Antifungal Pharmacology

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Andy Alspaugh, MD
Sevtap Arikan, MD
John Rex, MD
www.doctorfungus.org
Objectives

• To recognize the clinical significance of fungal infections
• To identify the antifungal agents and their mechanisms of action
• To evaluate potential antifungal drug interactions and toxicities
• To differentiate the mechanisms of antifungal drug resistance
Lecture outline

- Clinical significance of fungal infection
- Fungal cell structure and targets
- Antifungal agents and mechanism of action
- Antifungal drug interactions & nephrotoxicity
- Mechanisms of antifungal resistance
- Summary
Mycology Resources

- http://www.doctorfungus.org
- http://mycology.cornell.edu/
- http://www.mycology.net/
Lecture outline

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Medical Problems Caused by Fungi

1) Allergic disease

2) “Mushroom” poisoning

3) Mycotoxins
   Secondary metabolites
   --many have industrial uses
   --Fusarium mycotoxin -- USSR after WWII
   --A. flavus “aflatoxin”

4) Mycoses -- infection and resulting disease cause by fungi
The Fungi

- 100,000 validly described species of fungi
- 150 primary human fungal pathogens
- Fungi yet to be discovered

-Kwon-Chung and Bennett, 1992
The Fungi

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150 primary human fungal pathogens

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100,000
Validly described species of fungi

150 primary human fungal pathogens

Fungi yet to be discovered

Candida,
Aspergillus,
Crypto, Blasto, Histo,
Cocci, Dermatophytes
Recent events in fungal diseases

Problems of today:

a. Growing population of immunocompromised
   i. Modern medical practices: transplantations, indwelling catheters, surgeries, anti-cancer therapies, broad-spectrum antibacterials, immunosuppressants; mycoses: especially candidiasis and aspergillosis
   ii. Natural diseases: AIDS; mycoses: especially candidiasis and cryptococcosis
   iii. Mean age of population is increasing; mycoses: especially candidiasis

b. Special problems associated with immunocompromised.
   i. Mycoses often are more severe, difficult to treat and diagnose.
   ii. Number of disease-causing fungi has increased.

c. Mobile population.
   i. People commonly travel through areas of endemic mycoses, which presents diagnostic challenges.

Advances in research:

a. New antifungal agents and treatment options

b. Tremendous increase in understanding of molecular basis of pathogenesis
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• Clinical significance of fungal infection
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Fungal cell

Cell membrane and cell wall

- Mannoproteins
- β-(1,6)-glucan
- β-(1,3)-glucan
- Chitin
- Phospholipid bilayer of cell membrane

Ergosterol

β-(1,3)-glucan synthase

Ergosterol Synthesis Pathway

Squalene

DNA/RNA Synthesis
ANTIFUNGAL DRUGS targets

- Membrane disrupting agents
  Amphotericin B, nystatin
- Ergosterol synthesis inhibitors
  Azoles, allylamines, morpholine
- Nucleic acid inhibitor
  Flucytosine
- Anti-mitotic (spindle disruption)
  Griseofulvin

- Glucan synthesis inhibitors
  Echinocandins
- Chitin synthesis inhibitor
  Nikkomycin
- Protein synthesis inhibitors
  Sordarins, azasordarins
Lecture outline

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ANTIFUNGAL DRUGS classes

- **POLYENES**
  - Amphotericin B, nystatin

- **AZOLES**
  - Imidazoles: Ketoconazole
  - Triazoles: Fluconazole, itraconazole, voriconazole, posaconazole, ravuconazole

- **ALLYLAMINES**
  - Terbinafine, butenafine

- **MORPHOLINE**
  - Amorolfine

- **FLUORINATED PYRIMIDINE**
  - Flucytosine

- **ECHINOCANDINS**
  - Caspofungin, anidulafungin, micafungin

- **PEPTIDE-NUCLEOSIDE**
  - Nikkomycin Z

- **TETRAHYDROFURAN DERIVATIVES**
  - Sordarins, azasordarins

- **OTHER**
  - Griseofulvin
Polyenes

Cholesterol
Ergosterol
amphotericin B
Nystatin A₁
Binding to ergosterol, Intercalation of cell membrane

Leakage of intracellular cations and proteins
Amphotericin B

- **Mechanism**: binds sterols, preferentially ergosterol, and disrupts osmotic integrity of cell membrane

- **Complications**: fever, chills, myalgia, nephrotoxicity, thrombophlebitis

- **Pharmacokinetics**: poorly soluble in water
  - rapid uptake by RES, then redistributed
  - four formulations
    - ampho B colloidal dispersion (ABCD; Amphotec)
    - amphotericin B lipid complex (ABLC; Abelcet)
    - liposomal amphotericin B (L-AMB; Ambisome)
    - oral amphotericin B (poor absorption)

- **Indications**: broad range of activity, ABCD is mainstay of antifungal therapy
Azoles, allylamines & morpholines

ergosterol synthesis inhibitors

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Enzyme</th>
<th>Sterol Intermediate</th>
<th>Inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERG1</td>
<td>Squalene epoxidase</td>
<td>Squalene</td>
<td>Allylamines, Thiocarbamates</td>
</tr>
<tr>
<td>ERG7</td>
<td>Lanosterol Synthase</td>
<td>2,3-Oxidosqualene</td>
<td></td>
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<tr>
<td>ERG11</td>
<td>Lanosterol (C-14) Demethylase</td>
<td>Lanosterol</td>
<td>Azoles</td>
</tr>
<tr>
<td>ERG24</td>
<td>C-14 Sterol Reductase</td>
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<td>Morpholines</td>
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<td>ERG25</td>
<td>C-4 Sterol Demethylase Enzymes</td>
<td></td>
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</tr>
<tr>
<td>ERG6</td>
<td>C-24 Sterol Methyltransferase</td>
<td>Zymosterol</td>
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</tr>
<tr>
<td>ERG2</td>
<td>C-8 Sterol Isomerase</td>
<td>Fecosterol</td>
<td>Morpholines</td>
</tr>
<tr>
<td>ERG3</td>
<td>C-5 Sterol Desaturase</td>
<td>Episterol</td>
<td></td>
</tr>
<tr>
<td>ERG5</td>
<td>C-22 Sterol Desaturase</td>
<td></td>
<td>Azoles (?)</td>
</tr>
<tr>
<td>ERG4</td>
<td>C-24 Sterol Reductase</td>
<td>Ergosterol</td>
<td></td>
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</table>

Clin Microbiol Rev
1998; 11: 382
Azoles

fluconazole

itraconazole

voriconazole

posaconazole
Cell membrane

Ergosterol

Ergosterol Synthesis Pathway

Squalene

Accumulation of toxic sterols in cell membrane

Inhibition of 14-alpha-demethylase

Azole

Toxic sterols
Azoles

• **Mechanism**: block ergosterol synthesis via inhibition of cytochrome P450 dependent 14α-demethylase (Erg11)

• **Complications**: well-tolerated, hepatotoxicity, hypertension, headache, visual disturbances, resistance

• **Formulations**: poorly soluble in water, fungistatic
  - Fluconazole (Diflucan)
  - Voriconazole (Vfend)
  - Ravuconazole
  - Itraconazole (Sporanox)
  - Posaconazole
  - Ketoconazole (Nizoral)

• **Indications**: *Candida*, *Cryptococcus*, *Coccidioides*, *Histoplasma*, *Blastomyces*, some *Aspergillus* spp.
## Azoles, allylamines & morpholines

**Ergosterol synthesis inhibitors**

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*Clin Microbiol Rev*  
1998; 11: 382
**Allylaminones, morpholines**

- **Amorolfine**
- **Terbinafine**
- **Butenafine**
Allylamines, morpholines

- **Mechanism**: block ergosterol synthesis via inhibition of squalene epoxidase (allylamines), sterol reductase and isomerase activity (morpholines)

- **Complications**: mild gastrointestinal and skin reactions

- **Formulations**: poorly soluble in water, oral and topical, fungicidal
  - Terbinafine (Lamisil)
  - Amorolfine (Loceryl)
  - Butenafine (Mentax)

- **Indications**: dermatophytes, *Candida* (Mentax)
Echinocandins

- micafungin
- caspofungin
- anidulafungin
Mannoproteins

β(1,6)-glucan
β(1,3)-glucan

Chitin

Phospholipid bilayer of cell membrane

β(1,3) glucan synthase inhibitor

β(1,3) glucan synthase

Depletion of β(1,3) glucans in cell wall

Inhibition of β(1,3) glucan synthase
Echinocandins

- **Mechanism**: block cell wall synthesis via $\beta$-1,3 glucan synthesis inhibition

- **Complications**: well-tolerated, histamine release, no activity against *Cryptococcus*, *Fusarium* spp.

- **Formulations**: poorly soluble in water, fungicidal
  - Caspofungin (Cancidas)
  - Micafungin
  - Anidulafungin (Eraxis)

- **Indications**: *Candida*, *Aspergillus* spp.
Antimetabolites

flucytosine

griseofulvin
5-FC, 5-fluorocytosine; 5-FU, 5-fluorouracil; FdUMP, 5-fluorodeoxyuridine; FUMP, 5-fluorouridine monophosphate; FUDP, 5-fluorouridine diphosphate; FUTP, 5-fluorouridine triphosphate; dUMP, deoxyuridine monophosphate; dTMP, deoxythymidine monophosphate
Antimetabolites

• **Mechanism**: block fungal DNA and protein synthesis (Flucytosine), fungal mitosis (Griseofulvin)

• **Complications**: GI intolerance, bone marrow suppression, hepatotoxicity, headache, hallucinations, sedation, nausea

• **Formulations**: poorly soluble in water
  - Flucytosine (Ancobon)
  - Griseofulvin (Grifulvin V, Fulvicin U/F, Grisactin, Peninol)

• **Indications**: (Flucytosine): for resistant *Candida*, *Aspergillus* spp. and in combination with Ampho B for *Cryptococcus*; (Griseofulvin): dermatophytes
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Antifungal drug interactions

• Pharmacokinetic interactions: changes in absorption or elimination of interacting drug and the antifungal
  • Interactions of drug absorption
    • Ketoconazole, itraconazole require low pH for absorption (avoid antacids, vitamin supplements)
    • Pre-systemic clearance via membrane transporters (P-gp) & metabolic enzymes. Azoles can be both substrates and inhibitors of P-gp
  • Interactions of drug metabolism
    • Oxidation, reduction, hydrolysis, conjugation of lipophilic compounds
  • Interactions with cytochrome P450
    • Azoles are metabolized by CYP P450 system
    • Azoles are also reversible inhibitors of P450 enzymes
    • Co-administered metabolites are a concern
Azoles: Interactions in the GI Tract

Drug-pH

Portal vein

Absorption

Liver

Metabolism

To feces

Gut wall

P-gp efflux

Bioavailability
Antifungal drug interactions

- Pharmacokinetic interactions: changes in absorption or elimination of interacting drug and the antifungal
  
  - Interactions of drug absorption
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Proportion of Drugs Metabolized by CYP P450
Antifungal drug interactions

• Pharmacokinetic interactions: changes in absorption or elimination of interacting drug and the antifungal
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  • Oxidation, reduction, hydrolysis, conjugation of lipophilic compounds

• Interactions with cytochrome P450
  • Azoles are metabolized by CYP P450 system
  • Azoles are also reversible inhibitors of P450 enzymes
  • Co-administered metabolites are a concern
Inducers of CYP 3A4

Rifampin, Phenytoin, Carbamezepine, Phenobarbital

Ritonovir?

Reduction in levels:
- Ketoconazole (>90%)
- Fluconazole (~50%)
- Itraconazole (>90%)
- Voriconazole (~90%)
Azole Inhibition of CYP P450

Increased serum concentration of co-administered drug or metabolite
- Oral hypoglycemics
  - S-warfarin
  - R-Wafarin
- Cyclosporin
- Tacrolimus
- Sirolimus
- Phenytoin
- Carbamezepine
- Triazolam, alprazolam, midazolam
- Diltiazem
- Lovastatin
- Isoniazid
- Rifabutin
- Quinidine
- Protease inhibitors (saquinavir, ritonavir)
- Busulfan
- Vincristine
- Cyclophosphamide
- Digoxin
- Loratidine
- and others…
Nephrotoxicity

• Primarily due to Amphotericin B
• Two mechanisms:
  • Effects of ampho B on renal blood flow and glomerular filtration
    • Constriction of afferent arterioles decreases renal blood flow and GFR
    • Subsequent increase in serum creatinine and BUN
  • Direct toxic effect on distal tubules via membrane disruption
    • Cholesterol target
Constriction of the afferent arterioles leading to decreased glomerular filtration

Direct damage of distal tubular membranes leading to wasting of Na⁺, K⁺, and Mg²⁺

Tubular-glomerular feedback: Further constriction of arterioles
Lecture outline

• Clinical significance of fungal infection
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• Mechanisms of antifungal resistance
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Clinical Resistance is a Multifactorial Issue

<table>
<thead>
<tr>
<th>• <strong>HOST</strong></th>
<th>• <strong>FUNGUS</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune status</td>
<td>Initial MIC</td>
</tr>
<tr>
<td>Site of infection</td>
<td>Cell type: Yeast/hyphae..</td>
</tr>
<tr>
<td>Severity of infection</td>
<td>Genomic stability</td>
</tr>
<tr>
<td>Foreign devices</td>
<td>Biofilm production</td>
</tr>
<tr>
<td>Noncompliance with drug regimen</td>
<td>Population bottlenecks</td>
</tr>
</tbody>
</table>

• **DRUG**
- Fungistatic nature
- Dosing
- Pharmacokinetics
- Drug-drug interactions
Antifungal-drug-resistance mechanisms

Increased efflux
Overexpression of ABC (ATP-binding cassette) transporters or major facilitator proteins in the cell membrane. Often caused by mutations in transcriptional regulators. Represents a broad mutational target, as many non-synonymous changes in the regulators confer a resistant phenotype. See the second case study in BOX 3 for the example of the transcriptional regulator PDR1 of Saccharomyces cerevisiae.

Alteration of target enzyme
Changes in target protein either prevent binding of the antifungal drug or prevent the allostERIC inactivation of the target after the inhibitor binds. This is a relatively small mutational target, as only a few specific amino-acid changes confer resistance. Alternatively, the target protein might be overexpressed, resulting in sufficient activity in the presence of the drug.

Alteration of metabolism
Loss of enzyme activity prevents the accumulation of a toxic product in the presence of the drug. This is a relatively broad mutational target, as myriad amino-acid changes result in loss of function and a resistant phenotype. See experiment 2 (BOX 4) for the example of the loss of function in the sterol-biosynthesis gene ERG3 of S. cerevisiae.

Several excellent reviews\textsuperscript{1,3,4} have documented these mechanisms in detail. Known mechanisms of resistance do not account for all observed resistance. Additional mechanisms undoubtedly await discovery.
Resistance to Amphotericin B

- Technical difficulties in detection of resistance in vitro
- In vivo resistance is rare

\[ \text{C. lusitaniae, C. krusei} \]
\[ \text{C. neoformans} \]
\[ \text{Trichosporon spp.} \]
\[ \text{A. terreus} \]
\[ \text{S. apiospermum} \]
\[ \text{Fusarium spp.} \]
Mechanisms of Amphotericin B resistance

- Reduced ergosterol content (defective ERG2 or ERG3 genes)
- Alterations in sterol content (fecosterol, episterol: reduced affinity)
- Alterations in sterol to phospholipid ratio
- Reorientation or masking of ergosterol
- Stationary growth phase
- Previous exposure to azoles
- (?)
## Resistance to Azoles

- Well-known particularly for fluconazole
- Data available also for other azoles
- A significant clinical problem

### Resistance to Fluconazole

<table>
<thead>
<tr>
<th>PRIMARY</th>
<th>C. krusei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus</td>
<td></td>
</tr>
<tr>
<td>C. glabrata</td>
<td></td>
</tr>
<tr>
<td>C. norvegensis</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SECONDARY</th>
<th>C. albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. dubliniensis</td>
<td></td>
</tr>
</tbody>
</table>
Azole Resistance

• Single point mutation of ERG11 gene
  Altered lanosterol demethylase

• Overexpression of ERG11 gene
  Increased production of lanosterol demethylase

• Alterations in ERG3 or ERG5 genes
  Production of low affinity sterols

• Increase in mRNA levels of CDR1 or MDR1 genes
  Decreased accumulation of theazole in fungal cell

• Changes in sterol and/or phospholipid composition of fungal cell membrane (decreased permeability)
If your fungus is susceptible to azoles..

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If it isazole-resistant..
Resistance to Flucytosine

- **PRIMARY** non-albicans Candida
  - *C. neoformans*
  - *Aspergillus* (highest)

- **SECONDARY** *C. albicans*
  - *C. neoformans*

⚠️ Secondary resistance develops following flucytosine MONOtherapy.
Mechanisms of Resistance to Flucytosine

• Loss of permease activity

• Loss of cytosine deaminase activity

• Decrease in the activity of UPRTase
Resistance to Echinocandins

**PRIMARY**  
*C. neoformans*  
*Fusarium spp.*

**SECONDARY**  
(?) *Candida spp.*
Echinocandin Resistance
Molecular Aspects

• FKS1 encodes glucan synthase
• GNS1 encodes an enzyme involved in fatty acid elongation
• Resistance is observed following laboratory derived mutations in FKS1 or GNS1

• Other mechanisms (?)
Future Directions to Avoid Development of Resistance

• Proper dosing strategies
• Combination therapies
• Restricted and well-defined indications for prophylaxis with azoles

中美 Fungi will continue to develop NEW resistance mechanisms!..
Lecture outline

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Yeast in blood culture

Immunocompromised (transplant, BMT, AIDS)
- Start (lipid) polyene and wait for ID
  - Endemic mycosis
    - Continue (lipid) polyene
      - Until stable, then consider fluconazole or itraconazole as appropriate
  - Candida
    - Hemodynamically stable, no previous azoles
      - Start fluconazole, wait for ID and monitor response
        - If good response complete 14 days from first negative culture
        - If no response or clearly resistant isolate
    - Hemodynamically unstable previous azoles
      - Start echinocandin or (lipid) polyene, wait for ID and monitor response
        - If good response complete 14 days from first negative culture, may switch to fluconazole or voriconazole if stable and susceptible
        - If no response switch to other agent from the above classes

“Non-immunocompromised”
This AIDS patient failed fluconazole, amphotericin B, and itraconazole…

**Echinocandins: No cross-resistance**

**Baseline**

**After caspofungin**

Courtesy of John Rex, MD
# Recommendations

<table>
<thead>
<tr>
<th>Challenge</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid and accurate diagnosis is important, but difficult to achieve</td>
<td>Clinicians should aggressively pursue an accurate diagnosis, but pre-emptive therapy based on clinical criteria and diagnostics can be helpful</td>
</tr>
<tr>
<td>Too much or too little of a host immune response can be problematic</td>
<td>Individualized approaches are necessary to balance restoration of host immunity with risks of immune reconstitution inflammatory syndrome</td>
</tr>
<tr>
<td>Poor drug bioavailability, pharmacokinetic variability, drug interactions and toxicities can contribute to clinical failures</td>
<td>Clinicians should anticipate and manage these problems proactively to reduce risks of drug failure, toxicity and resistance</td>
</tr>
<tr>
<td>Removal of infected tissue can be critical to achieving clinical success, particularly in mould infections</td>
<td>Not all patients are candidates for surgery, but debridement of devitalized tissue and debulking large fungal burdens may be helpful for patients with mould infections</td>
</tr>
<tr>
<td>For many patients, fungal infection is a catastrophic event that renders them unable to receive treatment for their underlying disease such as chemotherapy</td>
<td>Prophylaxis with antifungal agents should be considered for high-risk patients. If break-through infections occur, an accurate diagnosis should be aggressively pursued and consistent antifungal drug strategies employed</td>
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<td>Combination therapy of invasive fungal infections is attractive from the perspective of synergistic potential, relative safety, and lack of overlapping toxicities. Randomized, controlled clinical trial data of combination antifungal therapy for mould infections are lacking</td>
<td>Single agents have been effective in treating the majority of invasive fungal infections if the patient’s underlying disease can be controlled; combination therapy with amphotericin B and flucytosine should be routinely employed in patients with cryptococcal meningitis; combination therapies for other fungal infections should be considered on a case-by-case basis until additional studies demonstrate benefits of this approach</td>
</tr>
</tbody>
</table>

**Combination antifungal therapy: what can and should we expect?**

MD Johnson¹ ² and JR Perfect¹ ²

¹Division of Infectious Diseases, Department of Medicine, Duke University Medical Center, Durham, NC, USA and ²Campbell University School of Pharmacy, Buies Creek, NC, USA
Ideal antifungal?
Not yet...

<table>
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<tr>
<td>The Holy Grail. Characteristics of the Ideal Antifungal Agent</td>
</tr>
</tbody>
</table>

- Broad antifungal activity
- Fungicidal
- Low frequency of resistance
  - Intrinsic resistance
  - Acquired resistance
- Available in IV and PO preparations
  - Ease of administration
- Low frequency of adverse events
- Minimal drug interactions
- Reasonable cost
The future of antifungals

TABLE 4
Search for the Ideal Antifungal Agent

<table>
<thead>
<tr>
<th>Area</th>
<th>Details</th>
</tr>
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<tbody>
<tr>
<td>A more complete understanding of the pathogenesis of invasive fungal infections</td>
<td>Virulence factors (e.g., adhesins) as targets for new antifungals and vaccines</td>
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<td>Identification of new compounds (natural and synthetic)</td>
<td>Identification of new targets by genomic analysis (signature)</td>
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<td>Traditional screening against fungal pathogens</td>
<td>Enhance efficacy and reduce toxicity of older antifungal drugs</td>
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<tr>
<td>Improved delivery systems (Lipid AMB, aerosols)</td>
<td>High-throughput and reproducible susceptibility testing</td>
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<td>A better understanding of the mechanisms of resistance to antifungal drugs</td>
<td>Combination therapy - synergy</td>
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<tr>
<td>Intrinsic vs Acquired</td>
<td>AMB + 5FC for cryptococcal meningitis</td>
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<tr>
<td></td>
<td>AMB + voriconazole for aspergillosis</td>
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<td></td>
<td>Echinocandins + voriconazole for filamentous moulds</td>
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<tr>
<td>Immune enhancement therapy</td>
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<tr>
<td>Monoclonal antibodies</td>
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<td>Manan binding lectins replacement</td>
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The effectiveness of the azole class of antifungals is based upon what structural feature of the fungus?

A. the chitin composition of the cell wall  
B. the cholesterol composition of the cell membrane  
C. the protein components of the cell membrane  
D. the ergosterol composition of the cell membrane
Fig. 1. Timeline of development of antifungal agents.