Module 1: Introduction to Fungi and Invasive Fungal Diseases

It should take approximately 1.5 hours to complete modules 1-3.

Purpose

Modules 1-3 will provide a comprehensive overview of systemic treatments for invasive fungal infections (IFIs), from mechanisms of action and patients at risk to treatment strategies and the latest guidelines in specific patient populations.

Learning objectives

In Module 1, you will learn to:

- Define basic terminology in mycology
- Explain the difference between yeast and mold in terms of such things as appearance and reproduction
- Identify key fungi and explain important details pertaining to, for example, yeast vs mold and/or individual and organ they primarily affect

1.1 Introduction to fungi

Fungi are eukaryotes, unlike bacteria which are prokaryotes. Two fundamental differences between eukaryotes and prokaryotes are that eukaryotic cells are much larger and have membrane-bound organelles, including a nucleus.[1]

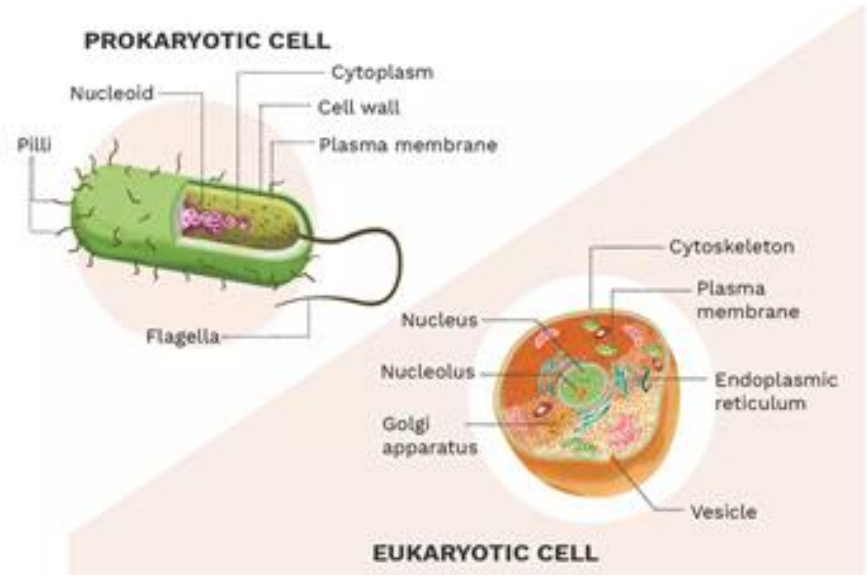

Figure 1. Prokaryotic vs Eukaryotic Cells. *(Image from https://www.thoughtco.com/what-are-prokaryotes-and-eukaryotes-129478)*

The study of fungi is known as Mycology. Fungi are also known as Eumycota (true fungi).[2]

Fungi form their own large and diverse kingdom, separate from plants and animals, and have features in common with both. Genetic studies have shown that fungi are more closely related to animals than plants.[1,2]

There are several features that make fungi distinct from plants and animals:

- Fungal cells have a rigid cell wall containing chitin (unlike animal cells which do not have a cell wall, and plant cell walls which contain cellulose).
- Their cell membranes contain ergosterol (unlike animal cell membranes which contain cholesterol).
- Unlike plant cells, they do not have chloroplasts or chlorophyll and so they cannot make their own food via photosynthesis.
- Fungi also cannot ingest food like animals – instead, they feed by absorbing nutrients from their surrounding environment.[2,3]
- Fungi are structurally simpler than plants and animals – the basic structural unit being either a hypha (chain of tubular cells), or a single cell.[4,5]

1.2 Morphology of fungi

Fungi show massive variation in terms of their size, shape and appearance. Individual fungal cells can range from 1 to 30µm. Fungi that exist mainly as single-celled organisms are referred to as **yeasts**, whereas those that form multicellular organisms are known as **molds**.[1,2] Many fungi are **dimorphic**, meaning that they can exist either as yeasts or molds depending on environmental conditions, the most important of which is the external temperature.[6]

1.2.1 Molds

Molds consist of aggregates of hyphae, (see **Figure 2a**) which are long, branching filaments formed from chains of cylindrical cells.[1,2] The process by which hyphae increase in length is known as apical elongation.[5] Hyphae can be septate (meaning that they have 'septa' or cross-walls separating the individual fungal cells) or they can be non-septate (lacking these cross-walls – see **Figure 4**).[3]

(a)

(b)

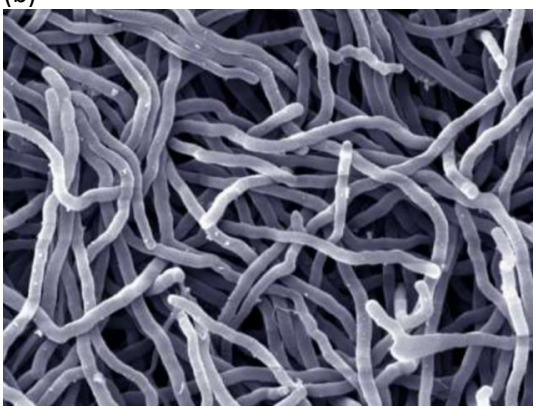

Figure 2. (a) Fungal hyphae, (b) Fungal mycelium. *(Image from https://www.slideshare.net/jlpatinho1972/fungi-kingdom-clil-natural-sciences)*

In most multi-cellular fungi, the vegetative state usually consists of a network of branching hyphae known as a mycelium (see **Figure 2b**).[3,4] Within a mycelium, vegetative hyphae penetrate the medium and are primarily involved in the absorption of nutrients. Aerial hyphae project above the surface of the medium. These aerial hyphae are involved in reproduction (see **Figure 3**).[2]

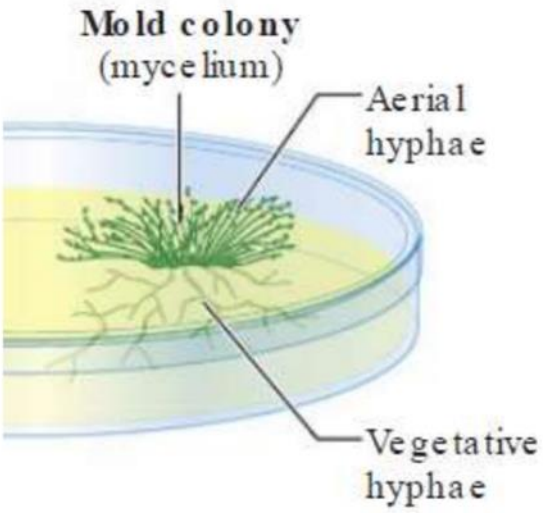

Figure 3. A mycelium showing aerial hyphae and vegetative hyphae. *(Image from https://www.slideshare.net/vsdvoet/microbiology-unit-5-fungi)*

1.2.2 Yeasts

Yeasts are microscopic, single-celled fungi. They usually reproduce by budding of new cells from their surface. This is an asexual process, with the bud initially forming as a protuberance from the parent cell that then detaches.[2,4] Sometimes a budding cell can remain attached and then itself form another bud. This may lead to chains of cells known as pseudohyphae (see **Figure 4**).[3]

The appearance of yeast colonies resembles those of bacterial colonies.[2]

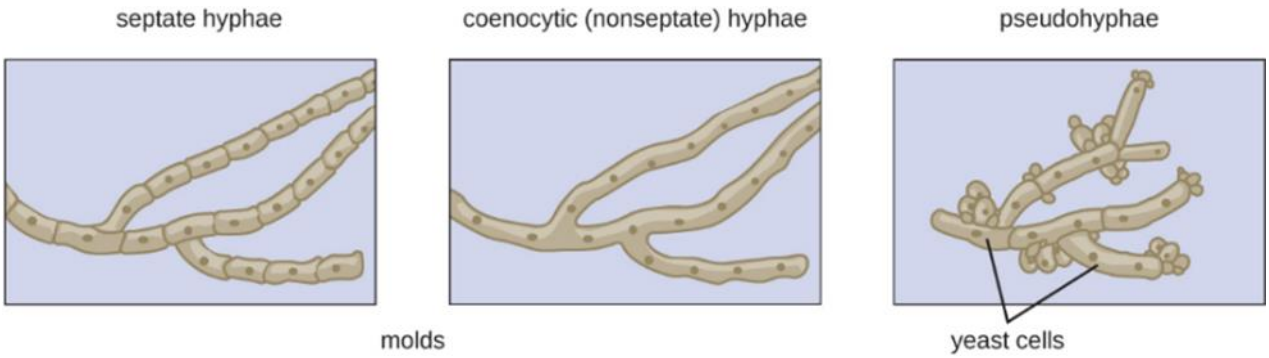

Figure 4. Molds form hyphae which may be septate (left) or non-septate (right). Yeasts may form pseudohyphae. *(Image from https://courses.lumenlearning.com/microbiology/chapter/fungi/)*

1.3 Physiology of fungi

1.3.1 Reproduction

Most species of fungi can reproduce both sexually and asexually; these are known as 'perfect fungi'.[2,6] Fungi that are unable to reproduce sexually are known as Deutoeromycetes (also referred to as 'imperfect fungi').[1,2] The majority of pathogenic fungi belong to the Deuteromycetes group.[2] An overview of the processes of both sexual and asexual reproduction is shown in **Figure 5**. Molds reproduce through the production of spores. The size and shape of the spores produced is a means of classifying fungi.[2,4]

1.3.1.1 Asexual reproduction

Asexual reproduction can occur through three main routes:

- ***Fragmentation***: Fragments of hyphae can separate and grow new colonies. Similarly, a fungal mycelium may split into fragments, with each part then growing into a separate mycelium.[6]
- ***Budding***: As described previously, during this process a new cell buds from a somatic yeast cell through mitosis, with the bud eventually separating from the parent cell.[6]
- ***Spore formation***: This is the most common mechanism of asexual reproduction. Asexual spores (genitally identical to the parent cell except for occasional mutations) are produced through mitosis.[6] There are several different types of asexual spores. Spores may be shed in large numbers inside a type of reproductive sac called a sporangium – these spores are referred to as sporangiospores. Spores that are not released inside a sporangium are known as conidia (or conidiospores).[1,6]

1.3.1.2 Sexual reproduction

Sexual reproduction introduces genetic variation which can help fungi adapt to new environments, and often occurs in response to adverse environmental conditions.[6,7]

Three stages are common to all variations of fungal sexual reproduction:[6]

- ***Plasmogamy*** is the process by which two haploid cells fuse. This results in a single cell with two separate haploid nuclei.[6,7]
- ***Karyogamy*** is the next stage, during which the two haploid nuclei fuse to form a diploid nucleus (containing two sets of chromosomes – one from each of the parent cells). This diploid cell is known as a zygote.[6,7] In most fungi, the zygote represents the only diploid cell within the fungal life cycle.[7]
- ***Meiosis*** is the final stage, during which haploid spores are produced. This occurs in the gametangia organs, resulting in sexual spores (gametes).[6,7]

Some fungi are homothallic (self-fertile), meaning that both male and female reproductive types exist within the same mycelium. However, the majority are heterothallic; sexual reproduction requires two different (but compatible) mycelia.[6] In some fungi, millions of spores are produced in large 'fruiting bodies', such as mushrooms.[4]

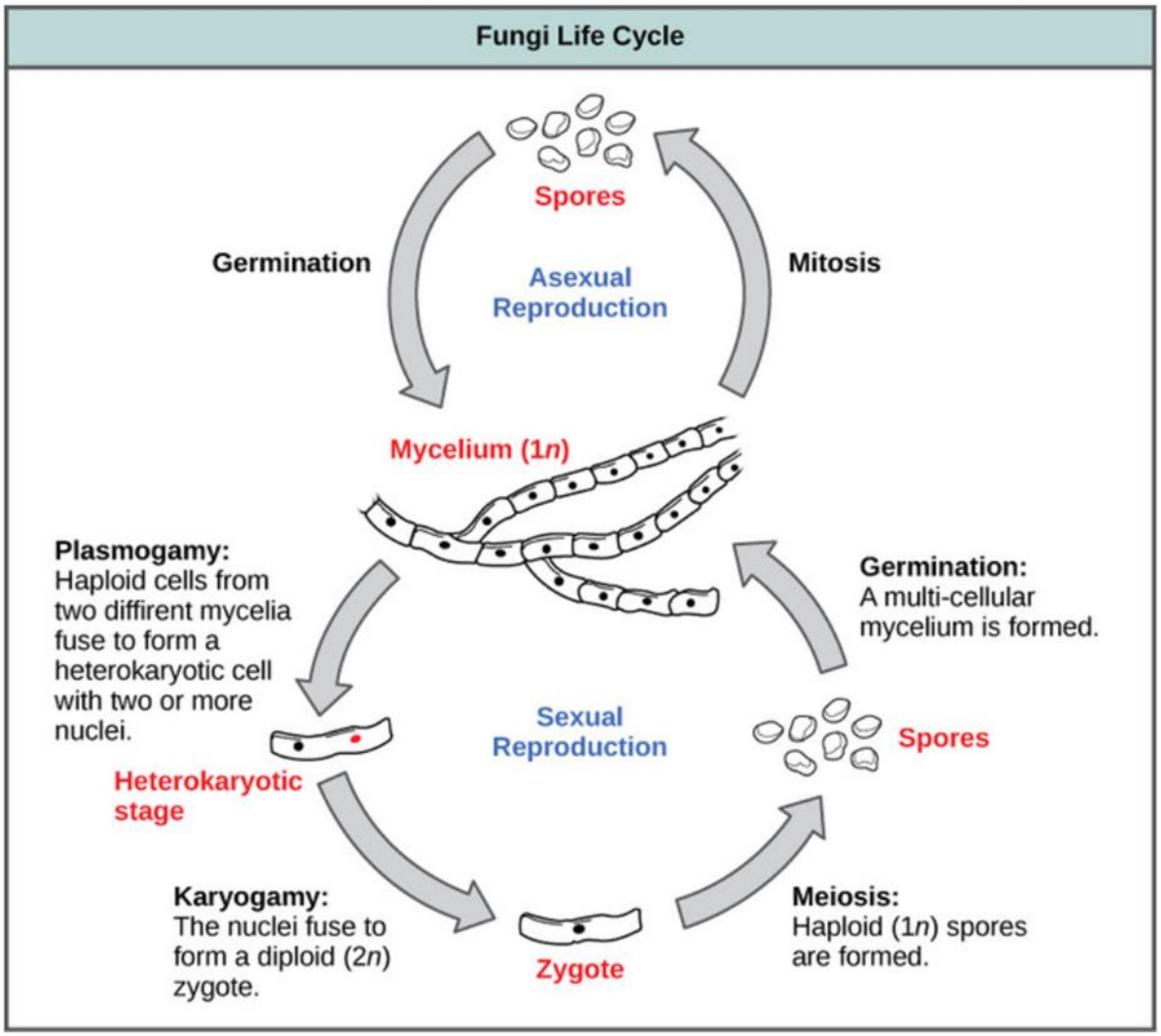

Figure 5. Types of fungal reproduction.[6] *(Image from https://courses.lumenlearning.com/boundless-biology/chapter/characteristics-of-fungi/)*

1.3.2 Nutrition

Fungi are heterotrophic; their nutrition comes from complex organic compounds. They achieve this by secreting enzymes (exoenzymes) that digest their food source externally and then absorbing the nutrients. The majority of fungi are saprophytes, meaning that they obtain their nutrients from dead or decaying organisms. As such, they fulfil a vital role in nature as decomposers, helping to return recycled nutrients to the soil.[2,3,6] Other species of fungi are parasites, feeding on other living organisms but not killing them.[2]

1.4 Classification of fungi

Conventionally, fungi were classified according to morphological and phenotypic features. Within recent years, molecular approaches to fungal identification have had a profound impact upon fungal classification.[4] The most recent taxonomy divides fungi into nine sub-kingdoms:

- Opisthosporidia
- Chytridiomycota
- Neocallimastigomycota
- Blastocladiomycota
- Zoopagomycota
- Mucoromycota
- Glomeromycota
- Ascomycota
- Basidiomycota [8]

Figure 6 gives an overview of these sub-kingdoms and the diversity of organisms within them.

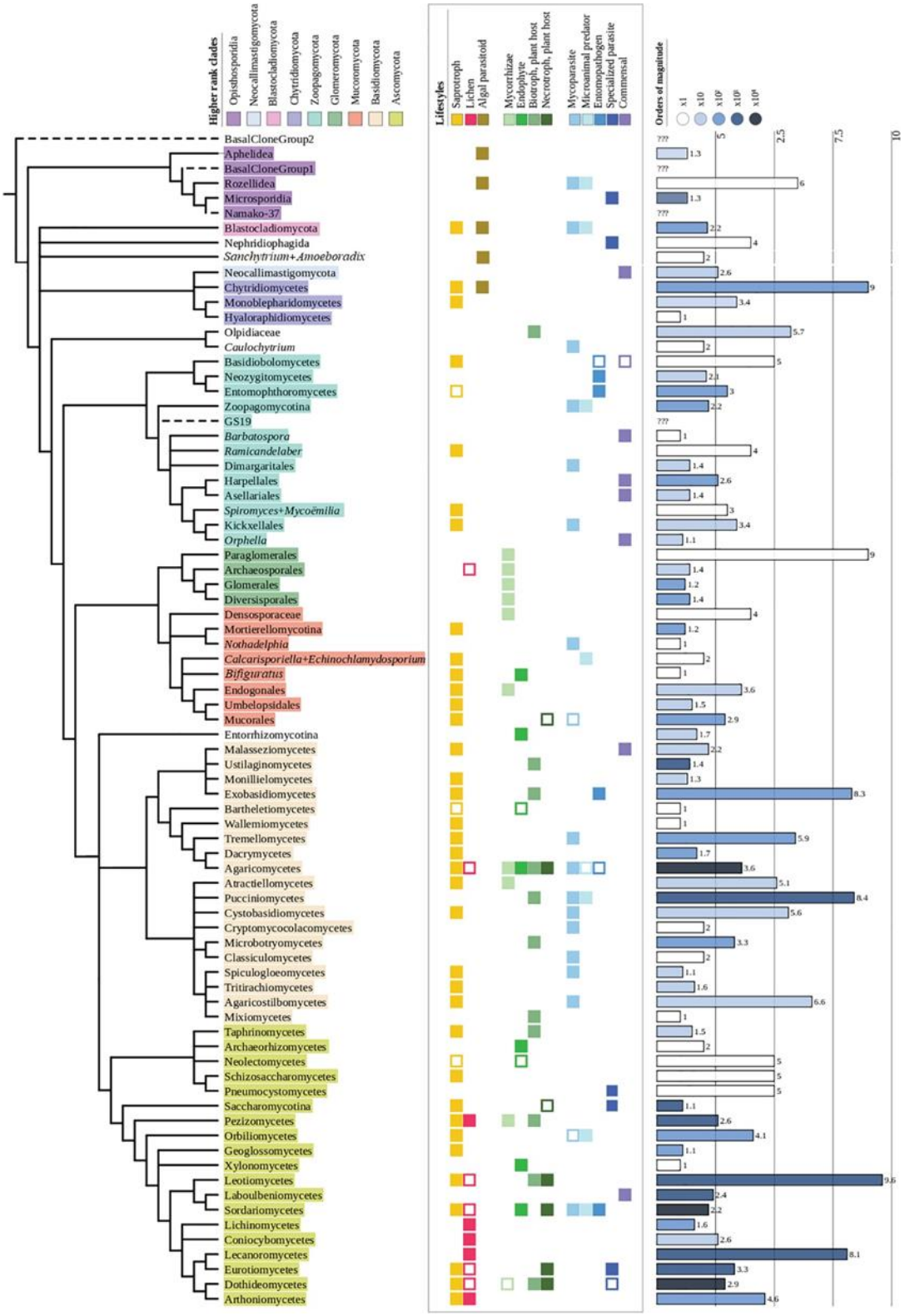

Figure 6. The fungal tree of life.[8] *(Image taken from Naranjo-Ortiz MA and Gabaldón T, 2019)*
The first column uses colours to cluster clades in corresponding phyla. The second column compiles the lifestyles present in each group. Empty squares indicate that the given lifestyle is anecdotic or hypothetical. The third column shows the number of described species in each group.

1.4.1 Nomenclature of fungi and fungal diseases

Fungi are named according to the International Code of Botanical Nomenclature. Confusingly, many fungi have been described by more than one name – typically having one name that refers to their sexual stage and another that refers to their asexual stage. This has generally happened when different stages were identified separately prior to the connection between them being established.[4]

Historically, fungal diseases were often named after the fungi that caused them (e.g., Candidosis being caused by *Candida* species). This has caused problems when the classification of fungi has changed (e.g., when the class *Zygomycetes* was abolished, the term Zygomycosis became obsolete – diseases previously falling into this category are now subdivided into Mucormycosis and Entomophtoromycosis).[4]

It can be more helpful when diseases are named after the pathology that they cause, with the individual organism specified afterwards. For example, the collective term 'Phaeohyphomycosis' describes infections due to brown-pigmented molds that appear in tissue as septate hyphae. Over 250 fungal species have been implicated in the etiology of this disease.[4]

1.5 Importance of fungi

In their role as decomposers, fungi fulfil a vital role in the breakdown of organic matter, enabling the return of nutrients to the environment.[2] Humans have utilized fungi for many years in everyday life, such as using yeast in the baking of bread or when brewing beer.[9] The discovery of penicillin, (produced by the mold Penicillium notatum) in 1928, is arguably the most important medical breakthrough of the last century.[10]

An important factor that makes fungi particularly useful is that they can be grown on an industrial scale in large bioreactors. For example, since 1919, citric acid has been produced commercially by fermentation with the filamentous fungus *Aspergillus niger*.[11]

These days, fungi are exploited in many different industries, as illustrated in **Table 1**.[11] However, this is just the tip of the iceberg, with a wealth of research and investment currently focusing on the potential for fungi to be harnessed in the production of more sustainable sources of food, fuels, textiles and construction materials (see **Figure 7**).[11]

Filamentous fungus	Important Product(s)
Acremonium chrysogenum	β-lactam antibiotics (cephalosporins)
Aspergillus niger	Enzymes (glucoamylase, proteases, phytases, glucose oxidase) Organic acids (citric acid, gluconic acid)
Aspergillus oryzae	Enzymes (amylases)
Aspergillus terreus	Enzymes (xylanases) Organic acids (itaconic acid) Secondary metabolites (lovastatin)
Blakeslea trispora	Vitamins (β-carotene)
Fusarium venenatum	Mycoprotein as meat alternative
Ganoderma lucidum	Composite materials (packaging material, construction material) Imitation leather
Mortierella alpina	Polyunsaturated fatty acids used as food additives

Mucor circinelloides	Polyunsaturated fatty acids used as food additives
Penicillium brevicompactum	Mycophenolic acid
Penicillium camemberti	Cheese production
Penicillium chrysogenum	β-lactam antibiotics (penicillins) Enzymes (glucose oxidase)
Penicillium nalgiovense	Mold-fermented salami
Penicillium roqueforti	Cheese production
Penicillium solitum	Mevastatin
Pleurotus ostreatus	Food Composite materials (packaging material, construction material)
Rhizopus oligosporus	Tempeh production
Thermothelomyces thermophilus	Enzymes (cellulases, phytases, laccases)
Trichoderma reesei	Enzymes (cellulases, hemicellulases)
Umbelopsis isabellina	Polyunsaturated fatty acids used as biodiesel

Table 1. List of established industrial uses of fungi.[11] *(Image taken from Meyer V, et al. 2020)*

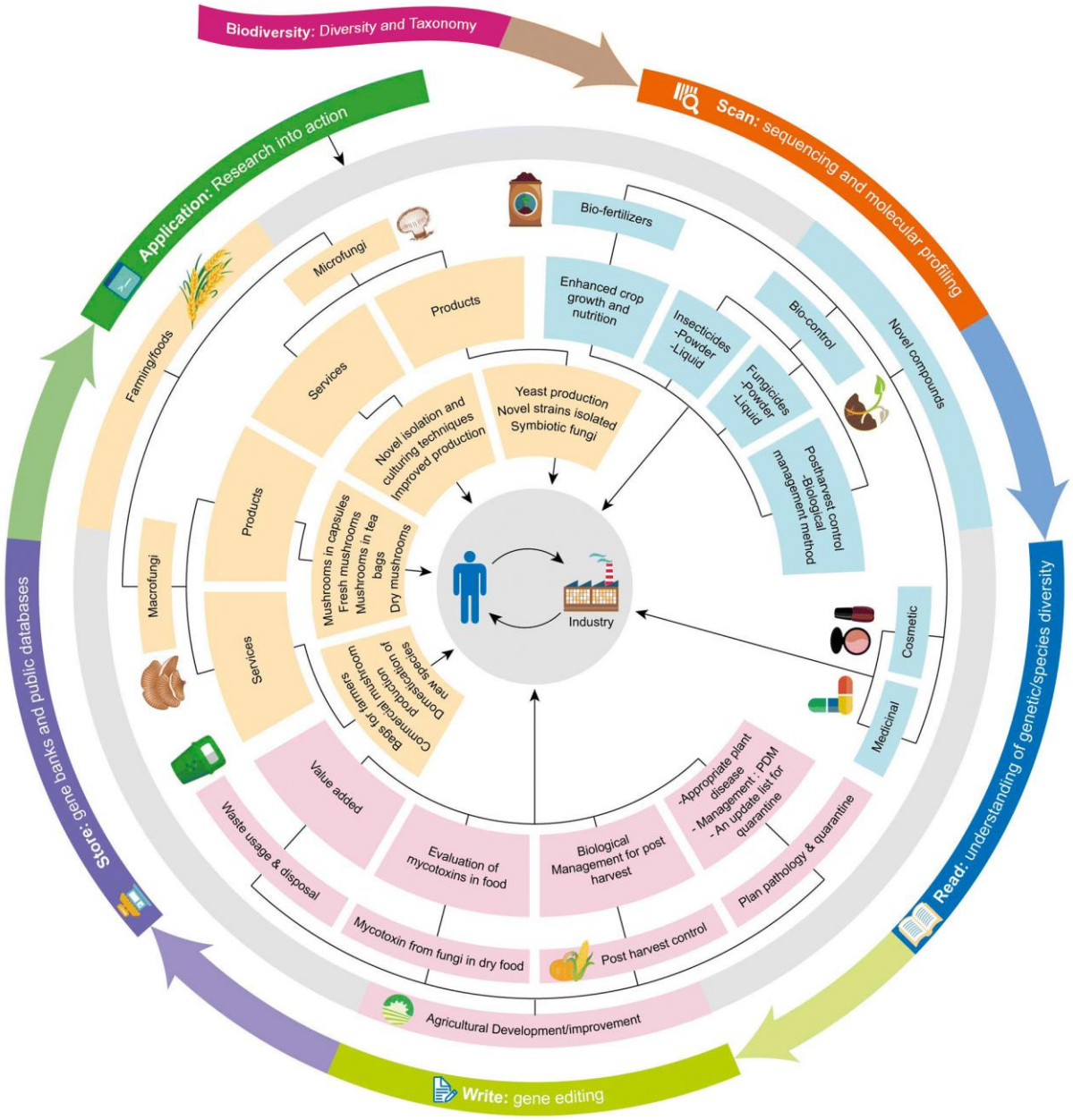

Figure 7. The potential use of fungi in biotechnology.[12] *(Image taken from Hyde K, et al. 2019)*

1.6 Key summary points

- Fungi are eukaryotes and form their own large and diverse kingdom
- Fungi can exist as single-celled yeasts or multicellular organisms known as molds
- Many medically important fungi are dimorphic, meaning that they can exist as either yeasts or molds depending on environmental conditions
- Many fungi are able to reproduce both sexually and asexually
- Fungi gain nutrition by secreting enzymes to digest their food and then absorbing the nutrients
- Most fungi are saprophytes, obtaining nutrients from dead or decaying matter, acting as decomposers
- Fungi have an ever-increasing variety of uses in food production, agriculture, pharmaceuticals, and industry

1.7 Multiple choice questions

Qu 1: Fungi that exist mainly as single-celled organisms are known as molds?

a) True

b) False

Qu 2: Spore formation is the most common mechanism of asexual reproduction?

a) True

b) False

Qu 3: All fungi are saprophytes, gaining their nutrition from dead or decaying organisms?

a) True

b) False

Qu 4: Dimorphism refers to the ability to reproduce by both sexual and asexual reproduction?

a) True

b) False

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Module 2: Pathogenesis and Diagnosis

It should take approximately 1.5 hours to complete modules 1-3.

Purpose

Modules 1-3 will provide a comprehensive overview of systemic treatments for invasive fungal infections (IFIs), from mechanisms of action and patients at risk to treatment strategies and the latest guidelines in specific patient populations.

Learning objectives

In Module 2, you will learn to:

- Understand the different types of fungal diseases in humans
- Describe the prevalence and epidemiology of the most common invasive fungal diseases
- Be aware of the risk factors which predispose individuals to invasive fungal diseases
- Understand how different invasive fungal diseases are diagnosed

2.1 Introduction to fungal diseases

Over 120,000 named species of fungi have been identified, but it is estimated that the total number of species that exist may be between 2.2 and 3.8 million.[1] Despite this, fewer than 500 species have been linked to causing disease in humans. Of these, only around 100 species can cause infection in otherwise healthy people (primary pathogens), with most fungal infections occurring in immunocompromised individuals (opportunistic pathogens).[2,3] A fungal 'infection' can be defined as "entry into body tissues followed by multiplication of the organism."[4] Some infections may lead to disease, whereas others may be asymptomatic.[4]

Most fungal infections in humans (mycoses) are caused by fungi that are ubiquitous in the environment (exogenous infections). Others are caused by human commensals, such as some Candida species (endogenous infections). Human infection can occur when immunocompromise or other host disability enables fungi to breach host barriers and multiply.[2,4] Some types of fungal diseases occur worldwide, whereas others are concentrated in certain geographical areas.[2]

2.2 Pathogenesis of fungal infection

Relatively little has been determined about the mechanisms by which fungi cause disease.[4] In healthy individuals, numerous mechanisms protect against fungal infections by preventing fungi entering the body. For example, the growth of fungi on intact skin is discouraged by various factors such as competition with normal bacterial flora and the presence of naturally occurring fatty acids on the skin.[4] When these protective mechanisms are breached or bypassed, fungi can enter host tissues.[4]

2.2.1 The role of dimorphism in the pathogenesis of fungal infections

Dimorphism (the ability to exist either as a yeast or a mold depending on environmental conditions) is important to the pathogenesis of many fungal infections.[5] Primary fungal pathogens that are capable of causing invasive disease exist in the soil as molds. However, when their spores are inhaled into the lungs, they undergo a temperature-sensitive change into the yeast form.[5] This can facilitate fungal pathogenesis; for example, the yeast form of *Histoplasma capsulatum* survives phagocytosis by macrophages in the lung alveoli as a means of entering the bloodstream.[5]

2.3 Types of fungal infection in humans

Fungal infections can be grouped based upon the initial site of infection into superficial, subcutaneous, and systemic mycoses.[2]

2.3.1 Superficial mycoses

These infections involve only the outer layers of the skin, hair, nails, and mucous membranes.[2] The primary infections that fall into this category are the dermatophytoses, superficial Candida infections and pityriasis versicolor (caused by commensal yeasts of the genus Malassezia).[2]

2.3.1.1 Dermatophytosis

Dermatophytoses (also called ringworm infections) are infections of the skin, hair, or nails by dermatophytes – a group of related filamentous fungi.[2] There are two main groups of causative organisms – Trichophyton species and Microsporidium species.[5] These fungi gain nutrition through breaking down keratin.[5] Infections are named based upon the part of the body involved.[2]

Figure 1 summarizes the different types of dermatophytosis and their clinical features.

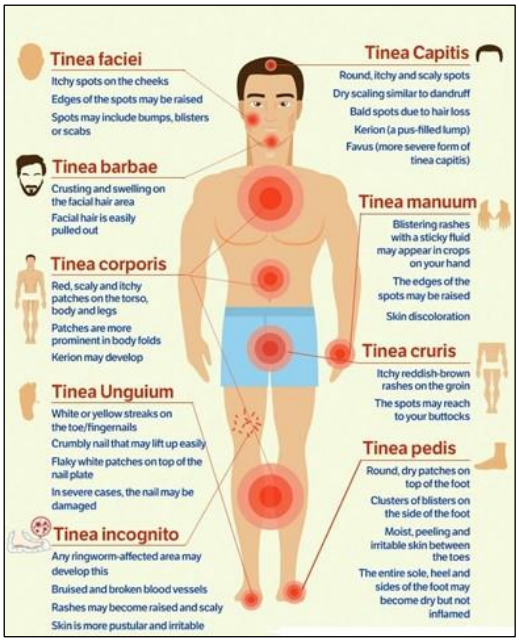


Figure 1. Types of dermatophytosis. *(Image from www.creative-biolabs.com/drug-discovery/therapeutics/dermatophytosis.htm)*

In contrast to most other fungal infections, dermatophytoses are contagious and can pass between individuals either by direct contact or indirectly; via towels, bedding, etc.[2] Where there are characteristic cutaneous lesions (**Figure 2**), diagnosis may be made clinically but should ideally be confirmed by microscopy and/or culture through taking samples of skin, hair or nails.[2]

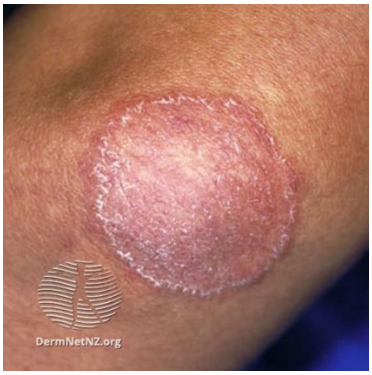


Figure 2: Typical rounded lesion seen with tinea corporis. *(Image © DermNetNZ)*

2.3.1.2 Superficial candidosis

Yeasts belonging to the genus *Candida* are opportunistic pathogens that often cause superficial infections of mucosae, skin, or nails but can sometimes cause invasive disease.[2] *Candida* spp. is often found as a commensal on the skin and in the mouth, gastrointestinal (GI) tract, and vagina. Changes in these areas that encourage fungal growth can lead to *Candida* multiplying and causing infection.[6] Most superficial *Candida* spp. infections are endogenous in origin, but there can be transmission between individuals. For example, oral candidosis can occur in neonates whose mothers have vaginal candidosis.[2] **Figures 3** summarizes the clinical features and risk factors for oropharyngeal and vaginal candidosis.

Clinical feature of oropharyngeal candidosis:

- White patches in mouth, tongue, and throat
- Erythema
- Soreness
- Loss of taste
- Pain while eating or swallowing
- Angular cheilitis

Clinical features of vaginal candidosis:

- Vaginal itching or soreness
- Dyspareunia
- Dysuria
- Abnormal vaginal discharge

Risk factors for oropharyngeal candidosis:

- Neonates
- Wearing dentures
- Diabetes
- Cancer
- HIV/AIDS
- Antibiotic use
- Corticosteroid use (including inhaled)
- Dry mouth
- Smoking

Risk factors for vaginal candidosis:

- Pregnancy
- Hormonal contraception
- Diabetes
- Immunosuppression
- Antibiotic use

Figure 3: Clinical features and risk factors for candidosis. [6] *(Image from https://cdn.britannica.com/61/130161-050-BE731814/Thrush-tongue-palate.jpg)*

2.3.2 Subcutaneous mycoses

This group of infections is generally a consequence of traumatic implantation of fungi from soil or vegetation, leading to infection of the dermis and subcutaneous tissues. Usually, there is a slow localized spread of infection, although widespread infection can occur in the immunocompromised. These fungi generally have a low degree of infectivity.[2,4,7] These types of infections are more common in rural parts of the world, where people often walk barefoot.[2] Two examples of subcutaneous mycosis are outlined in **figure 5** below.

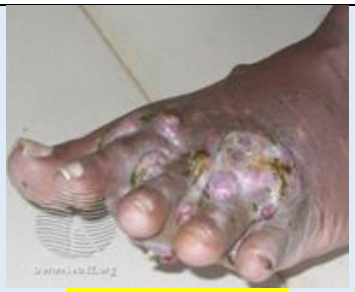
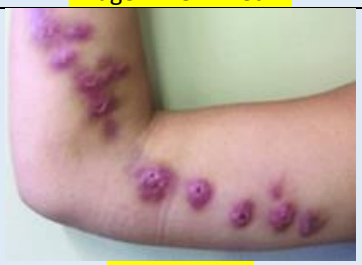
Mycetoma:[8] • Mainly found in rural equatorial parts of Africa, Latin America, and Asia • Fungi from soil and water enter tissues through breaks in the skin • Infection leads to abscesses containing firm masses of fungal filaments • These grow slowly and can develop discharging sores • Usually affects the feet or hands • Infection is usually painless but can be severely debilitating • Can also be caused by bacteria (actinomycetoma) [2,8]	 Image: ®DermNetNZ
Sporotrichosis:[9] • Caused by the dimorphic fungus Sporothrix schenckii • This fungus is found worldwide in soil and plant matter • Infection usually occurs through traumatic implantation • Most commonly presents as localized cutaneous or subcutaneous lesions • Can spread along lymphatic channels • Less commonly can cause lung pulmonary or disseminated disease [2,10]	 Image – ref [10]

Figure 5: Examples of subcutaneous mycosis.

2.3.3 Systematic mycoses

Invasive fungal disease (IFD) is proven when (a) there is proven tissue damage due to fungal elements and/or (b) the fungus is cultured from sterile clinical samples, e.g., blood, tissue, or cerebrospinal fluid (CSF).[6] There are various routes by which invasive fungal disease can occur:

- Invasion into the mucosa
- Inhalation of fungal spores from the environment
- Direct inoculation of fungal spores (for example, via intravenous catheters)[6]

Most common IFDs usually originate in the respiratory tract, often secondary to inhalation of fungal spores.[2] IFDs can be due to so-called 'true pathogens' or due to opportunistic infections.[2]

True pathogens can cause infection in apparently normal (healthy and immunocompetent) individuals. There are four main diseases in this category:[2]

- Blastomycosis
- Coccidioidomycosis
- Histoplasmosis
- Paracoccidioidomycosis

These diseases are often mild and of short duration in healthy individuals (and may be asymptomatic).[2] However, they can cause severe and potentially life-threatening infection in those who are immunocompromised.[2] For example, histoplasmosis and coccidioidomycosis have been frequently identified in patients with the human immunodeficiency virus (HIV) and are considered as acquired immunodeficiency syndrome (AIDS)-defining illnesses.[2]

The fungi implicated in opportunistic infections are generally only able to cause systemic infection in an immunocompromised host. There are five main diseases in this group:[2]

- Aspergillosis
- Candidosis
- Cryptococcosis (this is an exception as it can occur in immunocompetent individuals)
- Mucormycosis
- Pneumocystosis

When these fungi cause infection in immunocompromised individuals, they usually result in significant disease with high fatality rates.[2]

Four main criteria need to be met to allow fungi to cause invasive disease in humans:[6]

1. Ability to grow at or above human body temperature
2. Ability to breach host barriers to access internal tissues
3. Ability to break down tissues and absorb their components
4. Ability to evade the host immune system

Table 1 shows estimates of the incidence and mortality rates of the most common IFDs. **Figure 6** shows examples of the morphology of these organisms.

Mycosis	**Main etiologic agent**	**Cases per year**	**Mortality rate (%)**
Invasive candidiasis	*Candida albicans*	~750,000	~40
Invasive aspergillosis	*Aspergillus fumigatus*	>300,000	30–70
Pneumocystis pneumonia	*Pneumocystis jirovecii*	>400,000	10–60
Cryptococcal meningitis	*Cryptococcus neoformans*	~225,000	15–50
Disseminated histoplasmosis	*Histoplasma capsulatum*	~100,000	10–60
Mucormycosis	*Rhizopus oryzae*	>10,000	35–100

Table 1: ***Estimated incidence and mortality rates of the most frequent IFDs affecting humans.***[6]

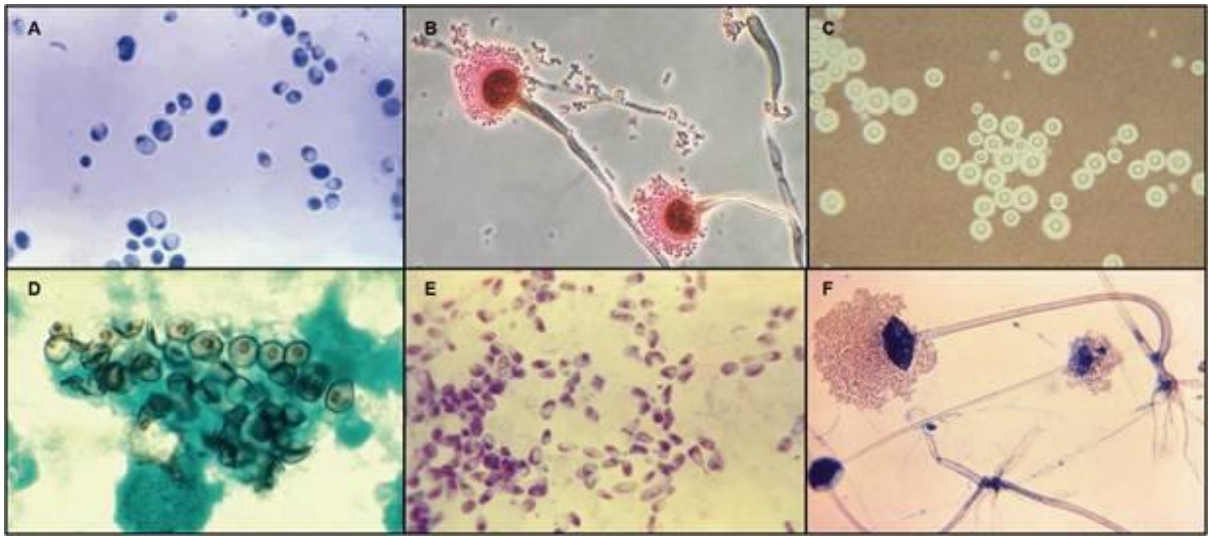


Figure 6: Photomicrographs of the most common fungal pathogens causing invasive diseases in humans*. A: Candida albicans in its yeast-phase, B: Filamentous conidiophores of Aspergillus fumigatus. C: Encapsulated yeasts of Cryptococcus neoformans. D: Pneumocystis jirovecii cysts. E: Histoplasma capsulatum in its yeast phase. F: Reproductive sporangia of Rhizopus oryzae*.[6]

2.3.4 Emerging causes of invasive fungal disease

In recent years, some fungal species previously considered harmless have been recognized as a cause of life-threatening infection in people who are immunocompromised (e.g., non-aspergillus molds such as *Zygomycetes* or the soil fungus, *Fusarium*).[2,11,12] These emerging pathogens are important as they frequently show resistance to amphotericin B, which is often used in the empirical treatment of suspected fungal infections in neutropenic patients.[2]

2.4 Epidemiology of Invasive fungal diseases

Over the last few decades, the global burden of IFDs has been increasing as a consequence of a rise in the number of at-risk individuals who are more susceptible to these infections. This at-risk population includes people with HIV, transplant recipients, cancer patients, and patients treated with other immunosuppressive medication.[2,13]

Worldwide, it is estimated that over 1.7 million people die from fungal infections each year.[14] Accurate calculation is hampered by various factors, including variable access to and reliability of diagnostic methods as well as a lack of established surveillance systems in the majority of countries.[15]

Most IFDs are attributable to opportunistic fungi such as *Aspergillus*, *Candida*, *Cryptococcus*, *Rhizopus*, and *Pneumocystis jirovecii*. These fungi have a worldwide distribution.[13] Infections due to dimorphic fungi endemic in North America (e.g., *Histoplasma capsulatum* and *Coccidioides spp*.) are also increasing in incidence. In addition, more frequent international travel has led to these infections being encountered outside of the regions where they are endemic.[2]

There is significant disparity between the pattern of invasive fungal disease seen in more developed countries compared with that seen in the developing world.[2] In the developed world, opportunistic infections are seen more often in the context of immunosuppressive therapies, whereas in developing countries, they are more likely to be AIDS-associated.[2]

2.5 Overview of the most common invasive fungal diseases

2.5.1 Invasive candidosis

Candida spp. can cause opportunistic invasive infections in those who are immunocompromised or otherwise debilitated.[2] **Table 2** summarizes the risk factors for development of invasive candidosis. The species most often implicated in both superficial and invasive *Candida* infections is *Candida albicans*.[2]

Patients more at-risk of invasive candidosis include those who:

- Have spent a prolonged time in intensive care
- Have central venous lines
- Have a weakened immune system
- Have had recent major surgery
- Have been treated with multiple courses of antibiotics in hospital
- Are receiving total parenteral nutrition
- Are on dialysis
- Have diabetes
- Pre-term babies
- Inject illicit drugs

Table 2: Summary of at-risk groups for invasive candidosis.[6]

Most cases of invasive infection are endogenous in origin, but transmission can occur between individuals. For example, outbreaks of *C. albicans* in intensive care patients have been transmitted via the hands of healthcare workers.[2]

Examples of how invasive candidosis may manifest clinically include:

- Chronic disseminated candidosis (also known as hepatosplenic candidosis): Can occur in patients with leukemia. Following colonization of the gastrointestinal tract, the organism is thought to invade the liver via the hepatic portal vein. Infection usually begins while the patient is neutropenic, but as their neutrophil count recovers with treatment, the infection continues, often leading to persistent fever and weight loss.[2]
- Candidaemia (isolation of *Candida* on blood culture) and acute multi-organ disseminated candidosis: Most common in seriously unwell patients, usually with at least one indwelling vascular line. In these patients, the route of infection is often thought to be via translocation of *Candida* across the wall of the intestine into the bloodstream.[2,5]
- Endocarditis: *Candida* is the most common fungal cause of endocarditis. Risk factors are similar to bacterial endocarditis (e.g., valvular heart disease, prosthetic heart valves, intravenous drug abuse), as is the clinical presentation.[2]

2.5.1.1 Candida auris

Candida auris is an emerging fungal pathogen first identified in Japan in 2009 and has now been isolated in over 35 countries.[16,17] It has raised significant concern as a global health threat for three main reasons:

1. It tends to be resistant to multiple classes of anti-fungal drugs
2. It can be hard to detect using standard laboratory methods
3. It has caused outbreaks in healthcare settings [17]

2.5.2 Invasive aspergillosis

Aspergillosis refers to infections caused by molds of the genus *Aspergillus*.[2] These molds have a worldwide distribution and can be identified in indoor and outdoor air.[2] Most people will inhale *Aspergillus* spores every day without developing any health problems.[18] However, disease can occur in those who are immunocompromised and in some individuals with underlying lung diseases.[18] The types of disease caused by *Aspergillus* are summarized in **Table 3**. Invasive aspergillosis has a high case fatality rate of 50-100% in immunocompromised patients.[2]

Allergic bronchopulmonary aspergillosis	Allergic response to *Aspergillus* in the lungs, leading to cough and wheeze
Allergic aspergillus sinusitis	Allergic response to *Aspergillus* affecting the sinuses
Azole-Resistant aspergillus fumigatus	Treatment-resistant infection with *Aspergillus fumigatus*
Aspergilloma	Growth of a 'fungus ball' in the lungs or sinuses
Chronic pulmonary aspergillosis	Cavitating lung disease secondary to *Aspergillus* infection in the lungs
Invasive aspergillosis	Mainly in the immunocompromised. It usually affects the lungs but can spread to other parts of the body.
Cutaneous aspergillosis	Usually secondary to traumatic implantation in the immunocompromised

Table 3: Types of Aspergillus infection.[18]

2.5.3 Pneumocystis pneumonia

Pneumocystis pneumonia (also known as PCP) is caused by infection with the *Pneumocystis jirovecii*, which is now considered a fungus after being previously classified as a protozoan parasite.[19] Inhalation of *P. jirovecii* can lead to life-threatening pneumonia in the immunocompromised – and particularly in those who have HIV/AIDS.[2,19]

Pneumocystis fungi are ubiquitous organisms that live in the lungs of mammals, with *P. jirovecii* being found in human lungs.[2] Pneumocystis is not known to have an environmental form.[2] Pneumocystis is thought to spread from person to person through the air.[19]

2.5.4 Cryptococcosis

Cryptococcosis refers to infections caused by yeasts from the *Cryptococcus* genus, of which the majority are caused by two species – *Cryptococcus neoformans* and *Cryptococcus gattii*.[2] These environmental organisms have a worldwide distribution.[2,20] Although cryptococcosis can occur in immunocompetent individuals, the majority of infections are in the immunocompromised.[2,20]

C. neoformans tends to be found in soil, on decaying wood, or in bird droppings.[20] *C. gattii* lives mainly on trees and in the soil around trees and is mostly isolated in tropical and subtropical regions.[20]

The route of infection is usually via inhalation. Some evidence suggests that cryptococcosis may occur most often as a result of reactivation of a latent infection.[2] Infection of the lungs tends to occur first and can cause a pneumonia-like illness, although it can be asymptomatic.[2,20] However, the most common clinical presentation is with meningitis (following the spread of the fungus from the lungs to the brain) and disseminated infection can occur.[2] Mass lesions – known as cryptococcomas – can occur in the brain.[2]

C. gattii infection occurs mostly in those who are immunocompetent, whereas *C. neoformans* is more associated with immunocompromise (with over 80% of cases being linked to HIV infection).[2]

2.5.5 Mucormycosis

Mucormycosis is caused by a group of molds known as mucormycetes.[21] The organisms most often implicated in human infections are *Rhizopus oryzae* and *Rhizopus microsporus*.[2] Molds associated with mucormycosis are often ubiquitous in the soil and decomposing matter and can be found in the air both indoors and outdoors.[2] **Table 4** outlines the different types of mucormycosis in humans.

Rhinocerebral mucormycosis:	Sinus infection which can spread to the brain
Pulmonary mucormycosis:	Most often found in those with cancer and transplant patients
Gastrointestinal mucormycosis:	Most often found in children, particularly premature and/or low birth weight neonates
Cutaneous mucormycosis:	Usually occurs after traumatic implantation and is the most common type of mucormycosis in immunocompetent individuals.
Disseminated mucormycosis:	Most commonly affects the brain

Table 4: Types of Mucormycosis infection.[21]

2.5.6 Summary of other types of fungal disease

Table 5 summarizes features of other endemic mycoses that tend to occur in geographically distinct parts of the world.

Disease	Geographical Distribution	Who is Affects	Type of Infections
Talaromycosis	East and South-East Asia	Immunosuppressed individuals – primarily those with HIV	Usually disseminated infection often affecting liver, skin, GI tract, lymph nodes, and bone marrow
Blastomycosis	United States and Canada	Immunocompetent and immunosuppressed	May be asymptomatic. Around 50% develop symptoms of lung infection. Disseminated disease more likely in the immunocompromised
Coccidiodomycosis	Southern United States, Central and South America	Immunocompetent and immunosuppressed	May be asymptomatic but can cause acute or chronic lung disease and sometimes disseminated disease
Paracoccidiodomycosis	Central and South America	Immunocompetent and immunosuppressed	Many remain asymptomatic. Reactivation of disease in adults can lead to chronic, progressive granulomatous disease of lungs, skin, and other organs. Juvenile form can occur – uncommon but has poor prognosis
Histoplasmosis	Primarily in North America but also found in parts of Central and South America, Africa, Asia, and Australia	Immunocompetent and immunosuppressed – but symptoms more likely if immunocompromised	Primary infection is usually subclinical. Symptomatic infection is more likely in the immunocompromised or those with high-level exposure. Symptoms are usually of acute pulmonary infection, but disseminated disease can occur. Latent infection can cause symptoms years after initial exposure

Table 5: Summary of other endemic mycoses.[2,22-26]

2.6 Risk factors and diagnosis of IDFs

There are three main elements involved in the diagnosis of invasive fungal disease:[27]

1. Clinical examination
2. Imaging
3. Laboratory confirmation of diagnosis

The European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium (EORTC/MSGERC) developed consensus definitions of IFDs in 2002.[28] These definitions were most recently updated in 2020.[29]

IFDs are categorized as classified as:[29]

- Proven (signs of infection and fungus identified by histopathology, cytopathology, or culture)
- Probable (based on host factors, clinical criteria, mycological evidence)
- Possible (based on host factors and clinical criteria)

The criteria for proven and probable IFD are outlined in **Tables 6, 7 and 8**. The criteria for proven IFD apply regardless of immune status. Conversely, the criteria for probable IFD are only applicable to immunocompromised patients. With the exception of endemic mycoses, probable IFD requires the presence of at least one feature from each of these categories: host factors; clinical feature; and mycologic evidence. Those cases where mycological evidence is not found are considered 'possible IFD'.[29]

Fungus	Microscopic analysis of sterile material [a]	Culture of sterile material	Blood culture	Serology	Tissue nucleic acid diagnosis
Molds	Specimen obtained by needle aspiration or biopsy. Hyphae or melanized yeast-like forms are seen with evidence of associated tissue damage	Culture of a specimen obtained by a sterile procedure from a normally sterile and clinically or radiologically abnormal site consistent with an infectious disease process. Excluding BAL fluid, a paranasal or mastoid sinus cavity specimen, and urine	Mold grown on blood culture in the context of a compatible infectious disease process Note – *Aspergillus spp*. in blood cultures usually represents contamination	Not applicable	Amplification of fungal DNA by PCR combined with DNA sequencing when molds are seen in formalin-fixed paraffin-embedded tissue
Yeasts	Specimen obtained by needle aspiration or biopsy from a normally sterile site (other than mucous membranes) showing yeast cells. E.g., *Cryptococcus spp*. indicating encapsulated budding yeasts, *Candida spp*. showing pseudohyphae or true hyphae	Culture of a sample obtained by a sterile procedure (including drain placed > 24 hours ago) from a normally sterile site showing a clinical or radiological abnormality consistent with an infectious disease process	Yeast or yeast-like fungi on blood culture	Cryptococcal antigen in CSF or blood confirms cryptococcosis	Amplification of fungal DNA by PCR combined with DNA sequencing when yeasts are seen in formalin-fixed paraffin-embedded tissue
Pneumocystis	*Pneumocystis* detected in tissue, BAL fluid, or expectorated sputum using conventional or immunofluorescence staining	Not applicable	Not applicable	Not applicable	Not applicable
Endemic mycoses	Specimens obtained from an affected site showing the distinctive form of the fungus	Culture of the fungus from specimens from an affected site	Blood culture that yields the fungus	Not applicable	Not applicable

Table 6: Criteria for proven invasive fungal disease*. Adapted from Donelly et al.*[29]

[a] Tissue and cells submitted for histopathologic or cytopathologic studies should be stained using Grocott-Gomori methenamine silver stain or periodic acid Schiff stain to facilitate inspection of fungal structures.

BAL, Bronchoalveolar lavage.

<table>
<tr><th>Host factors</th><th>Clinical features</th><th>Mycological evidence</th></tr>
<tr><td rowspan="4">• Recent neutropenia (<0.5 × 10^9/L for >10 days) temporally related to onset of invasive fungal disease
• Haematologic malignancy
• Solid organ or stem cell transplant
• Doses of corticosteroids of ≥0.3 mg/kg prednisone equivalent for ≥3 weeks in the past 60 days
• T-cell immunosuppressants during the past 90 days (E.g., calcineurin inhibitors, TNFα blockers)
• B-cell immunosuppressants (E.g., Ibrutinib)
• Inherited severe immunodeficiency
• Acute refractory graft-versus-host disease grade III or IV involving the gut, lungs, or liver</td><td><u>Pulmonary aspergillosis:</u>
Presence of 1 of the following four patterns on CT:
• Dense, well-circumscribed lesions(s) +/- a halo sign
• Air crescent sign
• Cavity
• Wedge-shaped and segmental or lobar consolidation</td><td>• Any mold cultured from sputum, BAL, bronchial brush, or aspirate
• Microscopical detection of fungal elements indicating a mold in sputum, BAL, bronchial brush, or aspirate</td></tr>
<tr><td><u>Other pulmonary mold diseases:</u>

As above but also including a reverse halo sign</td><td><u>Tracheobronchitis:</u>
• Aspergillus cultured from BAL or bronchial brush
• Microscopic detection of fungal elements in BAL or bronchial brush indicating a mold</td></tr>
<tr><td><u>Tracheobronchitis:</u>

Tracheobronchial ulceration, nodule, pseudomembrane, plaque, or eschar seen on bronchoscopic analysis.</td><td><u>Sino-nasal diseases:</u>
• Mold cultured from sinus aspirate samples
• Microscopic detection of fungal elements in sinus aspirate samples indicating a mold</td></tr>
<tr><td><u>Sino-nasal diseases:</u>
• Acute localized pain
• Nasal ulcer with black eschar
• Extension from the paranasal sinus across bony barriers, including into the orbit</td><td rowspan="2"><u>Aspergillosis only:</u>
• Galactomannan antigen detected in plasma, serum, BAL, or CSF
• Positive Aspergillus PCR (2 positive tests) from plasma, serum, whole blood or BAL
• Aspergillus species cultured from sputum, BAL, bronchial brush, or aspirate</td></tr>
<tr><td><u>CNS infection:</u>
1 of the following two signs:
• Focal lesions on imaging
• Meningeal enhancement on MRI or CT</td></tr>
</table>

Table 7: Criteria for probable invasive pulmonary mold disease*. Adapted from Donelly et al.*[29]

BAL, Bronchoalveolar lavage.

Disease	Host factors	Clinical features	Mycological evidence
Candidiasis	• Recent neutropenia (<0.5 × 10^9/L for >10 days) temporally related to onset of invasive fungal disease • Haematologic malignancy • Solid organ or stem cell transplant • Doses of corticosteroids of ≥0.3 mg/kg prednisone equivalent for ≥3 weeks in the past 60 days • T-cell immunosuppressants during the past 90 days (E.g., calcineurin inhibitors, TNFα blockers) • B-cell immunosuppressants (E.g., Ibrutinib) • Inherited severe immunodeficiency • Acute refractory graft-versus-host disease grade III or IV involving the gut, lungs, or liver	At least 1 of the following after an episode of candidemia in the preceding two weeks: • Small, target-like abscesses in the liver or spleen (bull's-eye lesions) or brain • Meningeal enhancement • Progressive retinal exudates or vitreal opacities	• Fungitell ® assay[a] ≥80 ng/L in ≥2 consecutive serum samples provided other etiologies excluded • Positive T2Candida[b]
Cryptococcosis	• HIV infection • Solid organ or stem cell transplant • Hematological malignancy • Antibody deficiency (e.g., CVID) • Immunosuppressive therapy (inc. monoclonal antibodies) • End-stage liver or renal disease • Idiopathic CD4 lymphopenia Note – can occur in immunocompetent hosts	• Meningeal inflammation • Radiological lesion consistent with cryptococcal disease	• Recovery of *Cryptococcus* from a specimen obtained from any non-sterile site
Pneumocystosis (excluding HIV-associated	• Low CD4 count for any reason (< 200) • Medication associated with T cell dysfunction (anti-neoplastic, anti-inflammatory, immunosuppressive) • Doses of corticosteroids of ≥0.3 mg/kg prednisone equivalent for ≥2 weeks in the past 60 days • Solid organ transplant	Any consistent radiographic features, including: • Bilateral ground-glass opacities, • Consolidations • Small nodules • Infiltrates (unilateral, lobar, multifocal)	• Fungitell ® assay ≥80 ng/L in ≥2 consecutive serum samples provided other etiologies excluded • Detection of *P jirovecii* DNA by quantitative RT-PCR in a respiratory tract specimen

		• Nodular infiltrate +/- cavitation • Miliary pattern Respiratory symptoms (cough, dyspnea, and hypoxemia) accompanying radiographic abnormalities.	
Endemic mycoses	Affect both immunocompetent and immunocompromised.	Evidence for exposure to the fungus and compatible clinical illness.	• *Histoplasma* or *Blastomyces* antigen in urine, serum, or body fluid • *Coccidioides* antibody in CCSR or a 2-fold rise in 2 consecutive serum samples

Table 8: Other probable invasive fungal diseases*. Adapted from Donelly et al.*[29]

[a] Fungitell ® assay – test for IFD (including *Candida, Aspergillus* and *Pneumocystis*), detects (1→3)-β-D-Glucan in serum

[b] T2Candida – U.S. Food and Drug Administration (FDA) approved for detection of various *Candida spp.* in blood

2.7 Pulmonary CT in the diagnosis of fungal infection

IFDs account for around 15% of severe respiratory infections in immunocompromised patients, with the most important causes being *Pneumocystis jirovecii*, *Aspergillus spp* and *Cryptococcus spp*.[30,31] The presentation of these infections is non-specific. Findings on pulmonary CT can provide clues to the diagnosis.[30,31] **Figure 7** shows some pulmonary CT findings associated with specific IFDs.

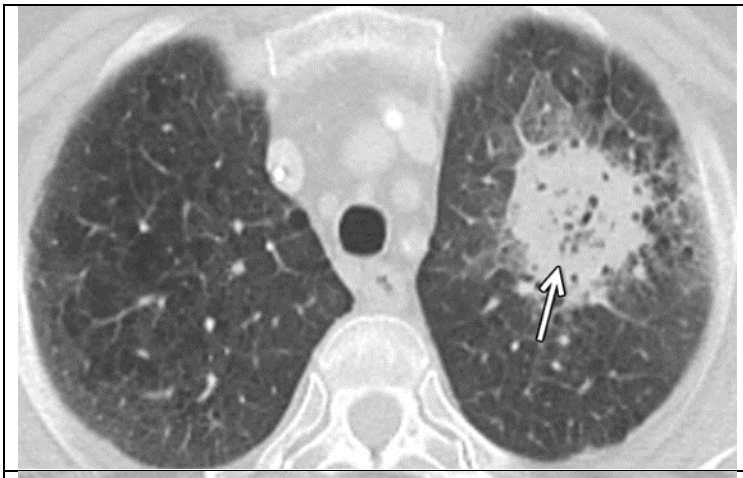
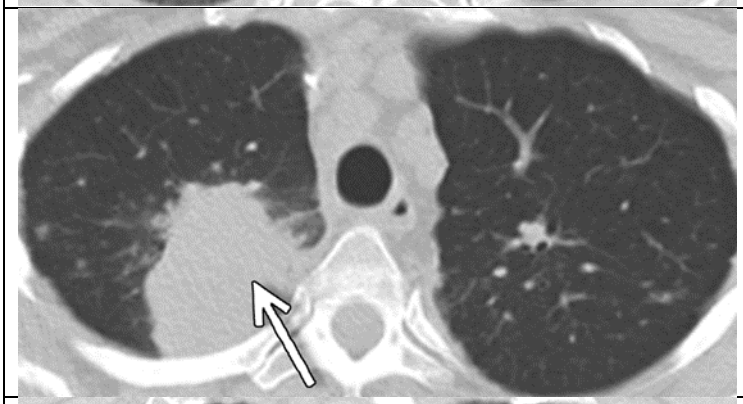
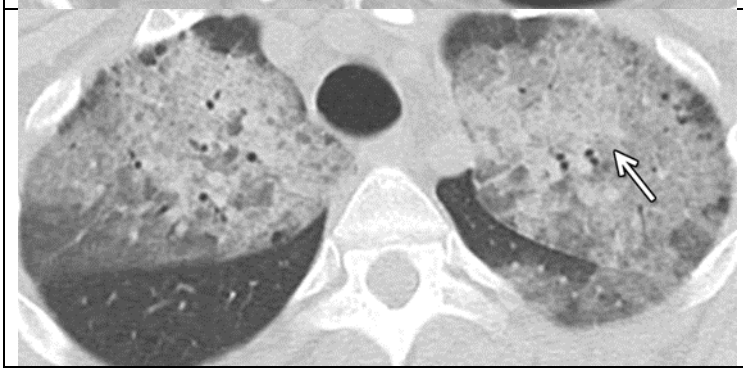
	a) Invasive pulmonary aspergillosis – arrow shows a mass-like area of airspace disease with air bronchograms and a halo of ground-glass opacity ('halo' sign – can also occur with *Candida* and *Cryptococcus* infections).
	b) Pulmonary cryptococcosis – arrow shows a right upper lobe mass with a surrounding area of ground-glass opacity and other scattered nodules.
	c) Pneumocystis pneumonia – main finding is patchy or ground-glass opacity with smooth septal line thickening ('crazy paving').

Figure 14: Pulmonary CT appearances in IFDs. *Images and text adapted from Orlowski et al.*[31]

2.8 Key summary points

- Fungal infections in humans can be superficial, subcutaneous, or systemic
- Only a small number of pathogenic fungi can cause invasive disease in an immunocompetent host
- The majority of IFDs are seen in immunocompromised individuals
- Fungal diseases are increasing in incidence due to a corresponding increase in the at-risk population
- Diagnosis of IFDs can be challenging as there is often significant clinical overlap with other conditions
- Earlier diagnosis of IFDs is important to allow timely and appropriate treatment

2.9 Multiple choice questions

Qu 1: How many species of fungi have been shown to cause disease in humans? Choose the closest number?

a) 20

b) 500

c) 2000

d) 10000

Qu 2: Dermatophyte infections cannot be transmitted between individuals?

a) True

b) False

Qu 3: Allergic bronchopulmonary aspergillosis only occurs in the immunocompromised?

a) True

b) False

Qu 4: Which of the following fungal infections is typically associated with a 'crazy paving' appearance on pulmonary CT?

a) Aspergillosis

b) Cryptococcosis

c) Pneumocystosis

d) All of the above

e) None of the above

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Module 3: Management of Invasive Fungal Infections

It should take approximately 1.5 hour to complete modules 1-3.

Purpose

Modules 1-3 will provide a comprehensive overview of systemic treatments for invasive fungal infections (IFIs), from mechanisms of action and patients at risk, to treatment strategies and latest guidelines in specific patient population.

Learning objectives

In Module 3 you will learn:

- To describe the targets for antifungal therapy and list the main types of antifungal drugs
- To understand the mechanisms of action of antifungal agents
- To develop knowledge of the common and unique toxicities of antifungal agents
- To describe the clinical landscape and key clinical trial data for various antifungal agents
- To understand the differences for the management of IFDs In the following patient populations:
    - Hemato-oncology patients
    - Critically ill patients in intensive care
    - Pediatric patients

3.1 Introduction to antifungal agents

The most commonly used antifungal agent classes used in clinical practice to treat invasive fungal infections (IFIs) include azoles, polyenes, echinocandins and occasionally pyrimidine analogs:[1,2]

- **Azoles**: these are small molecules that inhibit the membrane-bound enzyme lanosterol 14a-demethylase (Erg11), a member of the cytochrome P450 superfamily. Erg11 inhibition blocks ergosterol biosynthesis and causes deterioration in the fungal cell membrane.[1] Amphotericin B, is the only systemic polyene agent; nowadays it is primarily given as a liposomal formulation (L-AmB).[2]
- **Polyenes**: these are small molecules that bind irreversibly to ergosterol and destabilize the phospholipid membrane (e.g., by forming ion-leaking pores) and contribute to cell death (e.g., by inducing oxidative stress and DNA damage)[1,3]
- **Echinocandins**: these are large lipopeptide molecules that noncompetitively inhibit the enzyme β-(1,3)-glucan synthase, thus blocking the glucan synthesis pathway necessary for fungal cell wall formation.[1,4]
- **Pyrimidine analogs**: these block pyrimidine metabolism and interfere with fungal RNA and DNA synthesis.[1] Agents such as flucytosine are used in rare cases in combination therapies with other antimycotics.[2]

Figure 1 depicts the mechanisms by which common antifungal agents may attack the structure and function of the fungal cell.[1]

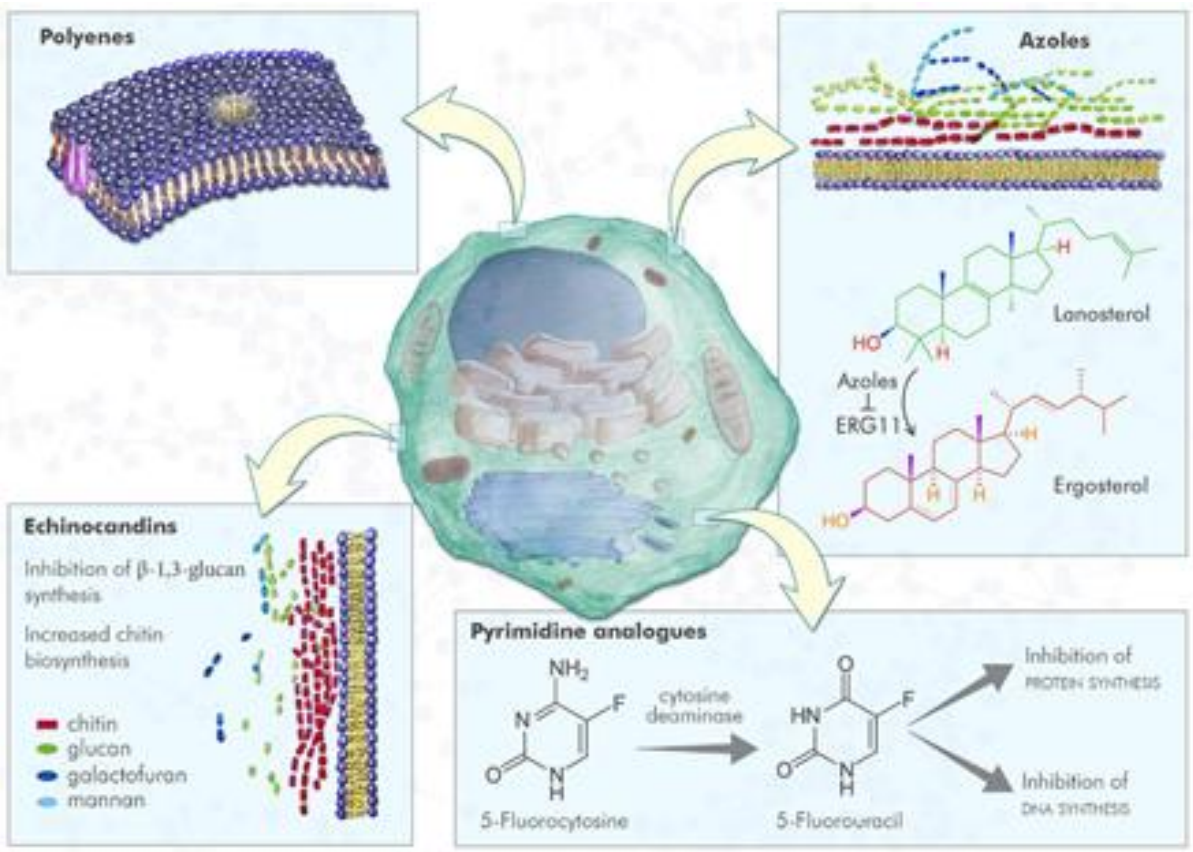


Figure 1. Mechanisms of action and biological targets of common antifungal agents. *Adapted from Di Mambro et al. (2019)*[1]

3.1.1 Common antifungal agents used in clinical practice

Table 1 lists the most commonly used Swiss-approved antifungal drugs for the treatment of IFIs. **Figure 2** summarizes the most common toxicities of antifungal agents.

Table 1. Common approved antifungal drugs in Switzerland for the treatment of invasive fungal infections.[5,6]

Antifungal class	Antifungal agent	Brand name	Common indication(s)
Azole	Fluconazole	Diflucan® Canesten®	Invasive infections due to susceptible *Candida* species; *Cryptococcosis* and *Coccidioides* infections
	Itraconazole	Itrazol®	Candidiasis: *Aspergillosis* when standard therapy is not an option or not tolerated; non-meningeal histoplasmosis; blastomycosis
	Voriconazole	Vfend®	Invasive *Aspergillosis;* non-neutropenic candidiasis; serious *Scedosporium* or *Fusarium* infections refractory to other agents
	Posaconazole	Noxafil®	Fusariosis resistant to amphotericin B; Chromoblastomycosis and mycetoma resistant to itraconazole; coccidioidomycosis resistant to amphotericin B, fluconazole or itraconazole
	Isavuconazole	Cresemba®	Invasive *Aspergillosis*
Polyene	Amphotericin B	Fungizone®	Life-threatening invasive fungal infections, including *Aspergillus*, *Blastomyces*, *Candida*, *Coccidioides*, *Cryptococcus*, *Histoplasma* as well as for pathogens of *Mucormycosis*
	Liposomal amphotericin B (L-AmB)	AmBisome®	Susceptible mycoses; empirical treatment for suspected fungal infections in patients with febrile neutropenia
Echinocandin	Caspofungin	Cancidas®	Invasive candidiasis including candidemia in neutropenic and non-neutropenic patients invasive *Aspergillosis* in refractory patients
	Micafungin	Mycamine®	Invasive candidiasis when other agents are not an option
	Anidulafungin	Ecalta®	Candidemia and invasive candidiasis when other systemic antifungals are not an option
Pyrimidine analog	Flucytosine	Ancotil®	In combination with amphotericin B for generalized candidiasis, *Cryptococcosis*, *Chromoblastomycosis* and *Aspergillosis*

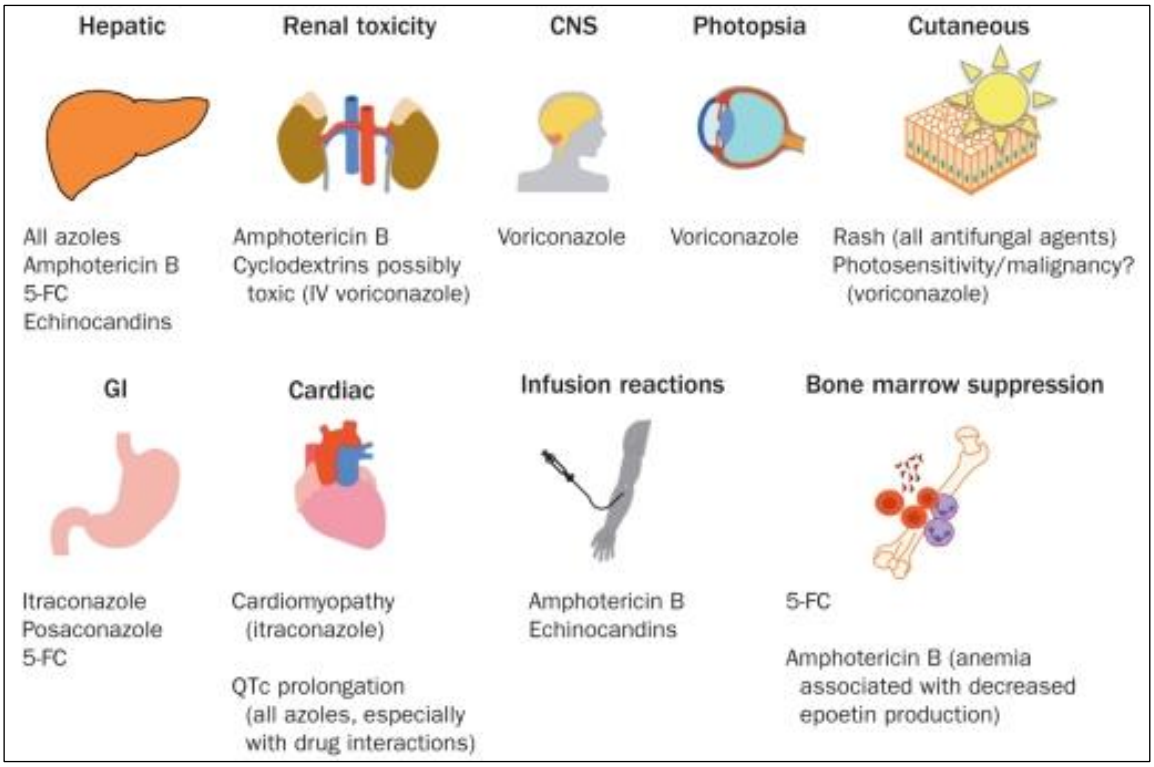


Figure 2. Toxicities of common antifungal agents.[7]
CNS, central nervous system; 5-FC, flucytosine; GI, gastrointestinal; IV, intravenous; QTc, corrected QT interval.

3.1.2 Novel antifungal agents in development

Several novel antifungal agents are currently under investigation in Phase 2 and 3 clinical trials (e.g., fosmanogepix, ibrexafungerp, olorofim, opelconazole, rezafungin).[8,9] These new agents are active against Aspergillus and Candida but lack activity against Mucormycosis.[8,9]

3.1.3 Treatment strategies

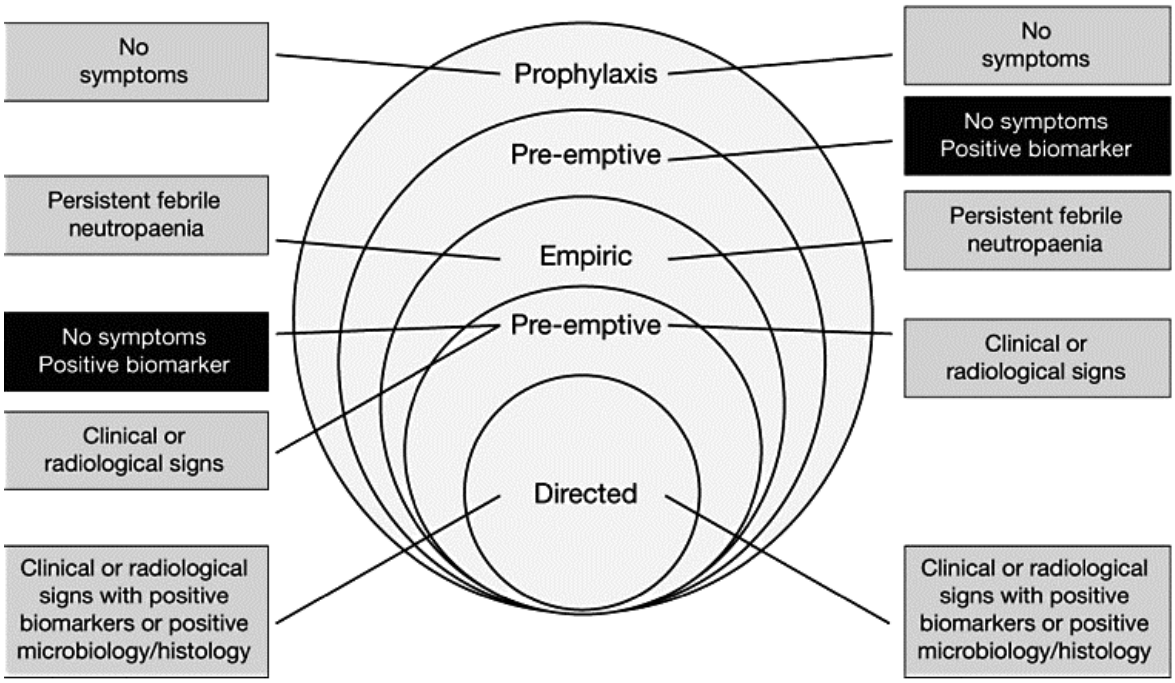
Antifungal agents can be used in different ways (**Figure 3**). IFIs can be classified as possible, probable or proven based on host, clinical and microbiological features using the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria.[10] In addition to the directed/targeted antifungal therapy for confirmed disease, approaches include prophylaxis, empirical therapy and pre-emptive/diagnostic-driven therapy.

- **Prophylaxis**: The administration of antifungals according to standard protocol in neutropenic patients with no definitive evidence of infection. The clinical efficacy of antifungal prophylaxis in the prevention of IFIs in high-risk patients has been shown in randomized controlled trials and is now recommended in international guidelines.[11,12] However, prophylaxis is not indicated in all hematologic patients.[11,12] Outside the setting of acute myelogenous leukemia (AML), myelodysplastic syndromes (MDS) and allogeneic hematopoietic stem cell transplant (HSCT), no formal recommendations can be made regarding antifungal prophylaxis.[11,12]
- **Empiric (fever-driven)**: An early approach in patients with persistent or recurrent febrile neutropenia unresponsive to broad-spectrum antibiotics. PTX3 (Pentraxin 3) and other such biomarkers may be used in the future to guide prophylaxis/empiric treatment.[13] However, this

strategy is controversial and has never been validated by solid evidence.[14-16] Indeed, empirical therapy of persistent febrile neutropenia can lead to overtreatment of many patients who experience fever not related to an invasive mold disease.[17]

- **Pre-emptive or diagnostic-driven therapy**: This strategy is usually based on the presence of specific clinical signs and fungal biomarkers, but there is no consensus on the definition and there may be overlap with empirical and targeted therapy.[17-19]

Figure 3. Variable definitions of treatment strategies and positions within the treatment continuum. Adapted from Drgona et al.[19]



3.1.4 Pivotal clinical studies (first-line treatment of IFIs)

3.1.4.1 Invasive candidiasis

Supporting evidence for the use of echinocandins as first-line antifungal therapy for invasive candidiasis in hematological patients can be found in three head-to-head, randomized, Phase 3 studies that evaluated micafungin,[20] anidulafungin[21] and caspofungin.[22]

- Micafungin is non-inferior to L-AmB for treating invasive candidiosis

Kuse et al. (2007) reported that micafungin was as efficacious as L-AmB for the treatment of candidaemia and invasive candidosis in a Phase 3 head-to-head randomised controlled trial.[20] Of the 531 patients randomized, 16% had a hematological disorder and 11.7% were neutropenic.[20] Assessment of overall treatment success at the end of the trial was defined as a complete or partial resolution of symptoms and mycological response as eradication or presumed eradication.[20] Results showed that micafungin was as effective as L-AmB and caused fewer adverse events (**Figure 4**).[20] Moreover, efficacy of micafungin was not affected by Candida species, site of infection or prognostic

factors (i.e., neutropenia, APACHE II score or catheter status).[20] Infusion-related adverse events were statistically more frequent in patients treated with L-AmB, but overall numbers of adverse events, serious adverse events and adverse events leading to treatment discontinuation were not significantly different between the two drugs.[20]

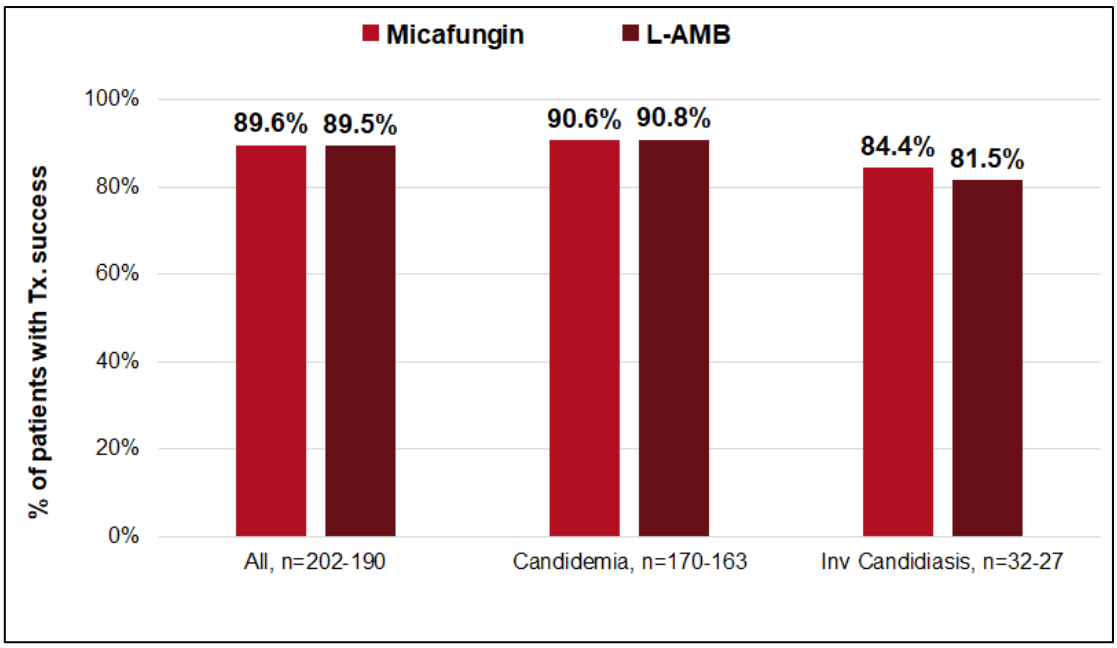


Figure 4. Treatment success demonstrating non-inferiority of micafungin versus L-AmB.[20] *Figure shows ITT population. L-AmB, liposomal amphotericin B; ITT, intent-to-treat; all randomized patients who received study drug; Tx, treatment.*

- Anidulafungin versus fluconazole for invasive candidiasis

A Phase 3, randomized, double-blind study compared anidulafungin with fluconazole as primary treatment of systemic candidiasis in adult patients (N=261) infected with any Candida species, except C. krusei.[21] Most of the patients (97%) were non-neutropenic. Global response rates at the end of the study treatment were significantly higher with anidulafungin (75.6%) than fluconazole (60.2%), demonstrating non-inferiority of anidulafungin.[21] At the end of all therapy survival was increased with anidulafungin treatment compared with fluconazole treatment (77% versus 69%, respectively; P = 0.13).[21]

- Caspofungin versus amphotericin B for invasive candidiasis

Mora-Duarte et al. compared the echinocandin caspofungin with amphotericin B in patients with invasive candidiasis.[22] Of the 224 patients included in the modified intent-to-treat analysis, 12.9% had a hematological malignancy and 10.7% were neutropenic.[22] Results demonstrated that caspofungin was at least as effective as amphotericin B for the treatment of invasive candidiasis; the proportion of patients with a favorable response at the end of intravenous therapy was 73.4% in the caspofungin group and 61.7% in the amphotericin B group.[22] Moreover, a significantly smaller percentage of patients treated with caspofungin had infusion-related adverse events, nephrotoxic effects, or hypokalemia compared to patients treated with amphotericin B.[22]

3.1.4.2 Invasive aspergillus

Supporting evidence for the use of azoles as first-line antifungal therapy for invasive aspergillosis in hematological patients can be found in two head-to-head, randomized, Phase 3 studies that evaluated voriconazole versus amphotericin B[23] and isavuconazole versus voriconazole.[24]

- Voriconazole for first-line treatment of invasive aspergillosis

In a prospective, Phase 3, randomized trial of voriconazole or amphotericin B in 277 immunocompromised patients with invasive aspergillosis, voriconazole demonstrated superior efficacy and a 22% relative survival benefit at 12 weeks compared with amphotericin B.[23] Switches to other licensed antifungal therapy were allowed in cases of insufficient clinical response or intolerance, as well as for long-term suppression after completion of initial randomized therapy.[23] Notably, the underlying condition for most of the patients enrolled in this study was allogeneic hematopoietic-cell transplantation (24.2%), acute leukemia (42.6%), or other hematologic diseases (12.6%). Furthermore, more than half of the patients (54.9%) were non-neutropenic at baseline.[23] Fewer severe adverse events that were potentially related to the study drug occurred in the voriconazole group (13.4%) than in the amphotericin B group (24.3%; P=0.008); the most frequent events was renal impairment (in 19 patients) in the amphotericin B group (n=19 vs n=2 in the voriconazole treatment group).[23]

- Isavuconazole for first-line treatment of invasive aspergillosis (SECURE study)

The Phase 3 SECURE trial compared isavuconazole and voriconazole for the treatment of invasive mold disease caused by Aspergillus spp. and other filamentous fungi.[24] Most of the randomized patients in this study (N=527) had hematologic malignancies (84%) and 66% were neutropenic.[24] All-cause mortality through day 42 in the intent-to-treat population (N=516), the primary endpoint, was 18.6% and 20.2% in the isavuconazole and voriconazole treatment groups, respectively (**Figure 5**). In this study, isavuconazole demonstrated non-inferior efficacy and was associated with fewer drug-related adverse events and treatment discontinuation compared to voriconazole.[24]

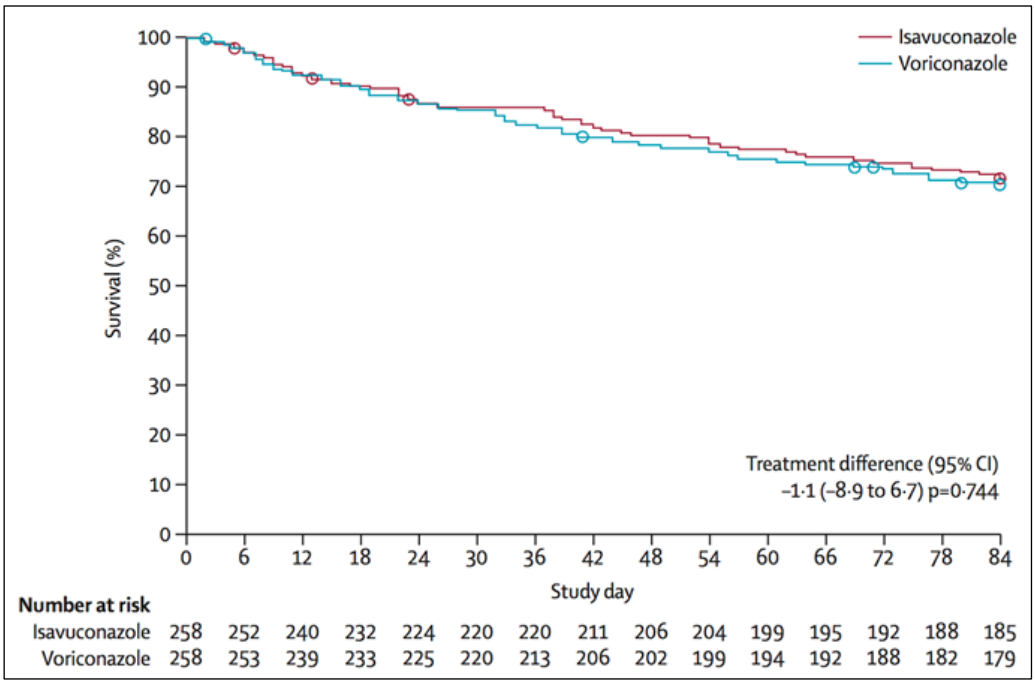


Figure 5. Survival curve demonstrating non-inferiority of isavuconazole versus voriconazole in SECURE study patients.[24] *Survival from first dose of study drug to day 84. Patients were censored on the day of their last known survival status, represented by the circles. Figure shows ITT population. ITT, intent-to-treat; all randomized patients who received study drug.*

3.1.4.3 Mucormycosis

- Isavuconazole for first-line treatment of mucormycosis

The VITAL study was an open-label, single-arm study of isavuconazole in adult patients with invasive aspergillosis and renal impairment or in patients with invasive fungal disease caused by other rare fungi. Invasive mucormycosis was proven or probable in 86% and 14% of patients, respectively.[25] The most common underlying condition was hematologic malignancy (59%) 27% of patients were neutropenic.[25] The VITAL study showed that isavuconazole was active as primary or salvage (refractory or intolerant to other antifungals) treatment for mucormycosis, with overall end-of-treatment complete and partial response of 32% for primary treatment and 36% for treatment of mucormycosis refractory to other antifungals;[25] these response rates are similar to those reported for L-AmB.[25]

3.1.5 Pivotal clinical studies (prophylaxis)

- Superior efficacy of posaconazole compared to fluconazole or itraconazole prophylaxis in patients with neutropenia

In 304 patients undergoing chemotherapy for AML or MDS, posaconazole prevented IFIs more effectively that either fluconazole or itraconazole and improved overall survival (P=0.04).[26] The absolute reduction of proven or probable IFIs in the posaconazole group was -6% (95% confidence interval, -9.7 to -2.5%; P<0.001), fulfilling statistical criteria for superiority.[26] Significantly fewer patients in the posaconazole group had invasive aspergillosis (1% vs. 20 7%; P<0.001).[26]

3.2 Management of IFIs in hemato-oncology patients

3.2.1 Overview

Treatment of invasive fungal infections (IFIs) is complex and often requires multiple treatment changes due to clinical efficacy or hepatotoxicity. Azole-based treatments are most commonly used in clinical practice and amphotericin-B products provide important broad-spectrum activity for breakthrough infections; however, options remain limited, which is a key unmet need.[11,12]

3.2.1.1 Epidemiology

The epidemiology of IFIs in hematological malignancy has evolved in recent years, driven by the development of novel antineoplastic treatments, changes in HSCT practices, and introduction of new antifungal agents for treatment and prophylaxis.

Historically, the two most common invasive fungal pathogens in high-risk haemato-oncological patients are *Candida* and *Aspergillus* species (spp.) but the incidence of non-*Candida albicans* and other organisms (e.g., *Mucorales*, *Trichosporon*, *Fusarium* spp.) is increasing.[27,28]

The introduction of antifungal prophylaxis with fluconazole and posaconazole in recent years has led to a substantial reduction of invasive candidiasis in Switzerland, whereas the incidence of aspergillosis has been increasing in this high-risk group.[28,29] Apart from *Aspergillus fumigatus*, the second most frequent cause of IFIs in Switzerland is *Mucormycosis*.[30] Azole-resistance in Aspergillus fumigatus are particularly prevalent in the Netherlands,[31] however, prevalence is currently low in Switzerland (approximately 1.3%), indicating a role for environmental and climate factors.

The incidence of invasive aspergillosis in AML patients ranges from 5% to 24%, while rates of candidaemia are less than 2%.[32] While the introduction of antifungal prophylaxis has reduced the number of IFIs and improved survival for patients with hematological malignancies, the number of breakthrough invasive fungal infections, particularly in patients using prophylaxis, is increasing and has management has thus become a matter of concern.[29,33,34] In addition, advances in treatment mean that higher-risk patients are living longer so will experience more breakthrough infections.

3.2.1.2 Patients at risk

In addition to classical risk factors for IFIs in immunocompromised patients (e.g., neutropenia, monocytopenia, increased age, etc.), environmental risk factors may play a significant role.[35] However, no clinical trial to date has demonstrated a strong association between environmental risk factors and IFIs in a prospective randomized setting.

Patients with hematological malignancies undergoing conventional chemotherapy, autologous or allogeneic hematopoietic stem cell transplantation are considered at high risk.[36] AML, MDS and more recently non-Hodgkin lymphomas (NHL), are hematologic malignancies more frequently associated with IFIs.[37] In particular, patients with long-term neutropenia following chemotherapy for AML are at risk of IFIs (**Table 2**).[38]

Furthermore, newer targeted immunotherapies such as tyrosine kinase inhibitors (TKI) and other immunomodulatory drugs put a broader spectrum of hemato-oncology patients at risk for IFIs.[12] In particular, inhibitors of bruton tyrosine kinase (BTK), e.g., ibrutinib,[39] mammalian target of

rapamycin (mTOR), janus kinase (JAK) and phosphatidylinositol 3 kinase (PI3K) delta may increase the risk of IFIs due to their impairment of critical components of the immune system.[12] An increasing number of patients with hematological malignancies historically considered low risk for IFIs have developed opportunistic infections caused by *Pneumocystis jirovecii*, *Cryptococcus neoformans*, and ubiquitous airborne filamentous fungi after receiving ibrutinib.[39] The individual risk of patients should be evaluated, and antifungal prophylaxis prescribed on a case-by-case basis.

Today, patients (traditionally stratified as low, intermediate and high risk) based on functional treatment options should be reclassified by disease risk.[36] It is yet unclear how all the above risk factors (disease, environmental, new populations, etc.) can be combined into a treatment algorithm for patients with hematological malignancies.

Table 2. Risk factors for IFIs in AML.[36]

Leukemia related	Host related	Treatment related	Environmental/ fungal exposure
• Lower Probability of CR (Adverse Cytogenetic/gene mutation profiles; WBC > 50.000/µL; Secondary AML Baseline neutropenia with ANC <500/µL for >7 d; MDS-related phagocytic dysfunction • Leukemia status: relapse-refractory > first induction > consolidation • Persistence of day 15 bone marrow blast cells • No CR by end of induction phase	• Age >65 years • Organ dysfunction with High comorbidity index or Poor Performance status (≥2) Chronic obstructive pulmonary disease • Active smoking • Immunity polymorphism • Pharmacogenomics of antineoplastic drugs	• Expected treatment related severe and prolonged neutropenia (ANC <100/µL for >10 d) • Highly mucotoxic regimen • Mucositis grade ≥3 for >7 days, especially if involving lower gut	• Rooms without HEPA filtration; Building constructions or renovations/ recent house renovation • Documented Airway Colonization By Aspergillus species • Prior Aspergillosis • Multisite colonization by Candida species • Jobs with high exposure (farming, gardening, construction work)

ANC, absolute neutrophils count; CR, complete remission; WBC, white blood cells.

3.2.2 Guidelines

Four European guidelines specific to the treatment of IFIs in high-risk patients with hemato-oncological disease are available, three of which have been published within the past five years and are considered current (**Table 3**). Guidelines/recommendations for empirical or pre-emptive antifungal therapy in neutropenic patients with cancer are partly outdated.

Table 3. Management guidelines for treatment IFIs in hemato-oncology patients

Guidelines	Year of publication	Organization	Reference
Primary prophylaxis of IFIs in patients with hematological malignancies	2018	AGIHO/DGHO	12
Primary antifungal prophylaxis in adult hematology patients	2018	ECIL/EBMT/EORTC/ICHS/ELN	11
Treatment of invasive candidiasis, aspergillosis and mucormycosis in leukemia and HSCT patients	2017	ECIL	40
Diagnosis and management of Candida infections in adults with hematological malignancies and after HSCT	2012	ESCMID	41

AGIHO/DGHO, Infectious Diseases Working Party of the German Society of Hematology and Oncology; EBMT, European Group for Blood and Marrow Transplantation; ECIL, European Conference on Infections in Leukemia; ELN, European LeukemiaNet; EORTC, European Organization for Research and Treatment of Cancer; ESCMID, European Society of Clinical Microbiology and Infectious Diseases; HSCT, hematopoietic stem cell transplant; ICHS, Immunocompromised Host Society.

3.2.3 Treatment

Note: A summary of the strength of recommendations and level of evidence are provided in Table A1 in the Appendix.

3.2.3.1 Primary prophylaxis

The ECIL guidelines recommend azoles as the first-choice prophylaxis for patients receiving intensive remission-induction chemotherapy for AML or MDS; notably, posaconazole remains the drug of choice when the incidence of invasive mold diseases exceeds 8% and in patients with neutropenia (Grade A, level of evidence I).[11,12]

Fluconazole as part of an integrated care strategy remains a valid option for patients at low-risk of invasive mold disease (incidence less than 8%; Grade B, level of evidence I).[11] Primary antifungal prophylaxis is not recommended in patients with low-to-intermediate MDS, MM, ALL, CLL, chronic myeloproliferative neoplasms (MPNs), as these are deemed low risk for IFIs[11] L-AmB has been more extensively studied.

3.2.3.2 Treatment for breakthrough infections

There is limited clinical experience and evidence from the literature regarding breakthrough IFIs. Use of the broad-spectrum triazole, posaconazole, is not yet associated with significant environmental pressure; however, rare molds may not be susceptible to it. Amphotericin-B products remain important for the treatment of breakthrough invasive mold infections.

3.2.3.3 Primary targeted/directed treatment

The sixth European Conference on Infections in Leukemia (ECIL-6) provides recommendations for the management of IFIs in patients with hematologic malignancies or HSCT recipients (Grade A, level of evidence II).[40]

***Invasive candidiasis**:* Echinocandins (e.g., micafungin, anidulafungin and caspofungin) are considered the preferred first-line choice for invasive Candida infections in the empiric setting before species identification in neutropenic and non-neutropenic patients.[40,42,43] L-AmB should be considered an alternative in case of contraindication to echinocandins (Grade A, level of evidence II).[40,42]

Fluconazole and voriconazole are recommended as possible alternatives in patients without prior azole exposure.[40,42]

***Invasive aspergillus**:* The azoles voriconazole and isavuconazole are recommended as first-line treatments (Grade A, level of evidence I).[42] More recently, in a setting high triazole resistance of *Aspergillus*, combination therapy with L-AmB was selected the preferred primary treatment strategy against invasive aspergillosis;[44] however, first-line combination antifungal therapy is not recommended in the 2017 ECIL guidelines.[40]

***Mucormycosis**:* A multidisciplinary approach is required, including surgery, control of underlying conditions and antifungal therapy (Grade A, level of evidence II). There is evidence of a beneficial role of L-AmB combined with surgery (level of evidence BII for a 5 mg/kg daily dose).[40,45] In vitro studies have demonstrated that amphotericin B, posaconazole and isavuconazole are the most potent agents.[40]

3.2.6 Key summary points

- Treatment of IMIs is complex
- The most frequent causes of IFIs in Switzerland are *Aspergillus fumigatus* and *Mucormycosis*
- Azole-resistance in *Aspergillus* (spp.) is currently low in Switzerland
- New populations at-risk of IFIs are emerging, including patients treated with newer biologic agents or small-molecule kinase inhibitors such as ibrutinib
- Guidelines/recommendations for antifungal therapy in hemato-oncology patients are partly outdated.
- Echinocandins are the preferred first-line antifungal therapy for invasive candidiasis
- Azoles are the preferred first-line treatment for invasive aspergillosis
- Prophylaxis with posaconazole is preferred when the incidence of IFIs exceeds 8% and in patients with neutropenia
- Amphotericin-B products remain important for the treatment of unspecified IFIs

3.3 Management of IFIs in ICU patients

3.3.1 Overview

IFIs are increasing among patients admitted to ICUs, attributable to the growing use of complex surgical procedures, invasive medical devices and long-term, broad-spectrum antibiotic therapy.[46] Two main life-threatening pathogens, invasive candidiasis and invasive aspergillosis, are the most important fungal diseases in terms of occurrence rates. Despite being the gold standard, culture-based microbiological methods have suboptimal sensitivity for Candida identification, missing almost 50% of cases.[47]

During the last decade, rare opportunistic pathogens, including yeast-like and other filamentous fungi, have emerged as additional causes of concern in the ICU.[48] Although the incidence of emerging IFIs such as *Mucorales*, *Saprochaete*, *Fusarium* and *Scedosporium* remains low in critically ill patients, at 4 per 1000 admissions, they are associated with high mortality rates (approximately 85%).[48] Furthermore, invasive pulmonary aspergillosis (IPA) has recently emerged as an additional complication for ICU patients, especially in patients with severe influenza, liver cirrhosis, COPD or Covid-19.[46,49,50]

3.3.1.1 Epidemiology

The two most common IFIs in non-neutropenic critically ill patients are invasive candidiasis and invasive aspergillosis. In Europe, the cumulative incidence of invasive candidiasis is approximately 7.07 episodes per 1000 ICU admissions.[51] It is also associated with high mortality (40-60%).[52] The most frequent clinical forms of invasive candidiasis in critically ill patients are candidemia and intra-abdominal candidiasis, which affect up to 5% of all ICU admissions.[53,54] A Swiss study reported that one-third of candidaemia occurs in the ICU.[55]

The incidence of Aspergillus spp. infections ranges from about 0.3% to 6.9% in ICU patients.[56] Invasive pulmonary aspergillosis is an emerging co-infection in patients with influenza who are admitted to the ICU and on mechanical ventilation.[57,58] More recently, the SARS-CoV2 infection has been has been associated with an increased risk of invasive pulmonary aspergillosis, named COVID-19-associated IPA (CAPA).[57] CAPA is a life-threatening complication with a reported incidence in the ICU of 4 to 35%.[49] The severe lung damage and immunologic derangement resulting from SARS-CoV-2 infection or its treatment predispose to superinfections with multiple pathogens, including bacteria, other viruses, and fungi.[59] Notably, many patients with CAPA do not show classic host risk factors, such as immunosuppression from organ transplant or neutropenia.[60] Prolonged invasive or non-invasive respiratory support, as well as the impact of corticosteroids and/or immunobiological therapies seem to play a critical role in increasing the risk of invasive pulmonary aspergillosis.[57] It is important to identify the onset of Aspergillus spp. Infections early in patients affected by COVID-19.[57]

3.3.1.2 Patients at risk

There are many risk factors associated with the development of invasive candidiasis for patients in the ICU (**Figure 6**).[61] In particular, the use of broad-spectrum antimicrobials, immunosuppressive drugs, and total parenteral nutrition alongside iatrogenic interventions which breach natural barriers to infection.[62] In addition, invasive procedures that disrupt natural skin or mucosal barriers, such as intravascular catheters, gastrointestinal tract surgery, and chemotherapy-associated mucositis, as

well as decreased host defenses, in particular neutropenia, facilitate local invasion and further candidemia.[61] **Table 4** summarizes the risk factors for developing invasive aspergillosis in the ICU.

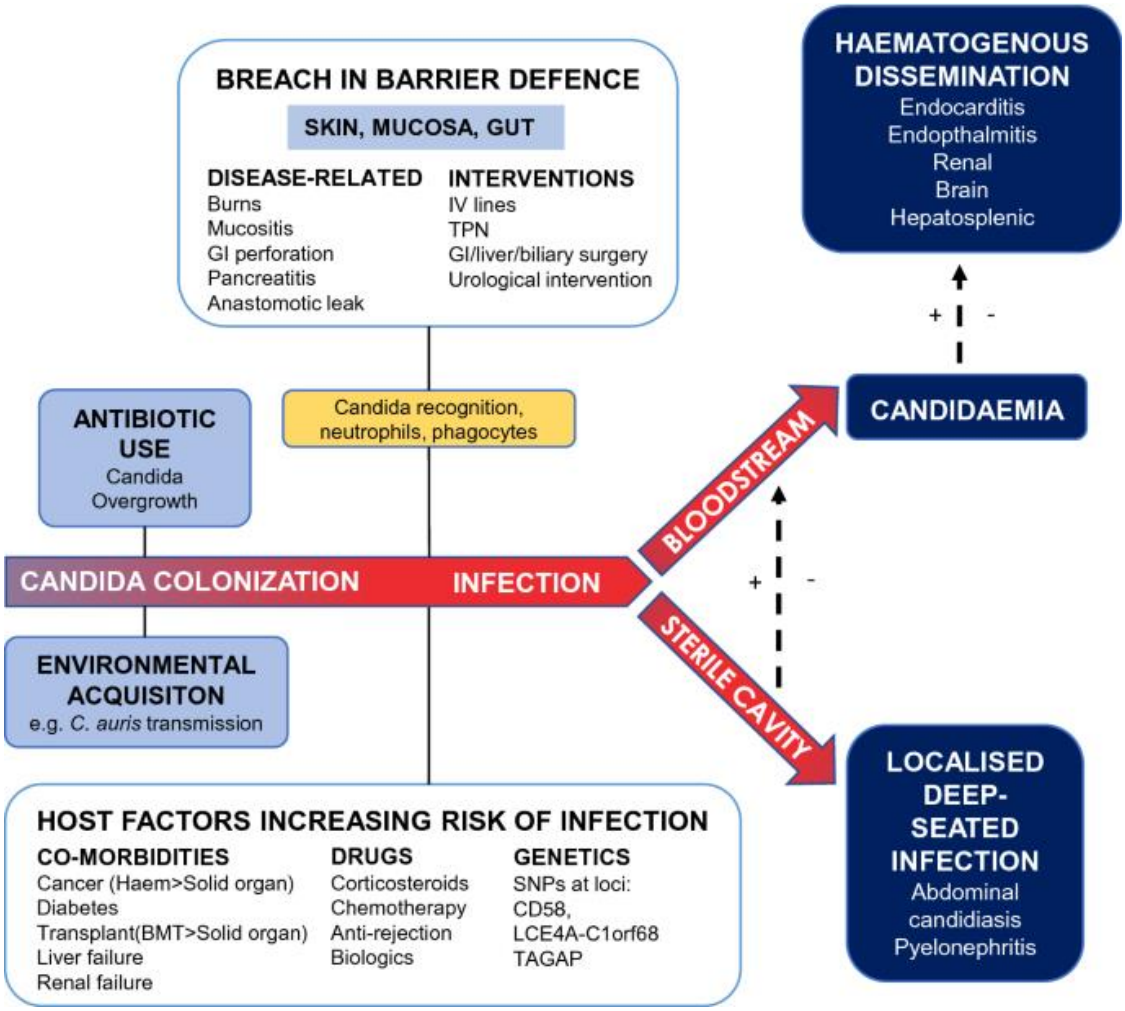


Figure 6. Risk factors for invasive candidiasis in ICU patients.[62]

Table 4. Risk factors for developing invasive aspergillosis in the ICU categorized by likelihood of risk. Adapted from Tunnicliffe et al.[63]

High risk	Intermediate risk	Low risk
• Neutropenia (<500 neutrophils/mm^3) • Hematological malignancy and allogeneic bone marrow transplantation	• Prolonged treatment with corticosteroids before admission to the ICU • Autologous bone marrow transplantation • Chronic obstructive pulmonary disease • Liver cirrhosis with a duration of stay in the ICU 17 days • Solid-organ cancer • HIV infection • Lung transplantation	• Severe burns • Other solid-organ transplant recipients (e.g., heart, kidney, or liver transplant recipients) • Steroid treatment with a duration of 7 days • Prolonged stay in the ICU (121 days) • Malnutrition • Post-cardiac surgery status

	• Systemic diseases requiring immunosuppressive therapy • anti-tumor necrosis factor therapy • Covid-19	

3.3.2 Guidelines

The ESICM/ESCMID (European Society of Intensive Care Medicine and the European Society of Clinical Microbiology and Infectious Diseases) have published guidelines for critically ill patients (**Table 5**).

Table 5. Management guidelines for treatment IFIs in ICU patients

Guidelines	Year of publication	Organization	Reference
ESICM/ESCMID task force on practical management of invasive candidiasis in critically ill patients	2019	ESICM/ESCMID	[64]
Management of invasive candidiasis and candidaemia in critically ill adults	2018	ESA IC scientific subcommittee	[65]

ESA IC, European Society of Anaesthesia Intensive Care Scientific Subcommittee; ESICM, European Society of Intensive Care Medicine; ESCMID, European Society of Clinical Microbiology and Infectious Diseases.

3.3.3 Treatment

Note: A summary of the strength of recommendations and level of evidence are provided in Table A1 in the Appendix.

3.3.3.1 Primary prophylaxis

Routine and universal administration of antifungal prophylaxis is not recommended in critically ill patients.[64] There is only weak-quality evidence (Grade C) to recommend the use fluconazole or an echinocandin to prevent invasive candidiasis in ICU patients.[64,65] A Cochrane analysis noted that the number needed to treat varied from 9 in high-risk patients to 188 in low-risk patients.[66] In view of this, antifungal prophylaxis is only recommended in high risk patients in ICUs with a high incidence of invasive candidiasis.[67,68]

3.3.3.2 Empiric therapy

Pre-emptive antifungal therapy is not recommended in critically ill patients (Grade C, level of evidence III).[64] Several observational studies suggest excessive unnecessary use of pre-emptive antifungal therapy.[64]

3.3.3.3 Primary targeted/directed treatment

Invasive candidiasis

Echinocandins should be used as the first treatment option in critically ill patients with septic shock and multisite organ failure (MOF) with IC (Grade C, level of evidence III).[64] Yang et al. found that caspofungin was more optimal than anidulafungin and micafungin, owing to its higher probability of a successful outcome against IC.[69] De-escalating from an echinocandin to fluconazole is recommended when the ICU patient is clinically stable and the isolate is susceptible to fluconazole (Grade A, level of evidence II).[64]

Amphotericin-B should not be used as a first-line treatment in critically ill patients with documented or suspected invasive candidiasis due to its significant nephrotoxicity. However, LAmB should be preferred over other lipid formulations when previous treatment with echinocandins and azoles has already failed (Grade A, level of evidence II).[64]

In settings with low fluconazole resistance, fluconazole should be considered as a first-line treatment option for ICU patients with low severity of disease (i.e., without septic shock and/or MOF) (Grade A, level of evidence III).[64]

Notably, a meta-analysis found no evidence of a therapeutic or survival benefit from choosing between echinocandins, voriconazole, or amphotericin B formulations as first-line therapy for ICU adults with invasive infection of the Candida species.[70]

3.3.4 Key summary points

- Candida species remain the leading cause of IFIs in the ICU[71]
- Emerging IFIs in ICU patients include invasive pulmonary aspergillosis and CAPA
- Prompt diagnosis and treatment, in conjunction with source control, are the key to improving outcomes[71]
- Evidence for prophylactic therapy for ICU patients remains weak
- Pre-emptive antifungal therapy is generally not recommended in critically ill patients
- Echinocandins are recommended as first-line therapy in candidaemia, with de-escalation to fluconazole when clinical stability is achieved

3.4 Management of IFIs in pediatric patients

3.4.1 Overview

There has been a significant increase in the number of pediatric patients at risk of IFIs, primarily because of the increasing use of immunosuppressive medications across many medical specialties.[72] However, there is a lack of pediatric specific evidence with respect to management strategies, prophylactic and targeted treatment as well the lack of dosing recommendations for the newer antifungals, which makes management of IFIs in pediatric patients challenging for clinicians.[73] Personalized assessment is indicated in individual patients based on specific individual risk factors and new treatments.[74]

3.4.1.1 Epidemiology

Candida spp are the most common invasive fungal disease among pediatric patients and *aspergillosis spp* and organisms from the *Mucorales* family are the most common causes of invasive mold disease in children.[72] *Candidemia* and invasive *aspergillosis* are associated with significant increases in hospital lengths of stay.[72] Overall in-hospital mortality rates are reported as 15.8% for pediatric patients with *candidemia* and 18% for children with invasive *aspergillosis*.[72] In recent years, there has been an increasing shift toward other non-Aspergillus molds such as *Fusarium*, *Scedosporium*, and *Mucorales*.[72]

3.4.1.2 Patients at risk

There is a broad range of pediatric patients vulnerable to IFIs and includes children who receive chemotherapy for a malignancy, pediatric hematopoietic stem cell transplantation (HCT) or solid organ transplant (SOT) recipients, children with a primary immunodeficiency (PID), children who receive immunomodulating therapy for an autoimmune condition, and those with an acquired immunodeficiency.[72] In addition, neonates and children hospitalized in an intensive care unit (ICU), among other groups, are also at risk for IFD.[72]

3.4.3 Guidelines

A few guidelines are available with recommendations for the management of IFIs in pediatric patients (**Table 6**). The most recent pediatric guidelines have been published by the 8th European Conference on Infections in Leukamia (ECIL-8).[74]

Table 6. Management guidelines for treatment IFIs in pediatric patients

Guidelines	Year of publication	Organization	Reference
Diagnosis, prevention, and treatment of invasive fungal diseases in paediatric patients with cancer or post-haematopoietic cell transplantation	2020	ECIL-8	[74]
Clinical Practice Guideline for Systemic Antifungal Prophylaxis in Pediatric	2020	Pediatric Oncology Group of Ontario	[75]

Patients With Cancer and HSCT Recipients			
Diagnosis and management of invasive aspergillosis in neonates and children	2019	ESCMID-ECMM	[76]
Prevention and management of invasive infections in neonates and children caused by Candida spp.	2012	ESCMID	[77]

ECIL, European Conference on Infections in Leukemia; ESCMID, European Society of Clinical Microbiology and Infectious Diseases; ECMM, European Confederation of Medical Mycology; HSCT, hematopoietic stem cell transplant.

3.4.4 Treatment

Note: A summary of the strength of recommendations and level of evidence are provided in Table A1 in the Appendix.

3.4.4.1 Primary prophylaxis

Primary antifungal prophylaxis is strongly recommended in pediatric patients who are at high risk of developing IFIs (Grade A, level of evidence II); notably, IFIs with an incidence of ≥10% are usually considered of high risk.[74] High-risk patients include those receiving intensive chemotherapy for AML, high-risk acute lymphoblastic leukemia, and recurrent acute leukemia, especially during prolonged courses of glucocorticosteroids or phases of long-lasting and profound granulocytopenia etc.[74]

Other options for pediatric patients with cancer or after hematopoietic cell transplantation include prophylaxis with fluconazole for patients with leukemia and allogeneic HCT in the pre-engraftment phase and posaconazole delayed-release tablets for patients aged 13 years or older (both Grade A, level of evidence II);[74] itraconazole for patients aged 2 years or older, 1 mg/kg L-AmB and voricanaole for patients aged 2 to 12 years or 12-14 years and weighing less than 50kg (Grade B, level evidence II).[74]

3.3.3.2 Empiric therapy

Empirical antifungal therapy can be considered in pediatric patients with persistent fever, low-risk conditions, profound and long-lasting granulocytopenia and severe mucosal damage (no grading) (Grade B, level of evidence II).[74]

Empiric caspofungin or L-AmB are recommended for pediatric patients without age restriction (Grade A recommendation, level of evidence I).[74]

Other antifungal agents such as itraconazole, voriconazole and micafungin are not approved in pediatric patients or not approved for empirical antifungal therapy, so their use cannot be recommended (Grade D recommendation, level of evidence II).[74]

3.4.4.3 Primary targeted/directed treatment

Invasive Candida

Recommendations for the management of invasive Candida spp are the same as for adults. Echinocandins (andidulafungin, caspofungin, micafungin) or L-AmB are the preferred agents before species identification (Grade A, level of evidence II). This recommendation applies to all pediatric patients, regardless of their absolute neutrophil count and hemodynamic status.[74]

Voriconazole and fluconazole are secondary options (Grade B recommendation, level of evidence II).[74]

Invasive Aspergillus

Primary treatment is the same as for adults. Voriconazole is recommended as first-line treatments (Grade A, level of evidence II).[74] Clinical trials in pediatrics with isavuconazole are ongoing.[74] L-AmB is also recommended as a first-line option if azole resistance is suspected or confirmed (Grade B, level of evidence II).[74]

Mucormycosis

L-AmB is the preferred first-line therapy, particularly for infections involving the central nervous system (CNS) or in pediatric patients with renal failure.[74]

3.4.5 Key summary points

- Pediatric patients with cancer and those undergoing allogeneic HSCT have an increased susceptibility to IFIs
- *Candida* spp are the most common IFIs among pediatric patients, but in recent years, there has been an increasing shift toward non-Aspergillus molds such as *Fusarium*, *Scedosporium*, and *Mucorales*
- There is a lack of phase 3 clinical trials to guide evidence-based interventions in pediatric patients
- The 8th European Conference on Infections in Leukaemia (ECIL-8) have published 2020 guidelines for the management of IFIs in pediatric patients
- Primary antifungal prophylaxis is strongly recommended in pediatric patients who are at high risk of developing IFIs
- Empirical antifungal therapy can be considered in pediatric patients with persistent fever, low-risk conditions, profound and long-lasting granulocytopenia and severe mucosal damage (no grading)
- Recommendations for targeted/directed therapy generally follow those for adult patients for approved antifungal agents

3.5 Multiple choice questions

Qu 1: To which class does the antifungal agent liposomal amphotericin B (L-AmB) belong?

a) Alylamines

b) Polyenes

c) Echinocandins

d) Azoles

e) Anti-metabolites

Qu 2: Which class of fungal agents inhibits fungal cell wall synthesis by inhibiting the enzyme 1,3 D-glucan synthase?

a) Alylamines

b) Polyenes

c) Echinocandins

d) Azoles

e) Anti-metabolites

Qu 3: Which of the following is considered the treatment of choice for Aspergillus infections in immunocompetent and immunocompromised patients?

a) Voriconazole

b) Voriconazole + caspofungin

c) Liposomal amphotericin B (3 mg/kg/d)

d) Amphotericin B + voriconazole

e) Liposomal amphotericin B (10 mg/kg/d)

Qu 4: In your opinion, which of the following is the best choice for *Candida* prophylaxis in adults?

a) Posaconazole in most cases

b) Fluconazole or itraconazole

c) Liposomal amphotericin B (L-AmB)

d) Voriconazole

e) None of the above

Qu 5. In a patient with *candidemia* without severe sepsis, which would be your preferred antifungal agent before knowing the species of *Candida*?

a) Wait for the full identification of the microorganism before starting any antifungal therapy

b) Voriconazole

c) An echinocandin or fluconazole

d) Liposomal amphotericin B

e) Posaconazole

Qu 6. In which of the following patients would you consider starting primary antifungal prophylaxis?

a) Low-risk patients with a hematological malignancy

b) Intermediate-risk patients with a hematological malognancy

c) Patients with chronic myeloproliferative neoplasms (MPNs)

d) In non-neutropenic patients when the incidence of IFIs is less than 8%

e) High-risk pediatric patients

Qu 7. The major problem with clinical evidence for empiric therapy has been what?

a) No clear benefit of one drug over another

b) Use of composite endpoint

c) Too few patients in studies to reach statistical significance

d) All of the above

Appendix

Table A1. Grade recommendations and Level of evidence.[11,74]

Strength of recommendation (Grade)		***Level of evidence***	
A	Strong/good evidence to support a recommendation for or against use	**I**	Evidence from at least one properly randomized controlled trial
B	Moderate evidence to support a recommendation for or against use	**II**	Evidence from at least one well-designed clinical trial without randomization; from cohort or case-controlled analytic studies (preferably from more than one center); multiple time series studies; or from dramatic results from uncontrolled studies
C	Poor/marginal evidence to support a recommendation	**III**	Evidence from opinions of respected authorities based on clinical experience, descriptive studies or reports from expert committees

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