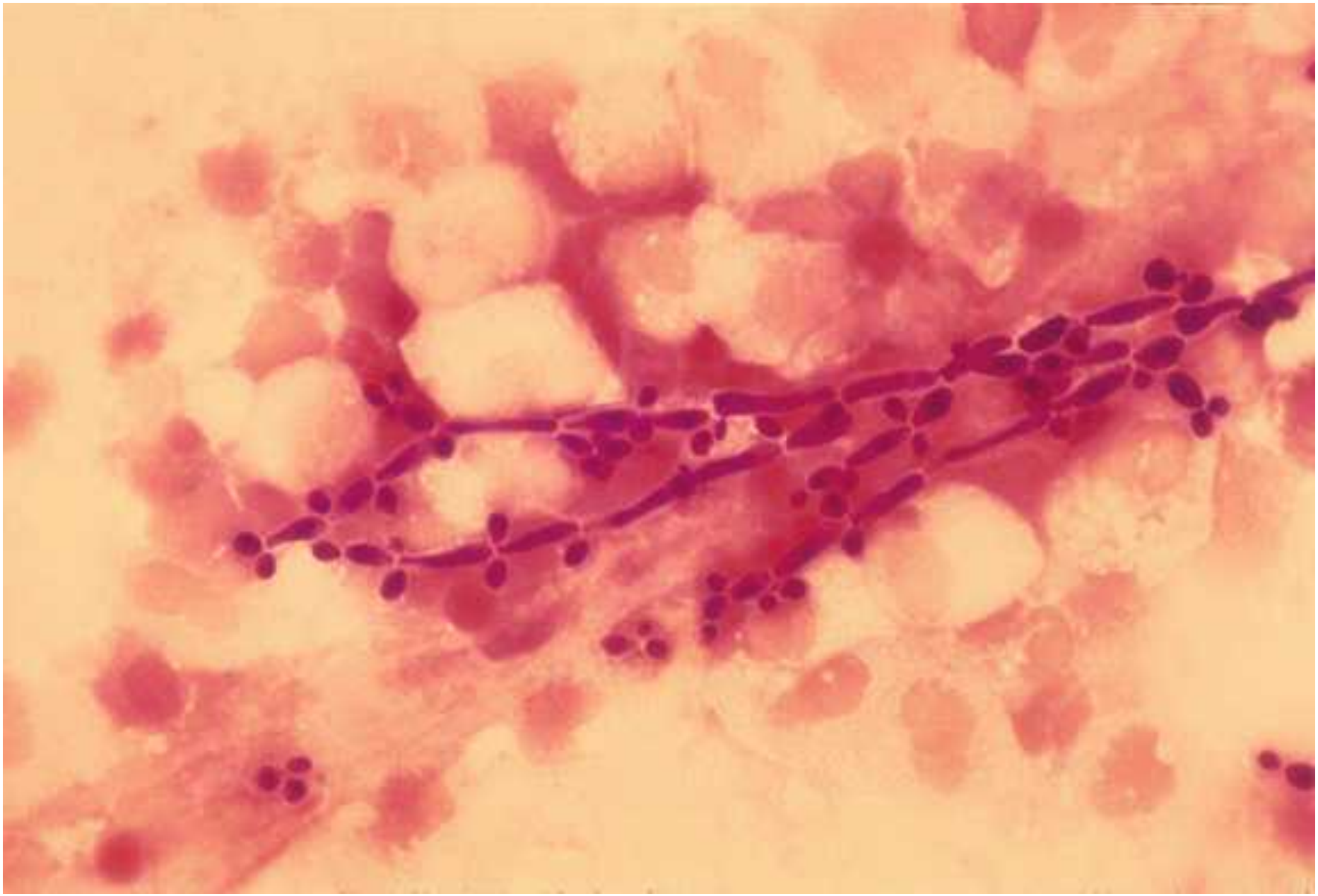
Primary cutaneous cryptococcosis from *Cryptococcus neoformans* var. *gattii*.



"Molluscum contagiosum" like lesions caused by *Cryptococcus neoformans*



Ulcerated skin lesion caused by *Cryptococcus neoformans* on the leg of an HIV+ patient



Direct smear of urine from a patient with candidiasis of the kidney