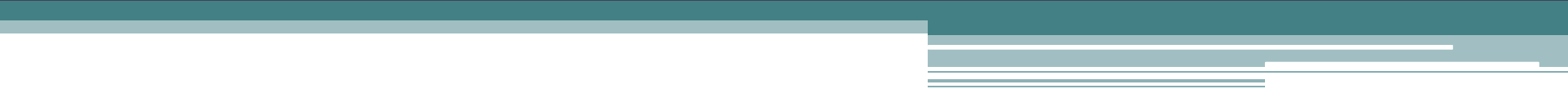


Relevance of Anti Fungals in ICU

A series of horizontal lines in teal and light blue colors, with varying lengths and offsets, creating a modern, layered effect across the middle of the slide.

Introduction

- Fungal infections are a worldwide global health problem.
- ≈ 1.5 million/year have disseminated or invasive fungal infections (IFIs), requiring advanced treatment and hospitalization
- Few fungal infections having mortality rates $\approx 90\% - 95\%$



Fungal infections

- Accounts for 10 % of all nosocomial infections.
- Fungal infections are difficult to diagnose because clinical symptoms, signs, and radiographic manifestations are unspecific.
- Immunocompromised hosts are at an increased risk of suffering from fungal infections

Eg. Organ transplant recipients
Lung diseases.



Common Fungal Pathogens

- The most important fungal pathogens are
 - *Aspergillus species*,
 - *Candida species*
 - *Cryptococcus species*,
 - *Pneumocystis jirovecii* , and
 - *Mucormycetes* (formerly *Zygomycetes*), with *Rhizopus species*, *Mucor species*, and *Lichtheimia species*



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Characteristics of fungal infections worldwide

Body location	Pathogen type	Organ	Most frequent genus	Estimated incidence of infection*
superficial	primary	Skin and hair	<i>Malassezia</i>	~140,000,000 cases/year
cutaneous	primary	Skin and nails	Trichophyton <i>Epidermophyton</i> <i>Microsporum</i>	~1,500,000,000 cases/year
mucosal	opportunistic	Vagina, digestive tract, urinary tract and eye	<i>Candida</i>	~75,000,000 cases/year ~9,500,000 cases/year
			<i>Aspergillus, Fusarium</i>	~1,000,000 cases/year
systemic	opportunistic	any organ (lungs, brain, bloodstream etc.)	<i>Candida</i>	~300,000 cases/year
			<i>Aspergillus</i>	~350,000 cases/year
			<i>Cryptococcus</i>	~1,000,000 cases/year
			<i>Histoplasma</i>	~500,000 cases/year
			<i>Pneumocystis</i>	>200,000 cases/year
			<i>Coccidioidomyces</i> and so on	up to 300,000 cases/year

Overview of laboratory methods available for the diagnosis of fungal diseases

- **Conventional methods**
 - Direct microscopy (KOH/Calcofluor white stains, Giemsa)
 - Histopathology
 - Culture
- **Serology**
 - Galactomannan test for *Aspergillus species*
 - Mannan test for *Candida species*
 - Antibody test for aspergillosis in immunocompetent hosts
 - β -1-3 D glucan panfungal test
 - Lateral device flow assays for *Aspergillus and Cryptococcus species*
 - Antigen and antibody based assays for non-European mycoses
- **Molecular methods**
 - Fluorescence *in situ* hybridization test
 - PCR assays

Specimens applied for diagnosing fungal infections

Assay	Specimens	Goal	Results	Time
Potassium hydroxide solution (KOH)	Skin scrapings, hair or nail clippings, tissue, vaginal secretions, body fluids, sputum	KOH dissolves non-fungal elements and reveals fungi on the microscope slide	Screening tool which detects fungal elements; does not inform about genus or species	Rapid, 40 min
Calcofluor white stain (CWS)	Skin scrapings, hair or nail clippings, body fluids, sputum, BALs	CWS binds to fungal elements and fluoresces under ultraviolet light; allows visualization sensitive visualizing fungal elements	Screening tool which detects fungal elements; does not inform about genus or species	Rapid, 40 min
Cultures	Skin, nail, hair, body fluids, tissue, swabs, sputum, blood	The specimen is placed on culture media	Tool to diagnose fungal infections, to perform species identification and antifungal susceptibility testing	Days to weeks

Specimens applied for diagnosing fungal infections

Assay	Specimens	Goal	Results	Time
Antifungal susceptibility testing	Fungal cultures	Follow-up to fungal culture; susceptibility testing may be ordered to guide antifungal therapy	To guide antifungal treatment, to monitor fungal epidemiology	3–4 days
Antigen testing	Blood, urine, CSF, body fluids	Detects antigens associated with specific fungi, a variety of tests is available	To diagnose and monitor infection	Rapid
Antibody testing	Blood, CSF, other body fluids	Detects immune response to a specific fungus; may be ordered on a single sample or on acute and convalescent samples collected 2–3 weeks apart	Diagnose current or recent infection by specific fungus; to monitor treatment	Days
Molecular tests for DNA and RNA	Fungus isolated in culture, or blood, CSF, body fluids, tissue	Detects genetic material of a specific fungus	Detects fungal DNA or RNA; not yet widely available, not yet validated, preferable in research settings	Days

Cultures

- Cultures represent the gold standard in diagnosing fungal diseases
- Positive cultures must be interpreted with caution, as the clinical significance depends on the type of fungus detected and the type of specimen investigated.
 - (a) For non-sterile sites, such as the skin, fungi identified may be disease-causing (pathogenic) or may be part of the normal skin flora; a mixture may be present.
 - (b) Sterile samples, such as blood or tissues that are properly collected will not be contaminated with normal flora, and a positive culture identifies the causing pathogen

Serological Tests

- Noninvasive diagnostic tests include the detection of fungal cell wall components and antibodies.
- However, the detection of serum antibodies is ineffective, as immunocompromised patients lack specific antibody response.

Method	Indication	Advantages	Disadvantages
Galactomannan (GM) Lateral flow assay	Early detection of invasive aspergillosis 2 Serum samples/ week Positive cutoff index > 0.5 BAL, positive cutoff index > 0.5 Detection of invasive aspergillosis Immunochromatographic dipstick assay for the qualitative and semiquantitative detection of <i>Aspergillus</i> antigen	A screening test to accompany conventional diagnostic methods in patients at high risk of IA: For neutropenic adults and children Consistent serum value > 1 is a sign of therapeutic failure Easy to handle, sensitivity and specificity is similar to GM tests	No good data for non-neutropenic patients Mould-active antifungal drugs have an impact on sensitivity Persistent GM antigenemia during therapy is a poor prognostic sign and should prompt a reassessment In some cases the stick is not easy to read, hence lack of result

Serological Tests

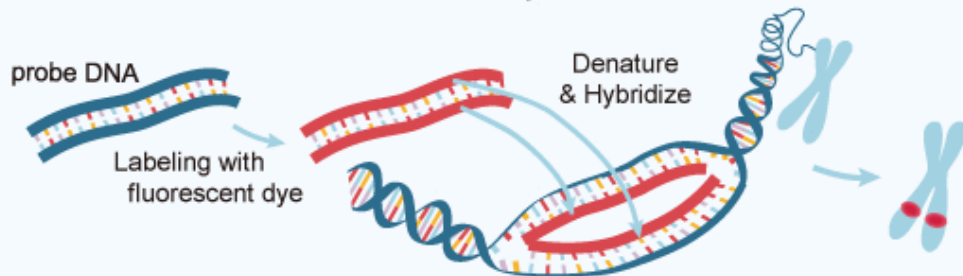
Method	Indication	Advantages	Disadvantages
Mannan plus anti-mannan	Candidemia	Good sensitivity and specificity when combined in ICU patients Early diagnosis prior to blood culture results ESCMID Diagnostic & Management Guideline for Candida Diseases 2012 recommend this test-combination because of high negative PV	Limited experience Non-mycological criterion The sensitivity and specificity were 87.5 and 85.5 % for (1 → 3)-β-D-glucan and 89.3 and 63.0 % for mannan antigen plus anti-mannan antibody <i>C. parapsilosis</i> and <i>C. guilliermondii</i> fungemias were not detected by the Platelia <i>Candida</i> Ag Plus assay
Molecular methods (polymerase chain reaction; PCR) Most experience of in-house tests	DNA detection mainly of <i>Aspergillus</i> species less experience for <i>Candida</i> species Suitable for blood, BAL, tissue	Early diagnosis (rapid techniques) with a high NPV High sensitivity (multicopy genes), capacity for rapid speciation and ability to quantitate fungal burden Low burden of organisms during bloodstream infections: <10 CFU/mL (in 25 % <1 CFU/mL) and intermittent nature of candidemia due to hepatic clearance of fungal cells and/or periodic release of cells from deep organ sites into circulation	Non-mycological Limited to reference laboratories (low availability) High costs, improve technical equipment Technical difficulties of efficient fungal DNA extraction from complex clinical samples

Molecular Detection of Fungi in Clinical Specimens

Fluorescence *In Situ* Hybridization (FISH) Test

- The FISH method uses PNA probes for the identification of *C. albicans* directly from positive blood culture.
- The *C. albicans* PNA FISH test (Advandx) is based on a fluorescein-labeled PNA probe that targets *C. albicans* 26S rRNA.
- The FISH results are unaffected by the type of blood culture system or broth formulation.
- This approach may provide a time saving of 24–48 h, compared with conventional laboratory identification methods used

Fluorescence In Situ Hybridization



Polymerase Chain Reaction (PCR)

- Detection of fungal DNA by means of PCR has been studied in detail for candidemia and invasive aspergillosis.
- The potential usefulness of DNA detection is evident, given that many opportunistic fungal pathogens grow slowly or are difficult to isolate.
- Clinical specimens include serum or whole blood for *Candida and Aspergillus species*, and respiratory samples (e.g., sputum, BAL fluid or tissue) for invasive aspergillosis.



Overview of commercially PCR test assays available

Name	Affigene <i>Aspergillus</i> tracer	MycAssay <i>Aspergillus</i>	<i>Aspergillus</i> spp. Q-PCR alert kit	MycReal <i>Aspergillus</i>
Company	Cepheid	Myconostica	Nanogen	Inogenetix
IVD accredited	Yes	Yes	Yes	No
Detection	Genus <i>Aspergillus</i>	Genus <i>Aspergillus</i>	Genus <i>Aspergillus</i>	<i>A. fumigatus</i> , <i>A. terreus</i> , <i>A. flavus</i> , <i>A. niger</i> , <i>A. nidulans</i> , and others
Species identification	No	No	No	Yes
Sample materials	Full blood, serum, plasma	BAL, sputum	BAL, sputum	Blood, liquor, BAL, puncture specimen, tissues, paraffin
DNA extraction kits	No kit/protocol	MycXtra	EXTRAcell	Protocol
Technology	Scorpions (FAM) amplification graphs	Molecular Beacons (FAM) amplification graphs	TaqMan-MGB (FAM) amplification graphs	HybProbes (LC640, 705) amplification and melting graphs
Target	18S-rRNA-gene	18S-rRNA-gene	18S-rRNA-gene	ITS-region
Internal control	Plasmid (ROX)	Plasmid (HEX)	Beta-globin-gene (VIC)	Plasmid (LC610)
PCR-plattform	Mx3000P and Mx300P iQ and iQ5 Rotor-Gene 3000	Light Cyclor 2.0 ABI 7500, smart cyclor MX3000	ABI 7500	Light Cyclor 2.0
Analysis	Automatically	Automatically	Manually	Manually
Sensitivity	0.54 Genome equivalents/μl	1.3 Copies of genome/PCR	10 Copies of target/PCR	3CFU/PCR
Specificity	Cross reaction (<i>Penicillium</i>)	Cross reaction (<i>Penicillium</i>)	Cross reaction (<i>Penicillium</i>)	Specific for <i>Aspergillus</i>

PCR and GM antigen testing

- PCR and GM antigen testing has recently been shown to improve sensitivity and specificity, when compared to either test alone.
- A systematic review and meta-analysis was done on the use of PCR tests for the diagnosis of invasive aspergillosis (IA).
- Data from more than 10,000 blood, serum, or plasma samples obtained from 1618 patients at risk for IA were retrieved from 16 studies.
- A single PCR-negative result is thus sufficient to exclude diagnosis of proven or probable IA.
- However, two positive tests are required to confirm the diagnosis because the specificity is higher than attained from a single positive test.

PCR and GM antigen testing

- Efforts are underway to devise a standard for *Aspergillus* PCR for screening which will help
 - enabling formal validation of the PCR, and
 - identify patients who are most likely to benefit from its implementation.
- For diagnosis of pneumocystis pneumoniae, PCR may be helpful, and is more sensitive than staining methods



Diagnostic performance based on fungal diseases present

Fungal diseases	Diagnostic performance					
	Microscopy	Culture	Xray/CT scan	Antigen	Antibody	DNA detection
<i>Candida</i> bloodstream	–	+++	+	+	+	+++
Cryptococcal meningitis	++	+++	+	+++	–	–
Invasive (pulmonary) aspergillosis	+	+	+++	++	–	++
Chronic aspergillosis	+	+	+++	–	+++	++
Allergic aspergillosis	+	+	++	–	+++	+
Coccidioidomycosis	+	++	++	–	+++	–
Histoplasmosis	+	++	+	++	–	–
Mucormycosis	+++	+	++ ^a	–	–	–
<i>Pneumocystis jirovecii</i>	+++	–	++	–	–	++
Unknown fungi in various specimens	+++	+++	–	–	–	– ^b

^aUsually no differentiation between *Aspergillus* and other moulds

^bA panfungal PCR may help in fungus identification

Antifungal Agents in Clinical Usage

Drug	Indication
<i>Polyenes</i>	
Amphotericin B	Life-threatening fungal infections, including cryptococcal meningitis, aspergillosis, blastomycosis and mucormycosis
<i>Azoles</i>	
Fluconazole	Invasive infections due to susceptible <i>Candida</i> species; cryptococcosis
Itraconazole	Blastomycosis, histoplasmosis, aspergillosis in patients refractory to Amphotericin B
Voriconazole	Invasive aspergillosis; non-neutropenic candidiasis; serious <i>Scedosporium</i> or <i>Fusarium</i> infections refractory to other agents
Posaconazole	Prevention of invasive fungal infections in neutropenic or HSC ¹ transplant recipients
Isavuconazole	Invasive yeast and mold infections, including aspergillosis and mucormycosis
<i>Echinocandins</i>	
Caspofungin	Candidemia; refractory aspergillosis
Micafungin	Candidiasis
Anidulafungin	Candidiasis (adjunctive therapy with voriconazole for aspergillosis)
<i>Anti-metabolites</i>	
Flucytosine	Adjunctive therapy in <i>Cryptococcus neoformans</i> meningitis and <i>Candida</i> septicemia and endocarditis (in combination with amphotericin B)

¹ HSC = hematopoietic stem cell.

Polyenes: Amphotericin B and Its derivatives

- Polyene antifungals target fungal plasma membrane.
- Broad spectrum and indicated for treatment of severe infections caused by *Candida* sp., *Cryptococcus* sp., Zygomycetes and as an alternative therapy for aspergillosis.
- Also used to treat many life-threatening IFIs due to other filamentous molds, as well as the thermally-dimorphic fungi, such as *Histoplasma*, *Coccidioides* and *Blastomyces*.
- Newer formulations , such as lipid-associated and liposomal formulations, demonstrate more selective fungal targeting and less host toxicity

Azoles and Triazoles

- Fluconazole is most widely-used yeast-active azole, and often very effective against *Cryptococcus* and *Candida* sp.
- Fluconazole resistance can present a significant clinical issue in systemic candidiasis:
 - Eg., *Candida* sp., such as *C. krusei*, are intrinsically resistant and other *Candida* isolates are often susceptible at high conc.
- The mold-active azoles include itraconazole, voriconazole, posaconazole and isavuconazole.

Azoles and Triazoles

- In addition to retaining activity against *Candida* and *Cryptococcus* yeasts, these agents also inhibit many filamentous fungi.
- Itraconazole was the first available azole with significant activity against molds, such as *Aspergillus fumigatus*.
- Isavuconazole (Cresemba®, Astellas Pharmaceuticals, Tokyo, Japan) is the most recently approved triazole antifungal drug.

Echinocandins

- Currently, three drugs are available for clinical usage: caspofungin, micafungin and anidulafungin.
- Echinocandins affect cell wall biosynthesis through the noncompetitive inhibition of -1,3-glucan synthase.
- Primarily used for the treatment of invasive candidiasis and as an alternative therapy for treatment of aspergillosis
- Echinocandins have low host toxicity and few drug interactions.

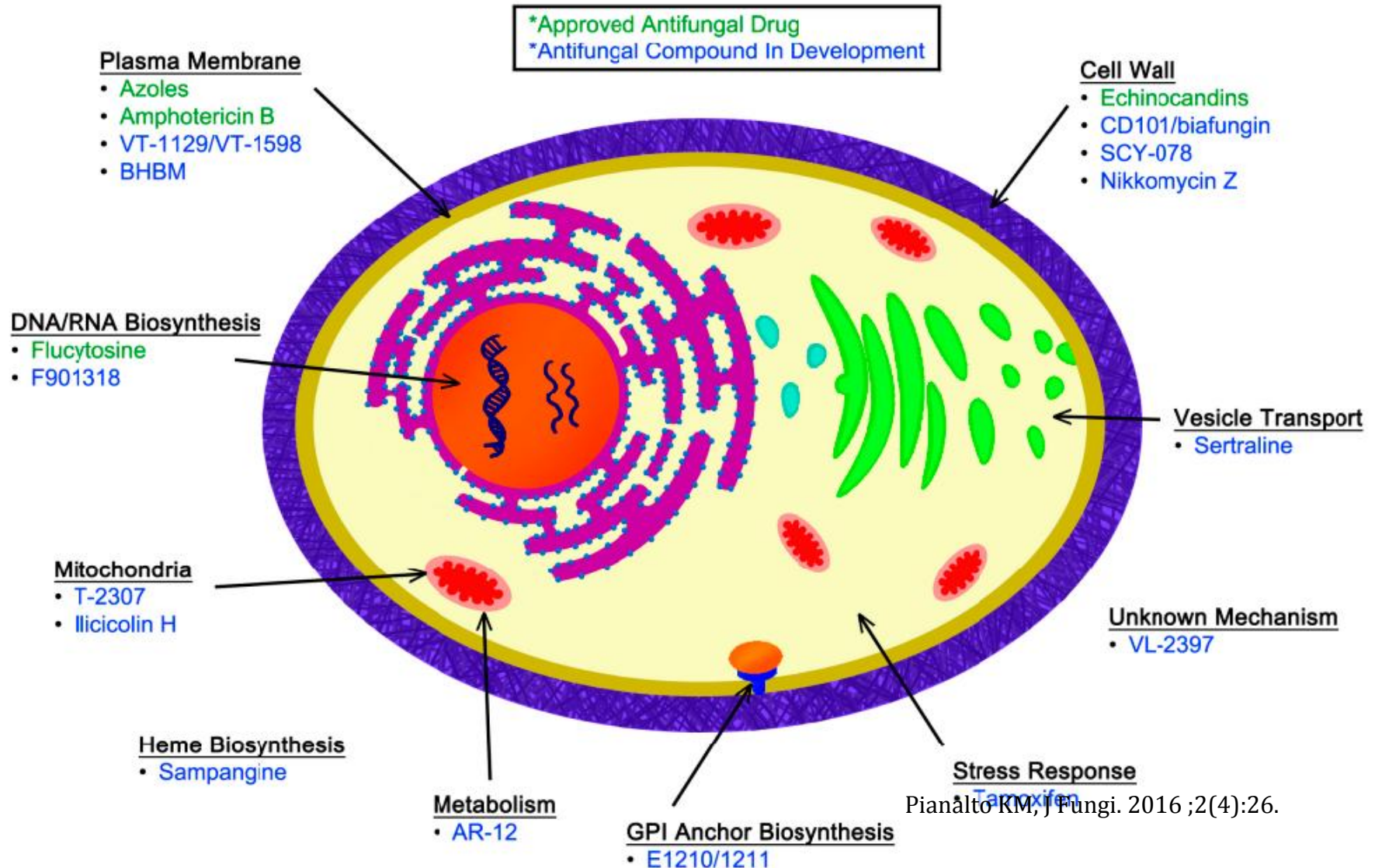
Nature, target, mode of action, and fungal resistance mechanisms of the major antifungal drugs used in human therapy

Antifungal agent	Mode of action and cellular target	Mechanism of resistance
polyenes	binding to ergosterol	absence of ergosterol (loss of function mutation in <i>ERG3</i> or <i>ERG6</i>) decrease of ergosterol content in cells
azoles	inhibition of cytochrome p450 function: 14 α -lanosterol demethylase (<i>ERG11</i>) sterol Δ^{22} desaturase (<i>ERG5</i>)	efflux mediated by multidrug transporters decrease of affinity in Erg11p by mutations upregulation of <i>ERG11</i> alterations in the ergosterol biosynthetic pathway
allylamines	inhibition of squalene epoxidase (<i>ERG1</i>)	unknown
morpholines	inhibition of sterol Δ^{14} reductase (<i>ERG24</i>) and the Δ^{7-8} isomerase (<i>ERG2</i>)	unknown
5-fluorocytosine	inhibition of nucleic acids synthesis	defect in cytosine permease deficiency or lack of enzymes implicated in the metabolism of 5-FC deregulation of the pyrimidine biosynthetic pathway
echinocandins	inhibition of β -1,3 glucan synthase (<i>FKS1&2</i>)	alteration of affinity of echinocandins for β (1,3)-glucan synthase

5-Fluorocytosine

- Fluoridated pyrimidine analog, which inhibits DNA and RNA synthesis
- It has been attributed with cytostatic effects and high rates of resistance developing during monotherapy.
- Therefore, flucytosine is rarely used as a single agent for the treatment of fungal infections.
- Multiple clinical trials have shown it to be highly effective in combination with amphotericin B against cryptococcal meningitis
- Indeed, amphotericin B plus flucytosine is the first-line treatment for *Cryptococcus* central nervous system (CNS) infections

Antifungal Agents in Development with Novel Modes of Action



New antifungal agents in development

Antifungal Compound	Indication(s) ¹	Activity (MIC)
AR-12	<i>Cryptococcus neoformans</i> <i>Candida albicans</i>	4 µg/mL 4 µg/mL
BHBM	<i>Cryptococcus neoformans</i> <i>Cryptococcus gattii</i> <i>Candida glabrata</i> <i>Blastomyces dermatitidis</i> <i>Histoplasma capsulatum</i> <i>Pneumocystis jirovecii</i>	0.25–8 µg/mL 0.5–2 µg/mL 0.125– >32 µg/mL 0.5–1 µg/mL 0.125–1 µg/mL 0.072–0.912 µg/mL ²
CD101	Candidemia ^{3,*} <i>Aspergillus</i> species ⁵	≤0.008–2 µg/mL ⁴ ≤0.008–0.03 µg/mL ⁴
E1210/1211	<i>Aspergillus</i> species ⁵ <i>Candida</i> species ⁶ <i>Scedosporium</i> species <i>Fusarium</i> species	≤0.008–0.25 µg/mL ≤0.002–0.25 µg/mL 0.03–0.25 µg/mL 0.015–0.25 µg/mL
F901318	<i>Aspergillus</i> species ⁵	<0.03 µg/mL
Illicicolin H	<i>Candida</i> species ⁷ <i>Aspergillus fumigatus</i> <i>Cryptococcus neoformans</i>	0.01–5 µg/mL 0.08 µg/mL 0.2–1.56 µg/mL
Nikkomycin Z	Coccidioidomycosis [*]	0.125 µg/mL

New antifungal agents in development

Sampangine	<i>Cryptococcus neoformans</i>	<0.05 µg/mL
	<i>Candida albicans</i>	3.1 µg/mL
	<i>Candida glabrata</i>	3.1 µg/mL
	<i>Candida krusei</i>	6.2 µg/mL
	<i>Aspergillus fumigatus</i>	6.2 µg/mL
SCY-078	Invasive candidiasis *	0.03–2 µg/mL ⁸
	<i>Aspergillus</i> species ⁵	0.03–0.25 µg/mL ⁴
Sertraline	<i>Cryptococcus</i> species *	2–6 µg/mL ⁴
T-2307	<i>Candida</i> species ⁹	0.00025–0.0078 µg/mL
	<i>Cryptococcus neoformans</i>	0.0039–0.0625 µg/mL
	<i>Aspergillus</i> species ^{5,10}	0.0156–2 µg/mL ⁴
	<i>Fusarium solani</i>	0.125 µg/mL
	<i>Mucor racemosus</i>	2 µg/mL
Tamoxifen	<i>Cryptococcus neoformans</i>	64 µg/mL
	<i>Candida albicans</i>	32 µg/mL
	<i>Candida glabrata</i>	8 µg/mL
VL-2397	Invasive aspergillosis *	1–4 µg/mL ^{4,9}
	<i>Candida glabrata</i>	≤2 µg/mL
	<i>Candida kefyr</i>	≤2 µg/mL
	<i>Cryptococcus neoformans</i>	≤2 µg/mL
VT-1129	Cryptococcal meningitis *	<0.0001–0.25 µg/mL ⁴
	<i>Candida</i> species ¹¹	<0.0001–1 µg/mL
VT-1598	Coccidioidomycosis	NA ¹²

Summary

- A precise mycological diagnosis is only possible with the help of laboratory testing.
- With improved diagnostics, confirmation of the cause of fungal infection is possible by serological and molecular testing.
- Conventional microbiological and microscopic techniques remain the cornerstone of diagnosis but lack sensitivity.
- Combined implementation of other available tools is therefore mandatory for proper diagnosis
- DNA and RNA-based methods hold promise for improved sensitivity and specificity, but these methods will require extensive validation in clinical studies

Summary

- Promising advances are being made in antifungal drug development, both through the
 - development of novel compounds with potent antifungal activity
 - repurposing of previously described compounds for new uses as antifungal agents
- Expanding insight into cellular processes required for fungal survival is now being translated to specific identification of new therapeutic targets.
- **Together, these efforts will greatly expand the currently limited number of drugs that we have to treat patients with life-threatening fungal infections.**

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Thank You