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Aspergillus-Human Interactions: From the Environment to Clinical Significance

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Abstract

Aspergillus species are ubiquitous fungi found in the environment worldwide. The most common *Aspergillus* species causing diseases in humans are *A. fumigatus*, *A. flavus*, *A. niger*, and *A. terreus*. However, species causing human infections are also depending on human immune status. Host immune status and previous underlying diseases are important factors leading to different clinical manifestations and different disease spectra of *Aspergillus* infections. The most severe form of *Aspergillus* infections is invasive aspergillosis in human tissue, especially invasive pulmonary aspergillosis (IPA), which has high morbidity and mortality in immunocompromised patients. ICU patients with influenza infections and COVID-19 infections are recently risk factors of invasive pulmonary aspergillosis. New diagnostic criteria include galactomannan antigen assays, nucleic acid amplification assays, and lateral flow assays for early and accurate diagnosis. Voriconazole and the newest azole, isavuconazole, are antifungals of choice in IPA. Nevertheless, azole-resistant *Aspergillus* strains are increasing throughout the world. The etiology and spreading of azole-resistant *Aspergillus* strains may originate from the widespread use of fungicides in agriculture, leading to the selective pressure of azole-resistant strains. Therefore, there is a necessity to screen *Aspergillus* antifungal susceptibility patterns for choosing an appropriate antifungal agent to treat these invasive infections. In addition, mutations in an ergosterol-producing enzyme, i.e., lanosterol 14- α demethylase, could lead to azole-resistant strains. As a result, the detection of these mutations would predict the resistance to azole agents. Although many novel azole agents have been developed for invasive *Aspergillus* infections, the rate of novel antifungal discovery is still limited. Therefore, better diagnostic criteria and extensive antifungal resistant *Aspergillus* screening would guide us to better manage invasive *Aspergillus* infections with our existing limited resources.

Keywords: *Aspergillus*, *Aspergillus*-human interactions, invasive aspergillosis, antifungal susceptibility test, azole, voriconazole, amphotericin B, influenza-associated pulmonary aspergillosis, COVID-19-associated pulmonary aspergillosis

1. Introduction

Aspergillus species are saprophytic ubiquitous filamentous fungi [1]. They are in Phylum Ascomycota with both sexual and asexual forms [1]. In their sexual form, they produce asci and ascospores within the appropriate environment, while they produce conidia, or asexual spores, on phialides surrounding their

vesicles at the tip of conidiophores in their asexual form [1]. *Aspergillus* conidia are different in size and shape depending on *Aspergillus* species, which affects the dispersion and infectivity properties of *Aspergillus* [1]. Their conidia can be found in the soil, decomposed piles, air, animals, and humans. They cause diseases in immunocompromised hosts, e.g., patients with acquired immunodeficiency syndrome (AIDS), allogenic hematopoietic stem cell transplant or solid organ transplant candidates, patients with immunosuppressive drugs, patients with prolonged neutropenia, and patients with other underlying diseases [2]. The common pathogenic *Aspergillus* species are *A. fumigatus*, *A. flavus*, *A. niger*, and *A. terreus* [3]. There are a wide variety of disease spectra of *Aspergillus* infections, i.e., invasive aspergillosis, chronic aspergillosis, and allergic forms of aspergillosis [1, 2]. The most severe form causing high morbidity and mortality rate, especially in immunocompromised hosts, is invasive aspergillosis (IA) [2, 4]. An increase of immunocompromised hosts would also increase patients with IA with a high mortality rate [4–14].

Invasive aspergillosis (IA) is recently increasing in patients with allogenic hematopoietic stem cell transplantation (HSCT) and solid organ transplantation [5, 8, 13, 15–22]. Underlying conditions of patients with IA are hematological malignancies, e.g., leukemia or lymphoma, bone marrow transplant, and solid-organ transplant patients [5, 8, 13, 15–22]. Recently, not only neutropenic patients are at risk for IA, but non-neutropenic patients with immunosuppressive agents, e.g., biologics, small-molecule kinase inhibitors (SMKIs), Chimeric Antigen Receptor (CAR) T cells, are also at risk [23–28]. In developing countries, poor-controlled diabetes mellitus is one of the critical risk factors of IA [10, 12]. Therefore, risk factors of IA are now patients with malignancy, autoimmune, inflammatory diseases, complex immune-metabolic diseases from aging, immunosuppressive treatment, previous septic conditions, novel biologic treatment, including patients with hematological malignancies receiving SMKIs, patients in ICU, patients with a cytokine storm syndrome from CAR-T cells treated with high-dose corticosteroids, patients in ICU with severe influenza or other viral infections [23–36]. In an era of Coronavirus Disease 2019 (COVID-19) infections, IA was recognized as a severe complication of patients with COVID-19 infections in ICU [37–46].

2. Pathogenesis of *Aspergillus* and its virulence factors

Among thousands of *Aspergillus* species, only less than twenty species could cause diseases in humans [47]. The pathogenic species usually possess virulence factors that help them survive and cause infections inside hosts. *Aspergillus fumigatus* was utilized as a model to study virulence factors in many studies (**Table 1**) [1].

To survive inside the host environment, *Aspergillus* species need to adapt to heat and hypoxic conditions inside hosts. For the heat stress, the trehalose pathway was shown to have a role in heat tolerance and virulence of *A. fumigatus* [47]. Heat shock proteins (HSPs), especially Hsp90, are chaperone proteins associated with stress tolerance, not only for heat [48–50]. In mammals, HIF1 α , as a common transcription factor, controls cellular homeostasis in hypoxic conditions [51]. In fungi, a homolog of HIF1 α , called the sterol regulatory element-binding protein (SREBP) or SrbA in *A. fumigatus*, is induced by hypoxia and iron starvation conditions [52–56]. SrbA protein is also associated with the virulence of *A. fumigatus* *in vivo* [52–54].

A. fumigatus possesses enzymes to protect itself against host reactive oxygen species (ROS), e.g., catalase, superoxide dismutases, thioredoxin, glutathione, including mitochondrial electron transport chain [57–62]. In some animal

Virulence factors	Characteristics
Stress tolerance	<ul style="list-style-type: none">• Thermotolerance• Hypoxic adaptation• pH/Reactive oxygen species (ROS) resistance• Secondary metabolites• Light response
Metabolism and nutrient uptake	<ul style="list-style-type: none">• siderophores, Zinc Magnesium Copper transporter, calmodulin, calcineurin, phosphate permeases
Cell components	<ul style="list-style-type: none">• Cell wall: β-glucan, chitin, rodlet• Galactosaminogalactan (GAG)• Melanin
Others	<ul style="list-style-type: none">• Biofilm• Cellular heterogeneity

Table 1.
Essential virulence factors in Aspergillus fumigatus requiring for causing infections inside humans [1].

models, e.g., an eye infection model, demonstrated that these fungal enzymes were essential for fungal virulence [63]. Secondary metabolites are also playing a role in fungal virulence [64–66]. *A. fumigatus* secondary metabolites are gliotoxin, fumigaclavine, tryptacidin, helvolic acid, fumitremorgin, fumagillin, and pseurotin, associated with host cellular toxicity [67–71]. However, the mechanisms behind this toxicity is still unclear and need to be further investigated *in vivo* [71]. *A. flavus* produces aflatoxins, which are important carcinogenic secondary metabolites, and other secondary metabolites, called Velvet complex, as environmental response mechanisms [72, 73]. Circadian rhythms or light response, which were studied thoroughly in the *Neurospora* model system, are essential to react with the environment [74]. Light-induced mycelial pigmentation and germination acted as a stress signaling pathway in *A. fumigatus* via transcription factor LreA and FphA, respectively [75–77].

For nutrient acquisition, exoenzymes or proteases are major enzymes produced by *A. fumigatus*, especially the alkaline protease Alp1 and the metalloprotease Mep1 [1, 78]. In *A. fumigatus*, a transcriptional repressor called CreA has a vital role in carbon catabolite repression. *AfCreA* regulates growth on different nitrogen, carbon, and lipid sources and has a role in amino acid transportation, nitrogen, and carbon assimilation, including glycogen and trehalose metabolism [79, 80]. Although CreA is not required for virulence, it is required for disease progression in invasive pulmonary aspergillosis (IPA) mouse models [79–81]. For nitrogen utilization, *AfRhbA*, a Ras-related protein in a nitrogen-regulated signaling pathway, and *AfAreA*, a GATA transcription factor requiring the expression of genes involving nitrogen utilization, are related to virulence in *A. fumigatus* [82–84]. *A. fumigatus* still needs divalent cations, i.e., iron, copper, magnesium, zinc, calcium, for its growth and virulence inside hosts via siderophores, calmodulin, calcineurin, specific importers, and exporters [85, 86].

Additionally, cell wall components of *Aspergillus fumigatus* are also essential virulence factors for fungal survival inside hosts and are important for host immune response [87–92]. Cell wall components consist of β -1,3-glucan, chitin, galactomannan, α -1,3-glucan, and melanin depending on different stages of *A. fumigatus*, i.e., conidial, or hyphal stage [91–95]. β -1,3-glucan, a central component of *Aspergillus* cell wall polysaccharide, is a pathogen-associated molecular

pattern (PAMP) recognized by host pattern recognition receptors (PRR), e.g., dectin-1 [88]. During its conidial stage, rodlet, or hydrophobins, and dihydroxynaphthalene (DHN) melanin are present to protect fungal conidia against host immune response by evading host pathogen-associated molecular patterns (PAMPs) recognition, including protecting fungi from unfavorable stress conditions [93–97]. Furthermore, in its hyphal stage, galactosaminogalactan (GAG), which is a water-insoluble polymer consisting of a pyranose-form galactose, galactosamine, and N-acetylgalactosamine (GalNAc), is present as an extracellular matrix on an outer layer of the cell wall [98]. GAG is associated with biofilm formation and immunosuppression properties by masking PAMP exposure and resisting neutrophil killing via neutrophil extracellular traps (NETs) [99–102]. The linkage between cell wall components and metabolic pathways is still unclear. Nevertheless, these components share the same building blocks, e.g., UDP-glucose, glucose 6-phosphate, with specific metabolic pathways, e.g., glycolysis, trehalose biosynthesis pathway [81, 103–105]. It is possible that the homeostasis of cell wall biosynthesis is involved with some metabolic pathways, e.g., the trehalose biosynthesis pathway. Disruption of one of these trehalose enzymes or building blocks would result in decreased virulence due to changes in cell wall compositions [81, 103–105]. Understanding this homeostasis would lead to the discovery of novel antifungal targets in the future.

3. Diagnosis of invasive *Aspergillus* infections: challenge in the field

Aspergillus infections are associated closely with host immune status [106, 107]. Severe asthma with fungal sensitization and allergic bronchial pulmonary aspergillosis (ABPA) are found in immunocompetent hosts with hypersensitivity, while aspergilloma and chronic pulmonary aspergillosis are found in immunocompetent hosts with previous structural diseases, such as lung cavity from previous tuberculosis infections [108]. In immunocompromised hosts, invasive aspergillosis is common and severe, causing high morbidity and mortality in patients [108, 109].

For invasive pulmonary aspergillosis, early diagnosis and prompt treatment are the keys to decrease the disease burden. Differentiation between *Aspergillus* colonization and invasive infections is still challenging [25, 92, 93]. Recently, the revised EORTC guideline for diagnosis of invasive fungal infections, including *Aspergillus* infections, recommended the diagnostic criteria including host factors, clinical, radiological, and microbiological criteria with new diagnostic methods (**Table 2**) [109]. Proven invasive aspergillosis is confirmed with histopathologic, cytopathologic, microscopic analysis, or nucleic acid analysis of sterile specimens or tissue or formalin-fixed paraffin-embedded tissue (FFPE), including culture recovered from sterile sites [109]. Species of common *Aspergillus* recovered from cultures are differentiated using macroscopic and microscopic morphology, but the nucleic acid analysis is necessary for the species complex (**Table 3**) [110]. For probable and possible invasive aspergillosis, host factors, clinical features, and mycological evidence are including for the diagnosis of invasive aspergillosis. Host factors include the history of neutropenia, which is less than 500 neutrophils/mm³, for more than ten days, hematological malignancy, allogenic stem cell transplantation, solid organ transplantation, therapeutic-dose corticosteroids at not less than 0.3 mg/kg for not less than three weeks during the previous 60 days, treatment with T-cell or B-cell immunosuppressants, inherited immunodeficiency, or acute graft-versus-host disease grade III or IV [109]. For clinical evidence of pulmonary aspergillosis, a chest high-resolution CT scan is recommended to observe any halo

Diagnosis of invasive aspergillosis.	Criteria
Proven	<ul style="list-style-type: none">• Microscopic analysis: from needle aspiration or biopsy OR• Culture: from sterile sites except for BAL fluid, paranasal sinuses, and urine OR• Tissue nucleic acid analysis from formalin-fixed paraffin-embedded tissue
Probable: 1 host factor + 1 clinical feature+1 mycological evidence	Host factors <ul style="list-style-type: none">• Recent neutropenia• Hematological malignancy• Receipt of an allogenic stem cell transplant• Receipt of a solid organ transplant• Prolonged use of corticosteroids• Use of T-cell immunosuppressants• Use of B-cell immunosuppressants• Inherited severe immunodeficiency• Acute GVHD grade III or IV Clinical features: pulmonary aspergillosis <ul style="list-style-type: none">• One of the following CT Chest patterns:<ul style="list-style-type: none">◦ Dense well-circumscribed lesion with or without a halo sign◦ Air crescent sign◦ Cavity◦ Wedge-shaped and segmental or lobar consolidation Mycological evidence <ul style="list-style-type: none">• Culture positive from sputum, BAL, bronchial brush, or aspirate• Direct examination positive from sputum, BAL, bronchial brush, or aspirate• Galactomannan antigen: plasma serum BAL CSF: any of:<ul style="list-style-type: none">◦ Single serum or plasma ≥ 1◦ BAL fluid ≥ 1◦ Single serum or plasma ≥ 0.7 and BAL fluid ≥ 0.8◦ CSF ≥ 1• <i>Aspergillus</i> PCR: any of:<ul style="list-style-type: none">◦ Plasma, serum, or whole blood 2 or more consecutive PCR◦ BAL fluid 2 or more duplicate PCR◦ At least 1 PCR from plasma serum or whole blood & 1 PCR from BAL fluid
Possible: 1 host factor + 1 clinical feature	

Table 2.
Diagnosis of invasive aspergillus infections from revised EORTC/MSG criteria 2020 (BAL: bronchoalveolar lavage; CT: computed tomography; CSF: cerebrospinal fluid; GVHD: graft versus host disease; PCR: polymerase chain reaction) [109].

sign, air-crescent sign, cavity, or wedge-shaped and segmental or lobar consolidation [109, 111]. Probable invasive aspergillosis still needs at least one mycological evidence to support the diagnosis. Mycological evidence is including cultures recovered from sputum, bronchoalveolar lavage (BAL), bronchial brush, or

<i>Aspergillus</i> species	Macroscopic features	Microscopic features
<i>Aspergillus fumigatus</i>	Typical blue-green colony with suede-like surface	Columnar uniseriate conidial heads with phialides limited to upper two-thirds of its vesicles; short and smooth conidiophores; basipetal green, rough-walled globose to subglobose conidia
<i>Aspergillus flavus</i>	Bright to dark yellow-green colony with a granular, flat surface	Radiate biseriate conidial heads with phialides over the surface of mature vesicles; coarsely rough conidiophores; pale green, globose to subglobose conidia
<i>Aspergillus niger</i>	Dark brown to the black colony with white to yellow color at the reverse side of the colony	Globose, large, dark brown, biseriate, radiate conidial head with long metulae; smooth, hyaline conidiophores; dark brown, rough conidia
<i>Aspergillus terreus</i>	Cinnamon-brown colony with suede-like surface and yellow to deep brown color at the reverse side of the colony	Compact, columnar, biseriate conidial heads; hyaline, smooth conidiophores; hyaline to yellow, smooth-walled conidia

Table 3.
Macroscopic and microscopic features of clinical-relevant Aspergillus species (colony on Czapek Dox agar at 30°C) [110].

aspirate [109]. *Aspergillus* galactomannan antigen assays with different thresholds depending on specimens, including serum, BAL fluid, plasma, and cerebrospinal fluid (CSF), support the diagnosis of invasive aspergillosis [112–115]. However, decreased sensitivity of galactomannan antigen assay is observed in patients with anti-mold therapy [115]. In addition, *Aspergillus* PCR from blood and BAL fluid is introduced to confirm the diagnosis and identify specific *Aspergillus* species with certain mutations related to triazole resistance [109, 116–124].

Nonetheless, revised EORTC/MSG criteria for diagnosing invasive fungal infections may be applied mainly for neutropenic patients or immunocompromised patients. Therefore, specific guidelines for the diagnosis of invasive aspergillosis in non-neutropenic patients in ICU (Invasive pulmonary aspergillosis in ICU, AspICU) or patients with influenza (Influenza-associated pulmonary aspergillosis, IAPA) or Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) (COVID-19 associated pulmonary aspergillosis, CAPA) co-infections were developed and published for early and accurate diagnosis (Table 4) [31, 125–127].

4. Treatment of *Aspergillus* infections

IA also includes the infections of the lower respiratory system, sinuses, and skin as entry routes. In addition, the cardiovascular system, central nervous system, and other tissues could be infected from hematogenous dissemination or direct extension from adjacent infected tissues [2]. Infectious Diseases Society of America (IDSA, 2016) and ESCMID-ECMM-ERS (2017) recommended voriconazole (6 mg/kg, intravenous route every 12 hours for one day, and then 4 mg/kg every 12 hours; 200–300 mg every 12-hour, oral route) as a first-line treatment for invasive pulmonary aspergillosis (IPA) [2, 128]. For alternative treatment, liposomal amphotericin B (3–5 mg/kg/day, intravenous route) and isavuconazole (200 mg every 8 hours for three days and then 200 mg daily) [2]. For other invasive aspergillosis syndromes, i.e., invasive sinus aspergillosis, tracheobronchial aspergillosis, invasive aspergillosis of the central nervous system or cardiovascular system, *Aspergillus* osteomyelitis,

Diagnostic criteria of IPA	AspICU [125]	IPA with influenza (IAPA) [126]	IPA with SARS-CoV-2 (CAPA) [127]
Host factors	One of the following: <ul style="list-style-type: none">• Neutropenia ($<500/\text{mm}^3$) before or at ICU admission• Hematological or oncological malignancy with cytotoxic therapy• Glucocorticoid treatment with prednisolone equivalent >20 mg/day• Immunodeficiency	Entry criteria: influenza-like illness + positive influenza PCR or antigen + timing (7 days before and 96 hours after ICU admission)	Entry criteria: patients with COVID-19 infections (RT-PCR) in ICU with a temporal relationship to suspected IPA
Clinical features	One of the following: <ul style="list-style-type: none">• Fever with appropriate antibiotic treatment for at least three days• Recurrent fever after a fever-free period for at least 48 hours with antibiotics and without other apparent cause• Dyspnea• Hemoptysis• Pleuritic chest pain or pleural friction rub• Worsening respiratory failure with appropriate antibiotics and ventilator support	None	None
Radiological evidence	<ul style="list-style-type: none">• Any medical imaging by conventional chest X-ray or CT scan of lungs	<ul style="list-style-type: none">• Pulmonary infiltrate OR• Cavitating infiltrate (not from other causes)	<ul style="list-style-type: none">• Pulmonary infiltrate OR• Cavitating infiltrate (not from other causes)

Diagnostic criteria of IPA	AspICU [125]	IPA with influenza (IAPA) [126]	IPA with SARS-CoV-2 (CAPA) [127]
Microbiological evidence	<ul style="list-style-type: none">• <i>Aspergillus</i> recovered from the lower respiratory tract (LRT) (entry criterion)• <i>Aspergillus</i>-positive culture of BAL fluid without bacterial growth together with a positive microscopic analysis showing branching hyphae (if no host factor)	<ul style="list-style-type: none">• If pulmonary infiltrate presents, at least one of the following:<ul style="list-style-type: none">◦ Galactomannan (GM) antigen assay: serum >0.5 or BAL ≥ 1.0 or◦ positive culture from BAL• If lung cavity presents, at least one of the following: positive sputum culture or tracheal aspirate culture	<p>Probable CAPA: at least one of the following:</p> <ul style="list-style-type: none">• Microscopic detection of septate hyphae in BAL• Positive BAL culture• Serum GM >0.5 or serum LFA index >0.5• BAL GM ≥1.0 or BAL LFA index ≥1.0• Two or more positive <i>Aspergillus</i> PCR in plasma, serum, or whole blood or a single positive <i>Aspergillus</i> PCR in BAL (<36 cycles); or a single positive <i>Aspergillus</i> PCR in plasma, serum, or whole blood with a single positive in BAL fluid (any threshold cycle) <p>Possible CAPA: at least one of the following:</p> <ul style="list-style-type: none">• Microscopic detection of septate hyphae in non-BAL• Positive non-BAL culture• Single non-BAL GM >4.5• Non-BAL GM >1.2 twice or more• Non-BAL GM >1.2 plus another non-BAL PCR or LFA positive

Diagnostic criteria of IPA	AspICU [125]	IPA with influenza (IAPA) [126]	IPA with SARS-CoV-2 (CAPA) [127]
Categories	<ul style="list-style-type: none">• Proven IPA: similar to EORTC/MSG 2020 criteria• Putative IPA: <i>Aspergillus</i>-positive from LRT + Clinical evidence + Radiological evidence + (Host factors or <i>Aspergillus</i> culture from BAL with positive microscopic analysis)• Colonization: ≥ 1 criterion for a diagnosis of putative IPA is not fulfilled	<ul style="list-style-type: none">• Proven IAPA: entry criteria with tissue diagnosis similar to EORTC/MSG 2020 criteria• Putative IAPA: entry criteria + Radiological evidence + Microbiological evidence• Colonization: ≥ 1 criterion for a diagnosis of putative IPA is not fulfilled	<ul style="list-style-type: none">• Proven CAPA: entry criteria with tissue diagnosis similar to EORTC/MSG 2020 criteria• Probable CAPA: entry criteria + radiological evidence + probable criteria of microbiological evidence• Possible CAPA: entry criteria + radiological evidence + possible criteria of microbiological evidence

Table 4.
Diagnostic criteria for invasive pulmonary aspergillosis (IPA) of patients in ICU (AspICU) or with influenza (IAPA) or SARS-CoV-2 (CAPA) coinfections (PCR: polymerase chain reaction; ICU: intensive care unit; RT-PCR: Real-time polymerase chain reaction; BAL: bronchoalveolar lavage; GM: galactomannan; LFA: lateral flow assay) [31, 125–127].

Aspergillus endophthalmitis and keratitis, cutaneous aspergillosis, and *Aspergillus* peritonitis, intravenous voriconazole is still the first-line therapy [2]. For IPA in ICU patients, patients with hematological malignancies, or solid organ transplants, IAPA, and CAPA, voriconazole and isavuconazole are still recommended as the first-line treatment (**Table 5**).

Voriconazole is metabolized at the liver via CYP2C19 and CYP3A4 [135]. Medications with CYP2C19 and CYP3A4-dependent metabolism, antacids, proton pump inhibitors may affect serum voriconazole levels [136]. Adverse reactions and toxicity of voriconazole are associated with higher serum voriconazole levels [137]. Common adverse reactions include reversible visual disturbances, hepatotoxicity, photosensitivity, reversible visual or auditory hallucinations, tachyarrhythmias, and QT interval prolongations [137, 138]. Isavuconazole is a second-generation broad-spectrum triazole requiring a loading dose with a five-day half-life [139]. Isavuconazole has fewer adverse reactions in photosensitivity, hepatotoxicity, visual abnormality, and less drug–drug interaction [140–142]. Isavuconazole is a CYP3A4 inhibitor and can decrease the metabolism of sirolimus, tacrolimus, cyclosporine, and digoxin, leading to increased levels of these agents [142]. Furthermore, isavuconazole can induce dose-dependent QTc shortening [143]. Isavuconazole was shown to be non-inferior to voriconazole to treat invasive mold disease from the SECURE trial [144]. Posaconazole is also a broad-spectrum triazole used mainly for prophylaxis and salvage treatment of invasive fungal infections [145]. A suspension form of posaconazole has unpredictable bioavailability and needs a high-fat meal for better absorption [146]. However, tablet and IV formulations overcome this limitation. Posaconazole strongly inhibits CYP3A4 and is metabolized through UGT1A4 [145]. Using CYP3A4 substrates with posaconazole should be cautious [145]. The common adverse effects of posaconazole are gastrointestinal disturbances, hepatotoxicity, rashes, fever, hypokalemia, hypomagnesemia, and QTc prolongation [145].

Amphotericin B, a polyene antifungal agent binding to ergosterol in the fungal cell membrane, has many forms, i.e., conventional with deoxycholate and lipid-based form [2, 147]. Conventional amphotericin B has common adverse effects, including acute reactions after infusion (fever, chills, nausea), phlebitis, hypokalemia, hypomagnesemia, and nephrotoxicity (usually from renal tubular acidosis). The lipid-based form has less nephrotoxicity than the conventional form [2]. Nevertheless, acute infusion reactions may still present in liposomal amphotericin B [148]. In addition, hypokalemia, hypomagnesemia, mild bilirubin, alkaline phosphatase elevations are also present occasionally in lipid-based amphotericin B [2]. Lipid-based amphotericin B is recommended for alternative treatment of invasive aspergillosis in case that azoles cannot be used [2].

Echinocandins, e.g., caspofungin, micafungin, is a non-competitive β -1,3 D-glucan synthase inhibitor leading to loss of fungal cell wall's strength and integrity [149, 150]. Echinocandins have fewer adverse reactions and fewer drug–drug interactions [149, 150]. Echinocandins are recommended for salvage therapy or in azole-resistant *Aspergillus* infections combined with azoles for invasive aspergillosis treatment (**Table 5**) [2, 151–153].

Therapeutic drug monitoring (TDM) of azoles, e.g., voriconazole, posaconazole, isavuconazole, is necessary, especially in elderly patients, obese patients, critically ill patients, and patients with potential azole drug–drug interactions [2]. For treatment of IA, IDSA recommended TDM of voriconazole at a trough level of >1 – 1.5 $\mu\text{g/mL}$ but less than 5 – 6 $\mu\text{g/mL}$ to prevent neurotoxicity [2]. American Society of Transplantation Infection Diseases Community of Practice (AST) recommended TDM of posaconazole (suspension and tablet form) and isavuconazole at a trough level of >1 – 1.25 $\mu\text{g/mL}$ and 2 – 3 $\mu\text{g/mL}$, respectively [154]. Timing

Condition	First-line treatment	Prophylaxis
IPA in ICU patients [129, 130]	Voriconazole (6 mg/kg, intravenous route every 12 hours for one day, and then 4 mg/kg every 12 hours; 200–300 mg every 12 hours oral route) or Isavuconazole (200 mg every 8 hours for 3 days and then 200 mg daily) (Liposomal amphotericin B, 3–5 mg/kg/day, intravenous route, in ICU patients with severe liver insufficiency, cirrhosis Child-Pugh scores B, C)	In immunocompetent patients in ICU, prophylaxis is not recommended for IPA
IPA in patients with hematological malignancies [131–133]	Voriconazole or Isavuconazole (Liposomal amphotericin B as alternative treatment)	Posaconazole (oral solution 200 mg every eight hours or tablet/intravenous route 300 mg every 12 h on day one then 300 mg daily) (in AML and MDS undergoing intensive chemotherapy with the incidence of invasive mold diseases >8% or in graft-versus-host disease) Voriconazole (200 mg orally every 12 h) (in HSCT)
IPA in patients with solid organ transplantation [134]	Voriconazole or Isavuconazole (Liposomal amphotericin B in hepatotoxicity, drug–drug interaction, ≥10% environment azole-resistant isolates found)	Kidney and heart transplantation are not recommended Lung transplantation: voriconazole, nebulized liposomal amphotericin B
IAPA [31, 126]	Voriconazole or Isavuconazole	No current recommendation Need further studies
CAPA [127]	For azole sensitive: Voriconazole or Isavuconazole (for 6–12 weeks) For azole-resistant: - Suspected: voriconazole + echinocandin (Caspofungin 70 mg first day followed by 50 mg daily) or isavuconazole + echinocandin - Proven: Liposomal amphotericin B	No current recommendation Need further studies

Table 5.
Treatment of invasive pulmonary aspergillosis (IPA) in ICU patients, patients with hematological malignancies, or solid organ transplants, influenza-associated pulmonary aspergillosis (IAPA), and COVID-19 associated pulmonary aspergillosis (CAPA) (AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; HSCT: hematological stem cell transplantation).

for measuring serum trough concentration of voriconazole, posaconazole, and isavuconazole is at 5–7 days, after 5 days, and within 7 days, respectively [154]. For prophylaxis, International Society for Heart and Lung Transplantation (ISHLT) recommended TDM of voriconazole and posaconazole at a trough level of $\geq 1 \mu\text{g/mL}$ and $> 0.7 \mu\text{g/mL}$, respectively [155]. Additionally, in CAPA, ECMM/ISHAM

recommended weekly TDM of voriconazole and posaconazole at a trough level of 2–6 µg/mL and 1–3.75 µg/mL, respectively [127].

5. Azole-resistant *Aspergillus*

5.1 Etiology and clinical significance

Voriconazole and isavuconazole are the first-line therapy of invasive aspergillosis [2, 129, 130]. Furthermore, azoles, i.e., posaconazole and voriconazole, are also used as prophylaxis of invasive aspergillosis in patients with hematological malignancies and solid organ transplantation [131–134]. Therefore, azoles are important antifungal agents to combat invasive aspergillosis. Unfortunately, azole-resistant *Aspergillus fumigatus* strains are emerging and increasing, leading to increased treatment failure and mortality [156, 157]. The etiology of these emerging azole-resistant *A. fumigatus* (ARAF) may be from the environmental selective pressure associated with azole fungicides in the agricultural area, including Europe, Asia, Latin America, the Midwest, and Southeast states of the USA [158–161]. The supporting evidence of environment-derived ARAF is that ARAF strains were recovered from azole-naïve patients [158, 162–165]. In addition, the most common mutations at the *cyp51A* gene (encoding lanosterol 14- α demethylase) causing azole resistance in ARAF strains, which are TR₃₄/L98H and TR₄₆/Y121F/T289A mutations, were also recovered from patients' homes and surroundings [166–171].

Azole fungicides, i.e., bromuconazole, difenoconazole, epoxiconazole, enilconazole, metconazole, prochloraz, propiconazole, prothioconazole-desthio, and tebuconazole, play an important role in the development of environment-derived azole-resistant *Aspergillus* isolates leading to cross resistance to medical azoles [169, 172, 173].

Antifungal susceptibility tests (AST) of *Aspergillus* species are essential for screening azole-resistant *Aspergillus* isolates. The indications to perform *Aspergillus* AST are that the fungus is recovered from sterile sites in regions with high azole-resistant rates, including long-term azole treatment in chronic bronchopulmonary aspergillosis and breakthrough *Aspergillus* infections or recurrent or persistent infections [2, 128, 174].

The standard antifungal susceptibility testing of filamentous fungi to observe the minimum inhibitory concentration (MIC) using broth microdilution assays was described by the Clinical and Laboratory Standards Institute (CLSI) and the European Union Committee on Antimicrobial Susceptibility Testing (EUCAST) [175, 176]. To determine antifungal resistance of *Aspergillus* species, e.g., *A. flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, CLSI and EUCAST utilized two values, which are epidemiological cutoff values (ECVs or ECOFFs) and clinical breakpoints (BP) (**Table 6**). ECVs for CLSI and ECOFFs for EUCAST of each antifungal agent against each *Aspergillus* originate from MIC distribution of the wild-type *Aspergillus* population [175–178]. These values can divide *Aspergillus* strains into two groups, which are wild-type and non-wild-type strains. Non-wild-type strains may resist those antifungal agents [175, 176, 178]. Clinical breakpoints are based on antifungal pharmacodynamics, pharmacokinetics, data from clinical trials, and patient outcomes [175, 176, 178]. Resistance is determined by the MICs over R (resistant) (**Table 6**). For EUCAST, another value is the area of technical uncertainty (ATU), which is the value that needs to be addressed before reporting these results, i.e., repeating the test, using a genotypic test, changing the susceptibility category, or including ATU as a part of the report [176].

Aspergillus species	Antifungal agents	CLSI M59 & M61, 2020 (µg/mL)				EUCAST BP ECOFF v2.0, 2020 (µg/mL)			
		ECV	S	I	R	ECV	S≤	R>	ATU
A. flavus	Amphotericin B	4	—	—	—	4	—	—	—
	Caspofungin	0.5	—	—	—	—	—	—	—
	Isavuconazole	1	—	—	—	2	1	2	2
	Itraconazole	1	—	—	—	1	1	1	2
	Posaconazole	0.5	—	—	—	0.5	—	—	—
	Voriconazole	2	—	—	—	2	—	—	—
A. fumigatus	Amphotericin B	2	—	—	—	1	1	1	—
	Caspofungin	0.5	—	—	—	—	—	—	—
	Isavuconazole	1	—	—	—	2	1	2	2
	Itraconazole	1	—	—	—	1	1	1	2
	Posaconazole	—	—	—	—	0.25	0.125	0.25	0.25
	Voriconazole	1	≤0.5	1	≥2	1	1	1	2
A. niger	Amphotericin B	2	—	—	—	0.5	1	1	—
	Caspofungin	0.25	—	—	—	—	—	—	—
	Isavuconazole	4	—	—	—	4	—	—	—
	Itraconazole	4	—	—	—	4	—	—	—
	Posaconazole	2	—	—	—	0.5	—	—	—
	Voriconazole	2	—	—	—	2	—	—	—
A. terreus	Amphotericin B	4	—	—	—	8	—	—	—
	Caspofungin	0.12	—	—	—	—	—	—	—
	Isavuconazole	1	—	—	—	1	1	1	—
	Itraconazole	2	—	—	—	0.5	1	1	2
	Posaconazole	1	—	—	—	0.25	0.125	0.25	0.25
	Voriconazole	2	—	—	—	2	—	—	—

Table 6. Interpretation of antifungal susceptibility tests and epidemiological cutoff values (ECVs) of *Aspergillus* species according to CLSI M59 and M61, 2020 and EUCAST BP ECOFF version 2, 2020 (S: susceptible, I: intermediate, R: resistant, ATU: Area of Technical Uncertainty) [175–177].

Molecular methods to detect *CYP51A* mutations, e.g., TR₃₄/L98H, TR₄₆/Y121F, are established by using classic PCRs with sequencing, real-time PCRs, loop-mediated isothermal amplification (LAMP), or whole-genome sequencing (WGS) [179]. These molecular methods have a high negative predictive value to rule out these resistant strains' infections [179]. However, they had narrow coverage and mutations at this point depending on association data between mutations and anti-fungal resistance property. Furthermore, commercial tools are still not approved by the US FDA [179].

5.2 Management of azole-resistant *Aspergillus* and novel antifungal candidates

Overexpression with a tandem repeat in the promoter area (TR₃₄ or TR₄₆) and point mutations (L98H or Y121F/T289A) in the *cyp51A* gene, encoding azole's target called lanosterol 14-α demethylase, would lead to azole resistance in *Aspergillus*

fumigatus including voriconazole and isavuconazole [156, 178]. To treat these azole-resistant *Aspergillus* infections, monotherapy of each azole should be avoided, especially in areas with more than 10% of azole resistance prevalence [180]. In areas with high rates of azole resistance, liposomal amphotericin B and a combination of voriconazole and echinocandin should be considered [2, 127, 128, 156, 180]. Therefore, the prevalence of azole-resistant *Aspergillus* strains using conventional culturing methods together with broth microdilution assays or using molecular biology (RT-PCR) is essential to decide the optimal treatment and to choose suitable antifungal agents to get rid of these infections [156, 179].

From the increased speed of azole-resistant *Aspergillus* strains, novel antifungal agents with high efficacy and fewer side effects are crucial to combat these infections with very high mortality [156]. However, discovering these novel antifungal agents has many steps and methods to evaluate both *in vitro* and *in vivo* analyses for both antifungal activity and toxicity [181, 182]. The first step for screening antifungal activity has many methods depending on the screening purpose [181]. To observe the antifungal activity of novel antifungal candidates, the broth microdilution method is the standard method to provide the MICs [183]. This method is perfect for various compounds requiring high throughput assays [181]. Furthermore, this method requires a small number of compounds and can apply to different *Aspergillus* species simultaneously [181]. To observe combinatorial effects between novel antifungal candidates and current antifungal agents, checkerboard assays are used to determine the fractional inhibitory concentration index (FICI) [184, 185]. The FICI is calculated using the sum of the fractional inhibitory concentration (FIC₁) of the first compound, which is MIC₁₊₂ of the combination of the first and the second compounds divided by MIC₁ of the first compound alone, and the FIC₂ of the second compound [184, 185]. Synergistic, additive, indifferent, and antagonistic effects are defined by FICI ≤ 0.5; >0.5–1; >1–4; and > 4, respectively [184–186]. For the cytotoxicity effects on human epithelial cells, many *in vitro* colorimetric assays, including mammalian tissue culture systems and vital dyes, are used, such as Alamar blue, MTT, XTT (tetrazolium) assays [181]. Next steps after *in vitro* studies to prove the antifungal activity and toxicity, *in vivo* animal models are used to study pharmacodynamics and pharmacokinetics, including *in vivo* antifungal activity and *in vivo* toxicity [181]. Then, these antifungal candidates would follow through the clinical trial phase I (safety), phase II (checking effectiveness), phase III (confirming effectiveness, side effects), and get approved [181, 182, 187].

Many novel antifungal compounds against both classical targets and novel targets are in clinical trials (Table 7) [262]. Novel targets against *Aspergillus* species include glycosylphosphatidylinositol (GPI) anchor protein, dihydroorotate dehydrogenase in pyrimidine synthesis, fungal mitochondrial respiration chain, siderophore iron transporter, Heat shock protein 90 (Hsp90), calcineurin, histone deacetylase (HDAC), inositol phosphorylceramide (IPC) synthase, chitin synthase, and sphingolipid pathway (Table 7). Nevertheless, more clinical trials are on the way for these agents before using them in the clinical practice against antifungal-resistant *Aspergillus*/fungal strains.

In addition, enzymes in the *Aspergillus* trehalose biosynthesis pathway, i.e., trehalose-6-phosphate synthase, trehalose-6-phosphate phosphatase, trehalase enzymes, were identified as important virulence factors, including proteins related to the trehalose pathway, i.e., AfSsdA, AfTslA [103, 105, 263, 264]. The trehalose pathway in *A. fumigatus* is associated with cell wall integrity and fungal virulence *in vivo* [103, 264, 265]. However, inhibitors of this pathway are still lacking and under-investigated. Validamycin A is one of the inhibitors of trehalase enzymes and was first demonstrated its strong antifungal activity against a plant fungal

Name.	Target	Mechanism	Advantage	Administration	Clinical trial
Classical targets					
Enochleated amphotericin B (CAmB) [188–192]	Ergosterol	Renovated structure of amphotericin B with cochleated lipid-crystal nanoparticles	Oral administration, broad-spectrum, less toxicity	Oral	Phase I
Rezafungin (CD101) [192–203]	1,3-β-D-glucan synthase (FKS)	1,3-β-D-glucan synthase inhibitor	Improved stability, long half-life (once a week), activity against <i>A. fumigatus</i> , <i>A. terreus</i> , <i>A. flavus</i> , and <i>A. niger</i>	Intravenous	Phase III
Ibrexafungerp (SCY-078) [204–210]	1,3-β-D-glucan synthase (FKS)	1,3-β-D-glucan synthase inhibitor	activity against <i>A. fumigatus</i> , <i>A. terreus</i> , <i>A. flavus</i> , and <i>A. niger</i> , including itraconazole-resistant <i>Aspergillus</i>	Oral and intravenous	Phase III
VT-1598 [211, 212]	Lanosterol demethylase (CYP51)	Tetrazole, inhibiting lanosterol demethylase	Less drug–drug interactions, long half-life, broad-spectrum: <i>Candida</i> , <i>Aspergillus</i>	Oral	Phase I
VT-1161 (oteseconazole) [213, 214]	Lanosterol demethylase (CYP51)	Tetrazole, inhibiting lanosterol demethylase	Less drug–drug interactions, long half-life: activity against azole-resistant <i>Candida</i> , onychomycosis	Oral	Phase III
VT-1129 (quileconazole) [215–220]	Lanosterol demethylase (CYP51)	Tetrazole, inhibiting lanosterol demethylase	Less drug–drug interactions, long half-life, brain penetration, activity against <i>Cryptococcus</i> , <i>Candida</i>	Oral	Phase I
PC945 [221–227]	Lanosterol demethylase (CYP51)	Triazole, inhibiting lanosterol demethylase	Fungicidal, high lung exposure, activity against <i>A. fumigatus</i>	Inhalation	Phase II
Novel targets					
Fosmanogepix (APX001) [228–236]	Glycosylphosphatidylinositol (GPI) anchor protein synthesis (GWT1)	Inhibiting GPI	Fungal-specific target, broad-spectrum, activity against <i>A. fumigatus</i> , <i>A. terreus</i> , <i>A. flavus</i> , and <i>A. niger</i>	Intravenous and oral	Phase II
APX2096 [236]	Glycosylphosphatidylinositol (GPI) anchor protein synthesis (GWT1)	Inhibiting GPI	Strong activity against <i>Cryptococcus</i> , effective blood–brain barrier penetration	Intraperitoneal and oral	—
Olorofim (F901318) [237–239]	Dihydroorotate dehydrogenase in pyrimidine synthesis	Inhibiting pyrimidine synthesis	Activity against <i>A. fumigatus</i> , <i>A. terreus</i> , <i>A. flavus</i> , and <i>A. nidulans</i> , including azole-resistant <i>A. fumigatus</i>	Intravenous and oral	Phase III

Name.	Target	Mechanism	Advantage	Administration	Clinical trial
T-2307 [240–242]	Intracellular mitochondrial membrane respiration potential	Inhibiting mitochondrial respiration chain (arylamidine)	Uptaking more by fungal cells, fungicidal activity against <i>A. fumigatus</i> , <i>A. terreus</i> , <i>A. flavus</i> , <i>A. nidulans</i> , and <i>A. niger</i>	Subcutaneous	Phase I
VL-2397 (ASP2397) [243–245]	Unknown	Uptaking by siderophore iron transporter (Sit1)	Fungicidal, activity against <i>A. fumigatus</i> , <i>A. terreus</i> , <i>A. flavus</i> , and <i>A. niger</i>	Intravenous	Phase II
Geldanamycin [246–248]	Heat shock protein 90 (Hsp90)	Inhibiting Hsp90	Synergy to caspofungin	Intravenous	—
Tacrolimus (FK506) [249–251]	Calcineurin	Inhibiting calcineurin	Synergy to caspofungin, activity against <i>A. fumigatus</i>	Intravenous and oral	—
Cyclosporin A [249, 252]	Calcineurin	Inhibiting calcineurin	Activity against <i>A. fumigatus</i>	Intravenous, oral, and topical	—
FK506 analogs (9D31OD-FK506) [251]	Calcineurin	Inhibiting calcineurin	Synergy to azoles, decrease T-cell toxicity and host immunosuppression	Intravenous	—
Trichostatin A [253]	Histone deacetylase (HDAC)	Inhibiting HDAC	Synergy to caspofungin, activity against <i>A. fumigatus</i>	Intravenous	—
MGCD290 [254]	Histone deacetylase (HDAC)	Inhibiting HDAC	Synergy to caspofungin, azole, broad spectrum	Oral	Phase II
Aureobasidin A [255–258]	Inositol phosphorylceramide (IPC) synthase	Inhibiting IPC synthase	Synergy to caspofungin	Intravenous and oral	—
Nikkomycin [259]	Chitin synthase	Inhibiting chitin synthase	Broad-spectrum	Intravenous	—
BHBM D13 [260, 261]	Sphingolipid pathway	Acylhydrazone, inhibiting fungal sphingolipid glucosylceramide (GlcCer) synthesis	Broad-spectrum, specific to fungi, fungicidal, blood–brain barrier penetration, less toxicity	Intraperitoneal and oral	—

Table 7.
Summary of novel antifungal agents against classical targets and novel targets for *Aspergillus* infections.

pathogen, *Rhizoctonia solani* [266–269]. Furthermore, validamycin A has antifungal activity against *Candida albicans* and *Aspergillus flavus* [186, 270]. Validamycin A also possesses combinatorial effects with conventional amphotericin B against *A. flavus* [186]. Nevertheless, *in vivo* experiments are still necessary to verify an antifungal activity of validamycin A. Additionally, the high-osmolarity glycerol (HOG)-mitogen-activated protein kinase (MAPK) signaling pathway is associated with trehalose production and stress response in *A. fumigatus* [271–274]. This signaling pathway may be another good antifungal target to be developed in the future. Therefore, there are many more pathways involved with *Aspergillus* virulence, and there are so many unexplored areas in *Aspergillus* pathogenesis to develop novel antifungal candidates. With this knowledge, we could overcome the shortage of antifungal agents against many more antifungal-resistant *Aspergillus* strains to emerge very soon.

6. Conclusion

Aspergillus species are common fungi found everywhere around humans. They adapt and express many virulence factors to survive inside hosts and cause infections in immunocompromised hosts. Recently, new risk factors that cause severe invasive pulmonary aspergillosis are ICU patients with influenza infections or COVID-19 infections. The diagnosis of invasive aspergillosis, especially without proven tissue or culture evidence, is still challenging. New molecular methods, i.e., nucleic acid assays, lateral flow assays, are introduced for supporting the diagnosis of probable and possible invasive aspergillosis. Nevertheless, voriconazole and isavuconazole are the first-line therapy in IPA in ICU patients, patients with hematological malignancies, patients with IAPA, and CAPA. Furthermore, posaconazole is the principal antifungal agent for the prophylactic treatment of IPA in patients with hematological malignancies. Additionally, emerging azole-resistant *Aspergillus* strains are increasing, and the management against these azole-resistant *Aspergillus* strains is the combination therapy between azoles and echinocandins, including liposomal amphotericin B. Although novel antifungal agents against *Aspergillus* species are on their way, antimicrobial stewardship of existing antifungal agents is also crucial to prevent further breakthrough antifungal-resistant strains in the future. With our better understanding of *Aspergillus* pathogenesis, the shortage of antifungal agents against *Aspergillus* and its resistant strains would no longer be for the better lives of patients suffering from *Aspergillus* infections.

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Conflict of interest

The author declares no conflict of interest.

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References

- [1] Latge JP, Chamilos G. *Aspergillus fumigatus* and Aspergillosis in 2019. Clin Microbiol Rev. 2019;33(1).
- [2] Patterson TF, Thompson GR, 3rd, Denning DW, Fishman JA, Hadley S, Herbrecht R, et al. Practice Guidelines for the Diagnosis and Management of Aspergillosis: 2016 Update by the Infectious Diseases Society of America. Clin Infect Dis. 2016;63(4):e1-e60.
- [3] Sugui JA, Kwon-Chung KJ, Juvvadi PR, Latge JP, Steinbach WJ. *Aspergillus fumigatus* and related species. Cold Spring Harb Perspect Med. 2014;5(2):a019786.
- [4] Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC. Hidden killers: human fungal infections. Sci Transl Med. 2012;4(165):165rv13.
- [5] Fracchiolla NS, Sciume M, Orofino N, Guidotti F, Grancini A, Cavalca F, et al. Epidemiology and treatment approaches in management of invasive fungal infections in hematological malignancies: Results from a single-centre study. PLoS One. 2019;14(5):e0216715.
- [6] Slavin MA, Chakrabarti A. Opportunistic fungal infections in the Asia-Pacific region. Med Mycol. 2012;50(1):18-25.
- [7] Kriengkauykiat J, Ito JI, Dadwal SS. Epidemiology and treatment approaches in management of invasive fungal infections. Clin Epidemiol. 2011;3: 175-91.
- [8] Pfaller MA, Diekema DJ. Epidemiology of invasive mycoses in North America. Crit Rev Microbiol. 2010;36(1):1-53.
- [9] Lehrnbecher T, Frank C, Engels K, Kriener S, Groll AH, Schwabe D. Trends in the postmortem epidemiology of invasive fungal infections at a university hospital. J Infect. 2010;61(3):259-65.
- [10] Chakrabarti A, Chatterjee SS, Das A, Shivaprakash MR. Invasive aspergillosis in developing countries. Med Mycol. 2011;49 Suppl 1:S35-47.
- [11] Chakrabarti A, Chatterjee SS, Shivaprakash MR. Overview of opportunistic fungal infections in India. Nihon Ishinkin Gakkai Zasshi. 2008;49(3):165-72.
- [12] Thammahong A, Thayidathara P, Suksawat K, Chindamporn A. Epidemiology of invasive Aspergillosis in a tertiary-care hospital of Thailand, 2006-2011. Mycoses. 2012;55:230-.
- [13] Graf K, Khani SM, Ott E, Mattner F, Gastmeier P, Sohr D, et al. Five-years surveillance of invasive aspergillosis in a university hospital. BMC Infect Dis. 2011;11:163.
- [14] Gangneux JP, Camus C, Philippe B. Epidemiology of invasive aspergillosis and risk factors in non neutropaenic patients. Rev Mal Respir. 2010;27(8): e34-46.
- [15] Nucci M, Queiroz-Telles F, Tobon AM, Restrepo A, Colombo AL. Epidemiology of opportunistic fungal infections in Latin America. Clin Infect Dis. 2010;51(5):561-70.
- [16] Neofytos D, Fishman JA, Horn D, Anaissie E, Chang CH, Olyaei A, et al. Epidemiology and outcome of invasive fungal infections in solid organ transplant recipients. Transpl Infect Dis. 2010;12(3):220-9.
- [17] Kontoyiannis DP, Marr KA, Park BJ, Alexander BD, Anaissie EJ, Walsh TJ, et al. Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001-2006: overview of the Transplant-Associated

- Infection Surveillance Network (TRANSNET) Database. Clin Infect Dis. 2010;50(8):1091-100.
- [18] Pappas PG, Alexander BD, Andes DR, Hadley S, Kauffman CA, Freifeld A, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). Clin Infect Dis. 2010;50(8):1101-11.
- [19] Neofytos D, Horn D, Anaissie E, Steinbach W, Olyaei A, Fishman J, et al. Epidemiology and outcome of invasive fungal infection in adult hematopoietic stem cell transplant recipients: analysis of Multicenter Prospective Antifungal Therapy (PATH) Alliance registry. Clin Infect Dis. 2009;48(3):265-73.
- [20] Azie N, Neofytos D, Pfaller M, Meier-Kriesche HU, Quan SP, Horn D. The PATH (Prospective Antifungal Therapy) Alliance(R) registry and invasive fungal infections: update 2012. Diagnostic microbiology and infectious disease. 2012;73(4):293-300.
- [21] Robin C, Cordonnier C, Sitbon K, Raus N, Lortholary O, Maury S, et al. Mainly Post-Transplant Factors Are Associated with Invasive Aspergillosis after Allogeneic Stem Cell Transplantation: A Study from the Surveillance des Aspergilloses Invasives en France and Societe Francophone de Greffe de Moelle et de Therapie Cellulaire. Biol Blood Marrow Transplant. 2019;25(2):354-61.
- [22] Siopi M, Karakatsanis S, Roumpakis C, Korantanis K, Sambatakou H, Sipsas NV, et al. A Prospective Multicenter Cohort Surveillance Study of Invasive Aspergillosis in Patients with Hematologic Malignancies in Greece: Impact of the Revised EORTC/MSGERC 2020 Criteria. J Fungi (Basel). 2021;7(1).
- [23] Herbrecht R, Bories P, Moulin JC, Ledoux MP, Letscher-Bru V. Risk stratification for invasive aspergillosis in immunocompromised patients. Ann N Y Acad Sci. 2012;1272:23-30.
- [24] Ghez D, Calleja A, Protin C, Baron M, Ledoux MP, Damaj G, et al. Early-onset invasive aspergillosis and other fungal infections in patients treated with ibrutinib. Blood. 2018;131(17):1955-9.
- [25] Chamilos G, Lionakis MS, Kontoyiannis DP. Call for Action: Invasive Fungal Infections Associated With Ibrutinib and Other Small Molecule Kinase Inhibitors Targeting Immune Signaling Pathways. Clin Infect Dis. 2018;66(1):140-8.
- [26] Bazaz R, Denning DW. Subacute Invasive Aspergillosis Associated With Sorafenib Therapy for Hepatocellular Carcinoma. Clin Infect Dis. 2018;67(1):156-7.
- [27] Hill JA, Li D, Hay KA, Green ML, Cherian S, Chen X, et al. Infectious complications of CD19-targeted chimeric antigen receptor-modified T-cell immunotherapy. Blood. 2018;131(1):121-30.
- [28] Park JH, Romero FA, Taur Y, Sadelain M, Brentjens RJ, Hohl TM, et al. Cytokine Release Syndrome Grade as a Predictive Marker for Infections in Patients With Relapsed or Refractory B-Cell Acute Lymphoblastic Leukemia Treated With Chimeric Antigen Receptor T Cells. Clin Infect Dis. 2018;67(4):533-40.
- [29] Benjamim CF, Lundy SK, Lukacs NW, Hogaboam CM, Kunkel SL. Reversal of long-term sepsis-induced immunosuppression by dendritic cells. Blood. 2005;105(9):3588-95.
- [30] Taccone FS, Van den Abeele AM, Bulpa P, Misset B, Meersseman W, Cardoso T, et al. Epidemiology of invasive aspergillosis in critically ill patients: clinical presentation,

underlying conditions, and outcomes. *Crit Care*. 2015;19:7.

[31] Schauwvlieghe A, Rijnders BJA, Philips N, Verwijs R, Vanderbeke L, Van Tienen C, et al. Invasive aspergillosis in patients admitted to the intensive care unit with severe influenza: a retrospective cohort study. *Lancet Respir Med*. 2018;6(10):782-92.

[32] Huang L, Zhang Y, Hua L, Zhan Q. Diagnostic value of galactomannan test in non-immunocompromised critically ill patients with influenza-associated aspergillosis: data from three consecutive influenza seasons. *Eur J Clin Microbiol Infect Dis*. 2021.

[33] Waldeck F, Boroli F, Suh N, Wendel Garcia PD, Flury D, Notter J, et al. Influenza-associated aspergillosis in critically-ill patients-a retrospective bicentric cohort study. *Eur J Clin Microbiol Infect Dis*. 2020;39(10):1915-23.

[34] van de Veerdonk FL, Kolwijck E, Lestrade PP, Hodiament CJ, Rijnders BJ, van Paassen J, et al. Influenza-Associated Aspergillosis in Critically Ill Patients. *Am J Respir Crit Care Med*. 2017;196(4):524-7.

[35] Cornillet A, Camus C, Nimubona S, Gandemer V, Tattevin P, Belleguic C, et al. Comparison of epidemiological, clinical, and biological features of invasive aspergillosis in neutropenic and nonneutropenic patients: a 6-year survey. *Clin Infect Dis*. 2006;43(5):577-84.

[36] Jenks JD, Nam HH, Hoenigl M. Invasive aspergillosis in critically ill patients: Review of definitions and diagnostic approaches. *Mycoses*. 2021.

[37] Arastehfar A, Carvalho A, van de Veerdonk FL, Jenks JD, Koehler P, Krause R, et al. COVID-19 Associated Pulmonary Aspergillosis (CAPA)-From Immunology to Treatment. *J Fungi (Basel)*. 2020;6(2).

[38] Mohamed A, Rogers TR, Talento AF. COVID-19 Associated Invasive Pulmonary Aspergillosis: Diagnostic and Therapeutic Challenges. *J Fungi (Basel)*. 2020;6(3).

[39] Lai CC, Yu WL. COVID-19 associated with pulmonary aspergillosis: A literature review. *J Microbiol Immunol Infect*. 2021;54(1):46-53.

[40] Apostolopoulou A, Esquer Garrigos Z, Vijayvargiya P, Lerner AH, Farmakiotis D. Invasive Pulmonary Aspergillosis in Patients with SARS-CoV-2 Infection: A Systematic Review of the Literature. *Diagnostics (Basel)*. 2020;10(10).

[41] Marr KA, Platt A, Tornheim JA, Zhang SX, Datta K, Cardozo C, et al. Aspergillosis Complicating Severe Coronavirus Disease. *Emerg Infect Dis*. 2021;27(1).

[42] Machado M, Valerio M, Alvarez-Uria A, Olmedo M, Veintimilla C, Padilla B, et al. Invasive pulmonary aspergillosis in the COVID-19 era: An expected new entity. *Mycoses*. 2021;64(2):132-43.

[43] Costantini C, van de Veerdonk FL, Romani L. Covid-19-Associated Pulmonary Aspergillosis: The Other Side of the Coin. *Vaccines (Basel)*. 2020;8(4).

[44] Koehler P, Bassetti M, Chakrabarti A, Chen SCA, Colombo AL, Hoenigl M, et al. Defining and managing COVID-19-associated pulmonary aspergillosis: the 2020 ECMM/ISHAM consensus criteria for research and clinical guidance. *Lancet Infect Dis*. 2020.

[45] Chong WH, Neu KP. The Incidence, Diagnosis, and Outcomes of COVID-19-associated Pulmonary Aspergillosis (CAPA): A Systematic Review. *J Hosp Infect*. 2021.

- [46] Mitaka H, Kuno T, Takagi H, Patrawalla P. Incidence and Mortality of COVID-19-associated Pulmonary Aspergillosis: A Systematic Review and Meta-analysis. *Mycoses*. 2021.
- [47] Hohl TM, Feldmesser M. *Aspergillus fumigatus*: principles of pathogenesis and host defense. *Eukaryot Cell*. 2007;6(11):1953-63.
- [48] O'Meara TR, Cowen LE. Hsp90-dependent regulatory circuitry controlling temperature-dependent fungal development and virulence. *Cell Microbiol*. 2014;16(4):473-81.
- [49] Robbins N, Uppuluri P, Nett J, Rajendran R, Ramage G, Lopez-Ribot JL, et al. Hsp90 governs dispersion and drug resistance of fungal biofilms. *PLoS Pathog*. 2011;7(9): e1002257.
- [50] Schneider A, Blatzer M, Posch W, Schubert R, Lass-Flörl C, Schmidt S, et al. *Aspergillus fumigatus* responds to natural killer (NK) cells with upregulation of stress related genes and inhibits the immunoregulatory function of NK cells. *Oncotarget*. 2016;7(44): 71062-71.
- [51] Friedrich D, Fecher RA, Rupp J, Deepe GS, Jr. Impact of HIF-1 α and hypoxia on fungal growth characteristics and fungal immunity. *Microbes Infect*. 2017;19(3):204-9.
- [52] Barker BM, Kroll K, Vodisch M, Mazurie A, Kniemeyer O, Cramer RA. Transcriptomic and proteomic analyses of the *Aspergillus fumigatus* hypoxia response using an oxygen-controlled fermenter. *BMC genomics*. 2012;13:62.
- [53] Blatzer M, Barker BM, Willger SD, Beckmann N, Blosser SJ, Cornish EJ, et al. SREBP coordinates iron and ergosterol homeostasis to mediate triazole drug and hypoxia responses in the human fungal pathogen *Aspergillus fumigatus*. *PLoS Genet*. 2011;7(12): e1002374.
- [54] Chung D, Barker BM, Carey CC, Merriman B, Werner ER, Lechner BE, et al. ChIP-seq and in vivo transcriptome analyses of the *Aspergillus fumigatus* SREBP SrbA reveals a new regulator of the fungal hypoxia response and virulence. *PLoS Pathog*. 2014;10(11): e1004487.
- [55] Losada L, Barker BM, Pakala S, Pakala S, Joardar V, Zafar N, et al. Large-scale transcriptional response to hypoxia in *Aspergillus fumigatus* observed using RNAseq identifies a novel hypoxia regulated ncRNA. *Mycopathologia*. 2014;178(5-6):331-9.
- [56] Vodisch M, Scherlach K, Winkler R, Hertweck C, Braun HP, Roth M, et al. Analysis of the *Aspergillus fumigatus* proteome reveals metabolic changes and the activation of the pseurotin A biosynthesis gene cluster in response to hypoxia. *J Proteome Res*. 2011;10(5): 2508-24.
- [57] Shibuya K, Paris S, Ando T, Nakayama H, Hatori T, Latge JP. Catalases of *Aspergillus fumigatus* and inflammation in aspergillosis. *Nihon Ishinkin Gakkai Zasshi*. 2006;47(4): 249-55.
- [58] Paris S, Wysong D, Debeaupuis JP, Shibuya K, Philippe B, Diamond RD, et al. Catalases of *Aspergillus fumigatus*. *Infect Immun*. 2003;71(6):3551-62.
- [59] Lambou K, Lamarre C, Beau R, Dufour N, Latge JP. Functional analysis of the superoxide dismutase family in *Aspergillus fumigatus*. *Mol Microbiol*. 2010;75(4):910-23.
- [60] Kurucz V, Krüger T, Antal K, Dietl AM, Haas H, Pócsi I, et al. Additional oxidative stress reroutes the global response of *Aspergillus fumigatus* to iron depletion. *BMC genomics*. 2018;19(1):357.
- [61] Burns C, Geraghty R, Neville C, Murphy A, Kavanagh K, Doyle S.

Identification, cloning, and functional expression of three glutathione transferase genes from *Aspergillus fumigatus*. *Fungal Genet Biol.* 2005;42(4):319-27.

[62] Grahl N, Dinamarco TM, Willger SD, Goldman GH, Cramer RA. *Aspergillus fumigatus* mitochondrial electron transport chain mediates oxidative stress homeostasis, hypoxia responses and fungal pathogenesis. *Mol Microbiol.* 2012;84(2):383-99.

[63] Leal SM, Jr., Vareechon C, Cowden S, Cobb BA, Latge JP, Momany M, et al. Fungal antioxidant pathways promote survival against neutrophils during infection. *J Clin Invest.* 2012;122(7):2482-98.

[64] Macheleidt J, Mattern DJ, Fischer J, Netzker T, Weber J, Schroeckh V, et al. Regulation and Role of Fungal Secondary Metabolites. *Annu Rev Genet.* 2016;50:371-92.

[65] Valiante V. The Cell Wall Integrity Signaling Pathway and Its Involvement in Secondary Metabolite Production. *J Fungi (Basel).* 2017;3(4).

[66] Raffa N, Keller NP. A call to arms: Mustering secondary metabolites for success and survival of an opportunistic pathogen. *PLoS Pathog.* 2019;15(4): e1007606.

[67] Amitani R, Taylor G, Elezis EN, Llewellyn-Jones C, Mitchell J, Kuze F, et al. Purification and characterization of factors produced by *Aspergillus fumigatus* which affect human ciliated respiratory epithelium. *Infect Immun.* 1995;63(9):3266-71.

[68] Sugui JA, Pardo J, Chang YC, Zarembek KA, Nardone G, Galvez EM, et al. Gliotoxin is a virulence factor of *Aspergillus fumigatus*: gliP deletion attenuates virulence in mice immunosuppressed with hydrocortisone. *Eukaryot Cell.* 2007;6(9):1562-9.

[69] Spikes S, Xu R, Nguyen CK, Chamilos G, Kontoyiannis DP, Jacobson RH, et al. Gliotoxin production in *Aspergillus fumigatus* contributes to host-specific differences in virulence. *J Infect Dis.* 2008;197(3):479-86.

[70] Scharf DH, Heinekamp T, Remme N, Hortschansky P, Brakhage AA, Hertweck C. Biosynthesis and function of gliotoxin in *Aspergillus fumigatus*. *Appl Microbiol Biotechnol.* 2012;93(2):467-72.

[71] Brown R, Priest E, Naglik JR, Richardson JP. Fungal Toxins and Host Immune Responses. *Frontiers in microbiology.* 2021;12:643639.

[72] Amare MG, Keller NP. Molecular mechanisms of *Aspergillus flavus* secondary metabolism and development. *Fungal Genet Biol.* 2014;66:11-8.

[73] Amaike S, Keller NP. *Aspergillus flavus*. *Annu Rev Phytopathol.* 2011;49:107-33.

[74] Fuller KK, Dunlap JC, Loros JJ. Light-regulated promoters for tunable, temporal, and affordable control of fungal gene expression. *Appl Microbiol Biotechnol.* 2018;102(9):3849-63.

[75] Fuller KK, Ringelberg CS, Loros JJ, Dunlap JC. The fungal pathogen *Aspergillus fumigatus* regulates growth, metabolism, and stress resistance in response to light. *mBio.* 2013;4(2).

[76] Fuller KK, Cramer RA, Zegans ME, Dunlap JC, Loros JJ. *Aspergillus fumigatus* Photobiology Illuminates the Marked Heterogeneity between Isolates. *mBio.* 2016;7(5).

[77] Chen S, Fuller KK, Dunlap JC, Loros JJ. Circadian Clearance of a Fungal Pathogen from the Lung Is Not Based on Cell-intrinsic Macrophage Rhythms. *J Biol Rhythms.* 2018;33(1): 99-105.

- [78] Sriranganadane D, Waridel P, Salamin K, Reichard U, Grouzmann E, Neuhaus JM, et al. *Aspergillus* protein degradation pathways with different secreted protease sets at neutral and acidic pH. *J Proteome Res*. 2010;9(7):3511-9.
- [79] Ries LN, Beattie SR, Espeso EA, Cramer RA, Goldman GH. Diverse Regulation of the CreA Carbon Catabolite Repressor in *Aspergillus nidulans*. *Genetics*. 2016;203(1):335-52.
- [80] Beattie SR, Mark KMK, Thammahong A, Ries LNA, Dhingra S, Caffrey-Carr AK, et al. Filamentous fungal carbon catabolite repression supports metabolic plasticity and stress responses essential for disease progression. *PLoS Pathog*. 2017;13(4):e1006340.
- [81] de Assis LJ, Manfiolli A, Mattos E, Fabri J, Malavazi I, Jacobsen ID, et al. Protein Kinase A and High-Osmolarity Glycerol Response Pathways Cooperatively Control Cell Wall Carbohydrate Mobilization in *Aspergillus fumigatus*. *mBio*. 2018;9(6).
- [82] Panepinto JC, Oliver BG, Fortwendel JR, Smith DL, Askew DS, Rhodes JC. Deletion of the *Aspergillus fumigatus* gene encoding the Ras-related protein RhbA reduces virulence in a model of Invasive pulmonary aspergillosis. *Infect Immun*. 2003;71(5):2819-26.
- [83] Dietl AM, Amich J, Leal S, Beckmann N, Binder U, Beilhack A, et al. Histidine biosynthesis plays a crucial role in metal homeostasis and virulence of *Aspergillus fumigatus*. *Virulence*. 2016;7(4):465-76.
- [84] Hensel M, Arst HN, Jr., Aufauvre-Brown A, Holden DW. The role of the *Aspergillus fumigatus* areA gene in invasive pulmonary aspergillosis. *Molecular & general genetics: MGG*. 1998;258(5):553-7.
- [85] Blatzer M, Latge JP. Metal-homeostasis in the pathobiology of the opportunistic human fungal pathogen *Aspergillus fumigatus*. *Curr Opin Microbiol*. 2017;40:152-9.
- [86] Fleck CB, Schobel F, Brock M. Nutrient acquisition by pathogenic fungi: nutrient availability, pathway regulation, and differences in substrate utilization. *Int J Med Microbiol*. 2011;301(5):400-7.
- [87] Zacharias CA, Sheppard DC. The role of *Aspergillus fumigatus* polysaccharides in host-pathogen interactions. *Curr Opin Microbiol*. 2019;52:20-6.
- [88] Hatinguais R, Willment JA, Brown GD. PAMPs of the Fungal Cell Wall and Mammalian PRRs. *Curr Top Microbiol Immunol*. 2020;425:187-223.
- [89] Ahamefula Osibe D, Lei S, Wang B, Jin C, Fang W. Cell wall polysaccharides from pathogenic fungi for diagnosis of fungal infectious disease. *Mycoses*. 2020;63(7):644-52.
- [90] Fontaine T, Latge JP. Galactomannan Produced by *Aspergillus fumigatus*: An Update on the Structure, Biosynthesis and Biological Functions of an Emblematic Fungal Biomarker. *J Fungi (Basel)*. 2020;6(4).
- [91] Beauvais A, Latge JP. Special Issue: Fungal Cell Wall. *J Fungi (Basel)*. 2018;4(3).
- [92] Gow NAR, Latge JP, Munro CA. The Fungal Cell Wall: Structure, Biosynthesis, and Function. *Microbiol Spectr*. 2017;5(3).
- [93] Valsecchi I, Lai JI, Stephen-Victor E, Pille A, Beaussart A, Lo V, et al. Assembly and disassembly of *Aspergillus fumigatus* conidial rodlets. *Cell Surf*. 2019;5:100023.

- [94] Valsecchi I, Dupres V, Stephen-Victor E, Guijarro JI, Gibbons J, Beau R, et al. Role of Hydrophobins in *Aspergillus fumigatus*. *J Fungi (Basel)*. 2017;4(1).
- [95] Valsecchi I, Dupres V, Michel JP, Duchateau M, Matondo M, Chamilos G, et al. The puzzling construction of the conidial outer layer of *Aspergillus fumigatus*. *Cell Microbiol*. 2019;21(5):e12994.
- [96] Tsai HF, Wheeler MH, Chang YC, Kwon-Chung KJ. A developmentally regulated gene cluster involved in conidial pigment biosynthesis in *Aspergillus fumigatus*. *J Bacteriol*. 1999;181(20):6469-77.
- [97] Bayry J, Beaussart A, Dufrene YF, Sharma M, Bansal K, Kniemeyer O, et al. Surface structure characterization of *Aspergillus fumigatus* conidia mutated in the melanin synthesis pathway and their human cellular immune response. *Infect Immun*. 2014;82(8):3141-53.
- [98] Fontaine T, Delangle A, Simenel C, Coddeville B, van Vliet SJ, van Kooyk Y, et al. Galactosaminogalactan, a new immunosuppressive polysaccharide of *Aspergillus fumigatus*. *PLoS Pathog*. 2011;7(11):e1002372.
- [99] Briard B, Muszkieta L, Latge JP, Fontaine T. Galactosaminogalactan of *Aspergillus fumigatus*, a bioactive fungal polymer. *Mycologia*. 2016;108(3):572-80.
- [100] Gravelat FN, Beauvais A, Liu H, Lee MJ, Snarr BD, Chen D, et al. *Aspergillus* galactosaminogalactan mediates adherence to host constituents and conceals hyphal beta-glucan from the immune system. *PLoS Pathog*. 2013;9(8):e1003575.
- [101] Lee MJ, Geller AM, Bamford NC, Liu H, Gravelat FN, Snarr BD, et al. Deacetylation of Fungal Exopolysaccharide Mediates Adhesion and Biofilm Formation. *mBio*. 2016;7(2):e00252-16.
- [102] Lee MJ, Liu H, Barker BM, Snarr BD, Gravelat FN, Al Abdallah Q, et al. The Fungal Exopolysaccharide Galactosaminogalactan Mediates Virulence by Enhancing Resistance to Neutrophil Extracellular Traps. *PLoS Pathog*. 2015;11(10):e1005187.
- [103] Thammahong A, Caffrey-Card AK, Dhingra S, Obar JJ, Cramer RA. *Aspergillus fumigatus* Trehalose-Regulatory Subunit Homolog Moonlights To Mediate Cell Wall Homeostasis through Modulation of Chitin Synthase Activity. *mBio*. 2017;8(2).
- [104] Thammahong A, Puttikamonkul S, Perfect JR, Brennan RG, Cramer RA. Central Role of the Trehalose Biosynthesis Pathway in the Pathogenesis of Human Fungal Infections: Opportunities and Challenges for Therapeutic Development. *Microbiol Mol Biol Rev*. 2017;81(2).
- [105] Thammahong A, Dhingra S, Bultman KM, Kerkaert JD, Cramer RA. An Ssd1 Homolog Impacts Trehalose and Chitin Biosynthesis and Contributes to Virulence in *Aspergillus fumigatus*. *mSphere*. 2019;4(3).
- [106] Pirofski LA, Casadevall A. The damage-response framework of microbial pathogenesis and infectious diseases. *Adv Exp Med Biol*. 2008;635:135-46.
- [107] Park SJ, Mehrad B. Innate immunity to *Aspergillus* species. *Clin Microbiol Rev*. 2009;22(4):535-51.
- [108] Moldoveanu B, Gearhart AM, Jalil BA, Saad M, Guardiola JJ. Pulmonary Aspergillosis: Spectrum of Disease. *Am J Med Sci*. 2021;361(4):411-9.

- [109] Donnelly JP, Chen SC, Kauffman CA, Steinbach WJ, Baddley JW, Verweij PE, et al. Revision and Update of the Consensus Definitions of Invasive Fungal Disease From the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium. *Clin Infect Dis*. 2020;71(6):1367-76.
- [110] Walsh TJ, Hayden RT, Larone DH. Larone's medically important fungi: a guide to identification 2018.
- [111] Park SY, Kim SH, Choi SH, Sung H, Kim MN, Woo JH, et al. Clinical and radiological features of invasive pulmonary aspergillosis in transplant recipients and neutropenic patients. *Transpl Infect Dis*. 2010;12(4):309-15.
- [112] de Heer K, Gerritsen MG, Visser CE, Leeflang MM. Galactomannan detection in bronchoalveolar lavage fluid for invasive aspergillosis in immunocompromised patients. *Cochrane Database Syst Rev*. 2019;5:CD012399.
- [113] Leeflang MM, Debets-Ossenkopp YJ, Wang J, Visser CE, Scholten RJ, Hooft L, et al. Galactomannan detection for invasive aspergillosis in immunocompromised patients. *Cochrane Database Syst Rev*. 2015(12):CD007394.
- [114] Chong GM, Maertens JA, Lagrou K, Driessen GJ, Cornelissen JJ, Rijnders BJ. Diagnostic Performance of Galactomannan Antigen Testing in Cerebrospinal Fluid. *J Clin Microbiol*. 2016;54(2):428-31.
- [115] Duarte RF, Sanchez-Ortega I, Cuesta I, Arnan M, Patino B, Fernandez de Sevilla A, et al. Serum galactomannan-based early detection of invasive aspergillosis in hematology patients receiving effective antimold prophylaxis. *Clin Infect Dis*. 2014;59(12):1696-702.
- [116] Cruciani M, White PL, Mengoli C, Loffler J, Morton CO, Klingspor L, et al. The impact of anti-mould prophylaxis on *Aspergillus* PCR blood testing for the diagnosis of invasive aspergillosis. *J Antimicrob Chemother*. 2021;76(3):635-8.
- [117] Mikulska M, Furfaro E, De Carolis E, Drago E, Pulzato I, Borghesi ML, et al. Use of *Aspergillus fumigatus* real-time PCR in bronchoalveolar lavage samples (BAL) for diagnosis of invasive aspergillosis, including azole-resistant cases, in high risk haematology patients: the need for a combined use with galactomannan. *Med Mycol*. 2019;57(8):987-96.
- [118] Heldt S, Prattes J, Eigl S, Spiess B, Flick H, Rabensteiner J, et al. Diagnosis of invasive aspergillosis in hematological malignancy patients: Performance of cytokines, Asp LFD, and *Aspergillus* PCR in same day blood and bronchoalveolar lavage samples. *J Infect*. 2018;77(3):235-41.
- [119] Shokouhi S, Mirzaei J, Sajadi MM, Javadi A. Comparison of serum PCR assay and histopathology for the diagnosis of invasive aspergillosis and mucormycosis in immunocompromised patients with sinus involvement. *Curr Med Mycol*. 2016;2(4):46-8.
- [120] Dannaoui E, Gabriel F, Gaboyard M, Lagardere G, Audebert L, Quesne G, et al. Molecular Diagnosis of Invasive Aspergillosis and Detection of Azole Resistance by a Newly Commercialized PCR Kit. *J Clin Microbiol*. 2017;55(11):3210-8.
- [121] White PL, Wingard JR, Bretagne S, Loffler J, Patterson TF, Slavin MA, et al. *Aspergillus* Polymerase Chain Reaction: Systematic Review of Evidence for Clinical Use in Comparison With Antigen Testing. *Clin Infect Dis*. 2015;61(8):1293-303.
- [122] Freeman Weiss Z, Leon A, Koo S. The Evolving Landscape of Fungal

Diagnostics, Current and Emerging Microbiological Approaches. J Fungi (Basel). 2021;7(2).

[123] Loeffler J, Mengoli C, Springer J, Bretagne S, Cuenca-Estrella M, Klingspor L, et al. Analytical Comparison of In Vitro-Spiked Human Serum and Plasma for PCR-Based Detection of *Aspergillus fumigatus* DNA: a Study by the European *Aspergillus* PCR Initiative. J Clin Microbiol. 2015;53(9):2838-45.

[124] White PL, Barnes RA, Springer J, Klingspor L, Cuenca-Estrella M, Morton CO, et al. Clinical Performance of *Aspergillus* PCR for Testing Serum and Plasma: a Study by the European *Aspergillus* PCR Initiative. J Clin Microbiol. 2015;53(9):2832-7.

[125] Blot SI, Taccone FS, Van den Abeele AM, Bulpa P, Meersseman W, Brusselsaers N, et al. A clinical algorithm to diagnose invasive pulmonary aspergillosis in critically ill patients. Am J Respir Crit Care Med. 2012;186(1):56-64.

[126] Verweij PE, Rijnders BJA, Bruggemann RJM, Azoulay E, Bassetti M, Blot S, et al. Review of influenza-associated pulmonary aspergillosis in ICU patients and proposal for a case definition: an expert opinion. Intensive Care Med. 2020;46(8):1524-35.

[127] Koehler P, Bassetti M, Chakrabarti A, Chen SCA, Colombo AL, Hoenigl M, et al. Defining and managing COVID-19-associated pulmonary aspergillosis: the 2020 ECMM/ISHAM consensus criteria for research and clinical guidance. The Lancet Infectious Diseases. 2020.

[128] Ullmann AJ, Aguado JM, Arikan-Akdogan S, Denning DW, Groll AH, Lagrou K, et al. Diagnosis and management of *Aspergillus* diseases: executive summary of the 2017

ESCMID-ECMM-ERS guideline. Clin Microbiol Infect. 2018;24 Suppl 1:e1-e38.

[129] Cuenca-Estrella M, Kett DH, Wauters J. Defining standards of CARE for invasive fungal diseases in the ICU. J Antimicrob Chemother. 2019;74(Suppl 2):ii9-ii15.

[130] Azoulay E, Afessa B. Diagnostic criteria for invasive pulmonary aspergillosis in critically ill patients. Am J Respir Crit Care Med. 2012;186(1):8-10.

[131] Tissot F, Agrawal S, Pagano L, Petrikos G, Groll AH, Skiada A, et al. ECIL-6 guidelines for the treatment of invasive candidiasis, aspergillosis and mucormycosis in leukemia and hematopoietic stem cell transplant patients. Haematologica. 2017;102(3):433-44.

[132] Maertens JA, Girmenia C, Bruggemann RJ, Duarte RF, Kibbler CC, Ljungman P, et al. European guidelines for primary antifungal prophylaxis in adult haematology patients: summary of the updated recommendations from the European Conference on Infections in Leukaemia. J Antimicrob Chemother. 2018;73(12):3221-30.

[133] Wang J, Zhou M, Xu JY, Zhou RF, Chen B, Wan Y. Comparison of Antifungal Prophylaxis Drugs in Patients With Hematological Disease or Undergoing Hematopoietic Stem Cell Transplantation: A Systematic Review and Network Meta-analysis. JAMA Netw Open. 2020;3(10):e2017652.

[134] Garcia-Vidal C, Carratala J, Lortholary O. Defining standards of CARE for invasive fungal diseases in solid organ transplant patients. J Antimicrob Chemother. 2019;74(Suppl 2):ii16-ii20.

[135] Dolton MJ, McLachlan AJ. Voriconazole pharmacokinetics and exposure-response relationships:

assessing the links between exposure, efficacy and toxicity. *Int J Antimicrob Agents*. 2014;44(3):183-93.

[136] Mikus G, Scholz IM, Weiss J. Pharmacogenomics of the triazole antifungal agent voriconazole. *Pharmacogenomics*. 2011;12(6):861-72.

[137] Mitsani D, Nguyen MH, Shields RK, Toyoda Y, Kwak EJ, Silveira FP, et al. Prospective, observational study of voriconazole therapeutic drug monitoring among lung transplant recipients receiving prophylaxis: factors impacting levels of and associations between serum troughs, efficacy, and toxicity. *Antimicrob Agents Chemother*. 2012;56(5):2371-7.

[138] Elewa H, El-Mekaty E, El-Bardissy A, Ensom MH, Wilby KJ. Therapeutic Drug Monitoring of Voriconazole in the Management of Invasive Fungal Infections: A Critical Review. *Clin Pharmacokinet*. 2015;54(12):1223-35.

[139] Miceli MH, Kauffman CA. Isavuconazole: A New Broad-Spectrum Triazole Antifungal Agent. *Clin Infect Dis*. 2015;61(10):1558-65.

[140] Falci DR, Pasqualotto AC. Profile of isavuconazole and its potential in the treatment of severe invasive fungal infections. *Infect Drug Resist*. 2013;6:163-74.

[141] Livermore J, Hope W. Evaluation of the pharmacokinetics and clinical utility of isavuconazole for treatment of invasive fungal infections. *Expert Opin Drug Metab Toxicol*. 2012;8(6):759-65.

[142] Ellsworth M, Ostrosky-Zeichner L. Isavuconazole: Mechanism of Action, Clinical Efficacy, and Resistance. *J Fungi (Basel)*. 2020;6(4).

[143] Keirns J, Desai A, Kowalski D, Lademacher C, Mujais S, Parker B, et al.

QT Interval Shortening With Isavuconazole: In Vitro and In Vivo Effects on Cardiac Repolarization. *Clin Pharmacol Ther*. 2017;101(6):782-90.

[144] Maertens JA, Raad, II, Marr KA, Patterson TF, Kontoyiannis DP, Cornely OA, et al. Isavuconazole versus voriconazole for primary treatment of invasive mould disease caused by *Aspergillus* and other filamentous fungi (SECURE): a phase 3, randomised-controlled, non-inferiority trial. *Lancet*. 2016;387(10020):760-9.

[145] Van Daele R, Spriet I, Maertens J. Posaconazole in prophylaxis and treatment of invasive fungal infections: a pharmacokinetic, pharmacodynamic and clinical evaluation. *Expert Opin Drug Metab Toxicol*. 2020;16(7):539-50.

[146] Courtney R, Wexler D, Radwanski E, Lim J, Laughlin M. Effect of food on the relative bioavailability of two oral formulations of posaconazole in healthy adults. *Br J Clin Pharmacol*. 2004;57(2):218-22.

[147] Anderson TM, Clay MC, Cioffi AG, Diaz KA, Hisao GS, Tuttle MD, et al. Amphotericin forms an extramembranous and fungicidal sterol sponge. *Nat Chem Biol*. 2014;10(5):400-6.

[148] Roden MM, Nelson LD, Knudsen TA, Jarosinski PF, Starling JM, Shiflett SE, et al. Triad of acute infusion-related reactions associated with liposomal amphotericin B: analysis of clinical and epidemiological characteristics. *Clin Infect Dis*. 2003;36(10):1213-20.

[149] Chen SC, Slavin MA, Sorrell TC. Echinocandin antifungal drugs in fungal infections: a comparison. *Drugs*. 2011;71(1):11-41.

[150] Denning DW. Echinocandin antifungal drugs. *Lancet*. 2003; 362(9390):1142-51.

- [151] Wurthwein G, Cornely OA, Trame MN, Vehreschild JJ, Vehreschild MJ, Farowski F, et al. Population pharmacokinetics of escalating doses of caspofungin in a phase II study of patients with invasive aspergillosis. *Antimicrob Agents Chemother.* 2013;57(4):1664-71.
- [152] Hiemenz JW, Raad, II, Maertens JA, Hachem RY, Saah AJ, Sable CA, et al. Efficacy of caspofungin as salvage therapy for invasive aspergillosis compared to standard therapy in a historical cohort. *Eur J Clin Microbiol Infect Dis.* 2010;29(11):1387-94.
- [153] Heinz WJ, Buchheidt D, Ullmann AJ. Clinical evidence for caspofungin monotherapy in the first-line and salvage therapy of invasive *Aspergillus* infections. *Mycoses.* 2016;59(8):480-93.
- [154] Husain S, Camargo JF. Invasive Aspergillosis in solid-organ transplant recipients: Guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. *Clin Transplant.* 2019;33(9):e13544.
- [155] Husain S, Sole A, Alexander BD, Aslam S, Avery R, Benden C, et al. The 2015 International Society for Heart and Lung Transplantation Guidelines for the management of fungal infections in mechanical circulatory support and cardiothoracic organ transplant recipients: Executive summary. *J Heart Lung Transplant.* 2016;35(3):261-82.
- [156] Jeanvoine A, Rocchi S, Bellanger AP, Reboux G, Millon L. Azole-resistant *Aspergillus fumigatus*: A global phenomenon originating in the environment? *Med Mal Infect.* 2020;50(5):389-95.
- [157] Berkow EL, Nunnally NS, Bandea A, Kuykendall R, Beer K, Lockhart SR. Detection of TR34/L98H CYP51A Mutation through Passive Surveillance for Azole-Resistant *Aspergillus fumigatus* in the United States from 2015 to 2017. *Antimicrob Agents Chemother.* 2018;62(5).
- [158] Chowdhary A, Kathuria S, Xu J, Meis JF. Emergence of azole-resistant *aspergillus fumigatus* strains due to agricultural azole use creates an increasing threat to human health. *PLoS Pathog.* 2013;9(10):e1003633.
- [159] Toyotome T. Resistance in the Environmental Pathogenic Fungus *Aspergillus fumigatus*. *Med Mycol J.* 2019;60(3):61-3.
- [160] Resendiz-Sharpe A, Dewaele K, Merckx R, Bustamante B, Vega-Gomez MC, Rolon M, et al. Triazole-Resistance in Environmental *Aspergillus fumigatus* in Latin American and African Countries. *J Fungi (Basel).* 2021;7(4).
- [161] Toda M, Beer KD, Kuivila KM, Chiller TM, Jackson BR. Trends in Agricultural Triazole Fungicide Use in the United States, 1992-2016 and Possible Implications for Antifungal-Resistant Fungi in Human Disease. *Environ Health Perspect.* 2021;129(5):55001.
- [162] van der Linden JW, Snelders E, Kampinga GA, Rijnders BJ, Mattsson E, Debets-Ossenkopp YJ, et al. Clinical implications of azole resistance in *Aspergillus fumigatus*, The Netherlands, 2007-2009. *Emerg Infect Dis.* 2011;17(10):1846-54.
- [163] Chowdhary A, Kathuria S, Randhawa HS, Gaur SN, Klaassen CH, Meis JF. Isolation of multiple-triazole-resistant *Aspergillus fumigatus* strains carrying the TR/L98H mutations in the cyp51A gene in India. *J Antimicrob Chemother.* 2012;67(2):362-6.
- [164] Snelders E, van der Lee HA, Kuijpers J, Rijs AJ, Varga J, Samson RA,

- et al. Emergence of azole resistance in *Aspergillus fumigatus* and spread of a single resistance mechanism. PLoS Med. 2008;5(11):e219.
- [165] Meis JF, Chowdhary A, Rhodes JL, Fisher MC, Verweij PE. Clinical implications of globally emerging azole resistance in *Aspergillus fumigatus*. Philos Trans R Soc Lond B Biol Sci. 2016;371(1709).
- [166] Mellado E, Garcia-Effron G, Alcazar-Fuoli L, Melchers WJ, Verweij PE, Cuenca-Estrella M, et al. A new *Aspergillus fumigatus* resistance mechanism conferring in vitro cross-resistance to azole antifungals involves a combination of cyp51A alterations. Antimicrob Agents Chemother. 2007;51(6):1897-904.
- [167] Snelders E, Huis In 't Veld RA, Rijs AJ, Kema GH, Melchers WJ, Verweij PE. Possible environmental origin of resistance of *Aspergillus fumigatus* to medical triazoles. Applied and environmental microbiology. 2009;75(12):4053-7.
- [168] Mortensen KL, Mellado E, Lass-Florl C, Rodriguez-Tudela JL, Johansen HK, Arendrup MC. Environmental study of azole-resistant *Aspergillus fumigatus* and other aspergilli in Austria, Denmark, and Spain. Antimicrob Agents Chemother. 2010;54(11):4545-9.
- [169] Chowdhary A, Kathuria S, Xu J, Sharma C, Sundar G, Singh PK, et al. Clonal expansion and emergence of environmental multiple-triazole-resistant *Aspergillus fumigatus* strains carrying the TR(3)(4)/L98H mutations in the cyp51A gene in India. PLoS One. 2012;7(12):e52871.
- [170] Badali H, Vaezi A, Haghani I, Yazdanparast SA, Hedayati MT, Mousavi B, et al. Environmental study of azole-resistant *Aspergillus fumigatus* with TR34/L98H mutations in the cyp51A gene in Iran. Mycoses. 2013;56(6):659-63.
- [171] van der Linden JW, Camps SM, Kampinga GA, Arends JP, Debets-Ossenkopp YJ, Haas PJ, et al. Aspergillosis due to voriconazole highly resistant *Aspergillus fumigatus* and recovery of genetically related resistant isolates from domiciles. Clin Infect Dis. 2013;57(4):513-20.
- [172] Snelders E, Camps SM, Karawajczyk A, Schaftenaar G, Kema GH, van der Lee HA, et al. Triazole fungicides can induce cross-resistance to medical triazoles in *Aspergillus fumigatus*. PLoS One. 2012;7(3):e31801.
- [173] Jorgensen KM, Helleberg M, Hare RK, Jorgensen LN, Arendrup MC. Dissection of the Activity of Agricultural Fungicides against Clinical *Aspergillus* Isolates with and without Environmentally and Medically Induced Azole Resistance. J Fungi (Basel). 2021;7(3).
- [174] Bassetti M, Vena A, Bouza E, Peghin M, Munoz P, Righi E, et al. Antifungal susceptibility testing in *Candida*, *Aspergillus* and *Cryptococcus* infections: are the MICs useful for clinicians? Clin Microbiol Infect. 2020;26(8):1024-33.
- [175] CLSI. Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi. 2nd ed. CLSI supplement M61. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.
- [176] The European Committee on Antimicrobial Susceptibility Testing. Overview of antifungal ECOFFs and clinical breakpoints for yeasts, moulds and dermatophytes using the EUCAST E.Def 7.3, E.Def 9.3 and E.Def 11.0 procedures. Version 2, 2020. <http://www.eucast.org>.

- [177] CLSI. Epidemiological Cutoff Values for Antifungal Susceptibility Testing. 3rd ed. CLSI supplement M59. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.
- [178] Wiederhold NP, Patterson TF. Emergence of Azole Resistance in *Aspergillus*. *Semin Respir Crit Care Med*. 2015;36(5):673-80.
- [179] Garcia-Effron G. Molecular Markers of Antifungal Resistance: Potential Uses in Routine Practice and Future Perspectives. *J Fungi (Basel)*. 2021;7(3).
- [180] Verweij PE, Ananda-Rajah M, Andes D, Arendrup MC, Bruggemann RJ, Chowdhary A, et al. International expert opinion on the management of infection caused by azole-resistant *Aspergillus fumigatus*. *Drug Resist Updat*. 2015;21-22:30-40.
- [181] Scorzoni L, Sangalli-Leite F, de Lacorte Singulani J, de Paula ESAC, Costa-Orlandi CB, Fusco-Almeida AM, et al. Searching new antifungals: The use of in vitro and in vivo methods for evaluation of natural compounds. *J Microbiol Methods*. 2016;123:68-78.
- [182] Perfect JR. The antifungal pipeline: a reality check. *Nat Rev Drug Discov*. 2017;16(9):603-16.
- [183] CLSI. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi. 3ed ed. CLSI standard M38. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.
- [184] Odds FC. Synergy, antagonism, and what the checkerboard puts between them. *J Antimicrob Chemother*. 2003;52(1):1.
- [185] Meletiadis J, Pournaras S, Roilides E, Walsh TJ. Defining fractional inhibitory concentration index cutoffs for additive interactions based on self-drug additive combinations, Monte Carlo simulation analysis, and in vitro-in vivo correlation data for antifungal drug combinations against *Aspergillus fumigatus*. *Antimicrob Agents Chemother*. 2010;54(2):602-9.
- [186] Plabutong N, Ekronarongchai S, Niwetbowornchai N, Edwards SW, Virakul S, Chiewchengchol D, et al. The Inhibitory Effect of Validamycin A on *Aspergillus flavus*. *Int J Microbiol*. 2020;2020:3972415.
- [187] Umscheid CA, Margolis DJ, Grossman CE. Key concepts of clinical trials: a narrative review. *Postgrad Med*. 2011;123(5):194-204.
- [188] Aigner M, Lass-Flörl C. Encochleated Amphotericin B: Is the Oral Availability of Amphotericin B Finally Reached? *J Fungi (Basel)*. 2020;6(2).
- [189] Santangelo R, Paderu P, Delmas G, Chen ZW, Mannino R, Zarif L, et al. Efficacy of oral cochleate-amphotericin B in a mouse model of systemic candidiasis. *Antimicrob Agents Chemother*. 2000;44(9):2356-60.
- [190] Zarif L, Graybill JR, Perlin D, Najvar L, Bocanegra R, Mannino RJ. Antifungal activity of amphotericin B cochleates against *Candida albicans* infection in a mouse model. *Antimicrob Agents Chemother*. 2000;44(6):1463-9.
- [191] Lu R, Hollingsworth C, Qiu J, Wang A, Hughes E, Xin X, et al. Efficacy of Oral Encochleated Amphotericin B in a Mouse Model of Cryptococcal Meningoencephalitis. *mBio*. 2019;10(3).
- [192] Skipper CP, Atukunda M, Stadelman A, Engen NW, Bangdiwala AS, Hullsiek KH, et al. Phase I EnACT Trial of the Safety and Tolerability of a Novel Oral Formulation of Amphotericin B. *Antimicrob Agents Chemother*. 2020;64(10).

- [193] Kovacs R, Toth Z, Locke JB, Forgacs L, Kardos G, Nagy F, et al. Comparison of In Vitro Killing Activity of Rezafungin, Anidulafungin, Caspofungin, and Micafungin against Four *Candida auris* Clades in RPMI-1640 in the Absence and Presence of Human Serum. *Microorganisms*. 2021;9(4).
- [194] Ham YY, Lewis JS, 2nd, Thompson GR, 3rd. Rezafungin: a novel antifungal for the treatment of invasive candidiasis. *Future Microbiol*. 2021;16:27-36.
- [195] Miesel L, Cushion MT, Ashbaugh A, Lopez SR, Ong V. Efficacy of Rezafungin in Prophylactic Mouse Models of Invasive Candidiasis, Aspergillosis, and Pneumocystis Pneumonia. *Antimicrob Agents Chemother*. 2021;65(3).
- [196] Garcia-Effron G. Rezafungin- Mechanisms of Action, Susceptibility and Resistance: Similarities and Differences with the Other Echinocandins. *J Fungi (Basel)*. 2020;6(4).
- [197] Zhao Y, Perlin DS. Review of the Novel Echinocandin Antifungal Rezafungin: Animal Studies and Clinical Data. *J Fungi (Basel)*. 2020;6(4).
- [198] Wiederhold NP, Najvar LK, Jaramillo R, Olivo M, Wickes BL, Catano G, et al. Extended-Interval Dosing of Rezafungin against Azole-Resistant *Aspergillus fumigatus*. *Antimicrob Agents Chemother*. 2019;63(10).
- [199] Wiederhold NP, Locke JB, Daruwala P, Bartizal K. Rezafungin (CD101) demonstrates potent in vitro activity against *Aspergillus*, including azole-resistant *Aspergillus fumigatus* isolates and cryptic species. *J Antimicrob Chemother*. 2018;73(11):3063-7.
- [200] Sofjan AK, Mitchell A, Shah DN, Nguyen T, Sim M, Trojcek A, et al. Rezafungin (CD101), a next-generation echinocandin: A systematic literature review and assessment of possible place in therapy. *J Glob Antimicrob Resist*. 2018;14:58-64.
- [201] Helleberg M, Jorgensen KM, Hare RK, Datcu R, Chowdhary A, Arendrup MC. Rezafungin In Vitro Activity against Contemporary Nordic Clinical *Candida* Isolates and *Candida auris* Determined by the EUCAST Reference Method. *Antimicrob Agents Chemother*. 2020;64(4).
- [202] Pfaller MA, Carvalhaes C, Messer SA, Rhomberg PR, Castanheira M. Activity of a Long-Acting Echinocandin, Rezafungin, and Comparator Antifungal Agents Tested against Contemporary Invasive Fungal Isolates (SENTRY Program, 2016 to 2018). *Antimicrob Agents Chemother*. 2020;64(4).
- [203] Thompson GR, Soriano A, Skoutelis A, Vazquez JA, Honore PM, Horcajada JP, et al. Rezafungin versus Caspofungin in a Phase 2, Randomized, Double-Blind Study for the Treatment of Candidemia and Invasive Candidiasis- The STRIVE Trial. *Clin Infect Dis*. 2020.
- [204] Jallow S, Govender NP. Ibrexafungerp: A First-in-Class Oral Triterpenoid Glucan Synthase Inhibitor. *J Fungi (Basel)*. 2021;7(3).
- [205] Apgar JM, Wilkening RR, Parker DL, Jr., Meng D, Wildonger KJ, Sperbeck D, et al. Ibrexafungerp: An orally active beta-1,3-glucan synthesis inhibitor. *Bioorg Med Chem Lett*. 2021;32:127661.
- [206] Ghannoum M, Arendrup MC, Chaturvedi VP, Lockhart SR, McCormick TS, Chaturvedi S, et al. Ibrexafungerp: A Novel Oral Triterpenoid Antifungal in

Development for the Treatment of *Candida auris* Infections. Antibiotics (Basel). 2020;9(9).

Broad-Spectrum Antifungal Activity. Antimicrob Agents Chemother. 2017;61(7).

[207] Petraitis V, Petraitiene R, Katragkou A, Maung BBW, Naing E, Kavaliauskas P, et al. Combination Therapy with Ibrexafungerp (Formerly SCY-078), a First-in-Class Triterpenoid Inhibitor of (1 $\rightarrow$ 3)-beta-d-Glucan Synthesis, and Isavuconazole for Treatment of Experimental Invasive Pulmonary Aspergillosis. Antimicrob Agents Chemother. 2020;64(6).

[213] Elewski B, Brand S, Degenhardt T, Curelop S, Pollak R, Schotzinger R, et al. A phase II, randomized, double-blind, placebo-controlled, dose-ranging study to evaluate the efficacy and safety of VT-1161 oral tablets in the treatment of patients with distal and lateral subungual onychomycosis of the toenail. Br J Dermatol. 2021;184(2): 270-80.

[208] Davis MR, Donnelley MA, Thompson GR. Ibrexafungerp: A novel oral glucan synthase inhibitor. Med Mycol. 2020;58(5):579-92.

[214] Monk BC, Keniya MV, Sabherwal M, Wilson RK, Graham DO, Hassan HF, et al. Azole Resistance Reduces Susceptibility to the Tetrazole Antifungal VT-1161. Antimicrob Agents Chemother. 2019;63(1).

[209] Spec A, Pullman J, Thompson GR, Powderly WG, Tobin EH, Vazquez J, et al. MSG-10: a Phase 2 study of oral ibrexafungerp (SCY-078) following initial echinocandin therapy in non-neutropenic patients with invasive candidiasis. J Antimicrob Chemother. 2019;74(10):3056-62.

[215] Wiederhold NP, Xu X, Wang A, Najvar LK, Garvey EP, Ottinger EA, et al. In Vivo Efficacy of VT-1129 against Experimental Cryptococcal Meningitis with the Use of a Loading Dose-Maintenance Dose Administration Strategy. Antimicrob Agents Chemother. 2018;62(11).

[210] Larkin EL, Long L, Isham N, Borroto-Esoda K, Barat S, Angulo D, et al. A Novel 1,3-Beta-d-Glucan Inhibitor, Ibrexafungerp (Formerly SCY-078), Shows Potent Activity in the Lower pH Environment of Vulvovaginitis. Antimicrob Agents Chemother. 2019;63(5).

[216] Wiederhold NP, Najvar LK, Garvey EP, Brand SR, Xu X, Ottinger EA, et al. The Fungal Cyp51 Inhibitor VT-1129 Is Efficacious in an Experimental Model of Cryptococcal Meningitis. Antimicrob Agents Chemother. 2018;62(9).

[211] Garvey EP, Sharp AD, Warn PA, Yates CM, Atari M, Thomas S, et al. The novel fungal CYP51 inhibitor VT-1598 displays classic dose-dependent antifungal activity in murine models of invasive aspergillosis. Med Mycol. 2020;58(4):505-13.

[217] Schell WA, Jones AM, Garvey EP, Hoekstra WJ, Schotzinger RJ, Alexander BD. Fungal CYP51 Inhibitors VT-1161 and VT-1129 Exhibit Strong In Vitro Activity against *Candida glabrata* and *C. krusei* Isolates Clinically Resistant to Azole and Echinocandin Antifungal Compounds. Antimicrob Agents Chemother. 2017;61(3).

[212] Hargrove TY, Garvey EP, Hoekstra WJ, Yates CM, Wawrzak Z, Rachakonda G, et al. Crystal Structure of the New Investigational Drug Candidate VT-1598 in Complex with *Aspergillus fumigatus* Sterol 14alpha-Demethylase Provides Insights into Its

[218] Nielsen K, Vedula P, Smith KD, Meya DB, Garvey EP, Hoekstra WJ, et al. Activity of VT-1129 against *Cryptococcus neoformans* clinical isolates with high

fluconazole MICs. *Med Mycol.* 2017;55(4):453-6.

[219] Warrilow AG, Parker JE, Price CL, Nes WD, Garvey EP, Hoekstra WJ, et al. The Investigational Drug VT-1129 Is a Highly Potent Inhibitor of *Cryptococcus* Species CYP51 but Only Weakly Inhibits the Human Enzyme. *Antimicrob Agents Chemother.* 2016;60(8):4530-8.

[220] Lockhart SR, Fothergill AW, Iqbal N, Bolden CB, Grossman NT, Garvey EP, et al. The Investigational Fungal Cyp51 Inhibitor VT-1129 Demonstrates Potent In Vitro Activity against *Cryptococcus neoformans* and *Cryptococcus gattii*. *Antimicrob Agents Chemother.* 2016;60(4):2528-31.

[221] Murray A, Cass L, Ito K, Pagani N, Armstrong-James D, Dalal P, et al. PC945, a Novel Inhaled Antifungal Agent, for the Treatment of Respiratory Fungal Infections. *J Fungi (Basel).* 2020;6(4).

[222] Cass L, Murray A, Davis A, Woodward K, Albayaty M, Ito K, et al. Safety and nonclinical and clinical pharmacokinetics of PC945, a novel inhaled triazole antifungal agent. *Pharmacol Res Perspect.* 2021;9(1): e00690.

[223] Pagani N, Armstrong-James D, Reed A. Successful salvage therapy for fungal bronchial anastomotic infection after -lung transplantation with an inhaled triazole anti-fungal PC945. *J Heart Lung Transplant.* 2020;39(12): 1505-6.

[224] Rudramurthy SM, Colley T, Abdolrasouli A, Ashman J, Dhaliwal M, Kaur H, et al. In vitro antifungal activity of a novel topical triazole PC945 against emerging yeast *Candida auris*. *J Antimicrob Chemother.* 2019;74(10):2943-9.

[225] Colley T, Sehra G, Daly L, Kimura G, Nakaoki T, Nishimoto Y, et al.

Antifungal synergy of a topical triazole, PC945, with a systemic triazole against respiratory *Aspergillus fumigatus* infection. *Scientific reports.* 2019;9(1):9482.

[226] Kimura G, Nakaoki T, Colley T, Rapeport G, Strong P, Ito K, et al. In Vivo Biomarker Analysis of the Effects of Intranasally Dosed PC945, a Novel Antifungal Triazole, on *Aspergillus fumigatus* Infection in Immunocompromised Mice. *Antimicrob Agents Chemother.* 2017;61(9).

[227] Colley T, Alanio A, Kelly SL, Sehra G, Kizawa Y, Warrilow AGS, et al. In Vitro and In Vivo Antifungal Profile of a Novel and Long-Acting Inhaled Azole, PC945, on *Aspergillus fumigatus* Infection. *Antimicrob Agents Chemother.* 2017;61(5).

[228] Badali H, Patterson HP, Sanders CJ, Mermella B, Gibas CFC, Ibrahim AS, et al. Manogepix, the Active Moiety of the Investigational Agent Fosmanogepix, Demonstrates In vitro Activity against Members of the *Fusarium oxysporum* and *Fusarium solani* Species Complexes. *Antimicrob Agents Chemother.* 2021.

[229] Lee A, Wang N, Carter CL, Zimmerman M, Dartois V, Shaw KJ, et al. Therapeutic Potential of Fosmanogepix (APX001) for Intra-abdominal Candidiasis: from Lesion Penetration to Efficacy in a Mouse Model. *Antimicrob Agents Chemother.* 2021;65(4).

[230] Petraitiene R, Petraitis V, Maung BBW, Mansbach RS, Hodges MR, Finkelman MA, et al. Efficacy and Pharmacokinetics of Fosmanogepix (APX001) in the Treatment of *Candida* Endophthalmitis and Hematogenous Meningoencephalitis in Nonneutropenic Rabbits. *Antimicrob Agents Chemother.* 2021;65(3).

- [231] Shaw KJ, Ibrahim AS. Fosmanogepix: A Review of the First-in-Class Broad Spectrum Agent for the Treatment of Invasive Fungal Infections. *J Fungi (Basel)*. 2020;6(4).
- [232] Gebremariam T, Alkhazraji S, Alqarihi A, Wiederhold NP, Shaw KJ, Patterson TF, et al. Fosmanogepix (APX001) Is Effective in the Treatment of Pulmonary Murine Mucormycosis Due to *Rhizopus arrhizus*. *Antimicrob Agents Chemother*. 2020;64(6).
- [233] Alkhazraji S, Gebremariam T, Alqarihi A, Gu Y, Mamouei Z, Singh S, et al. Fosmanogepix (APX001) Is Effective in the Treatment of Immunocompromised Mice Infected with Invasive Pulmonary Scedosporiosis or Disseminated Fusariosis. *Antimicrob Agents Chemother*. 2020;64(3).
- [234] Gebremariam T, Alkhazraji S, Gu Y, Singh S, Alqarihi A, Shaw KJ, et al. Galactomannan Is a Biomarker of Fosmanogepix (APX001) Efficacy in Treating Experimental Invasive Pulmonary Aspergillosis. *Antimicrob Agents Chemother*. 2019;64(1).
- [235] Wiederhold NP, Najvar LK, Shaw KJ, Jaramillo R, Patterson H, Olivo M, et al. Efficacy of Delayed Therapy with Fosmanogepix (APX001) in a Murine Model of *Candida auris* Invasive Candidiasis. *Antimicrob Agents Chemother*. 2019;63(11).
- [236] Shaw KJ, Schell WA, Covel J, Duboc G, Giamberardino C, Kapoor M, et al. In Vitro and In Vivo Evaluation of APX001A/APX001 and Other Gwt1 Inhibitors against *Cryptococcus*. *Antimicrob Agents Chemother*. 2018;62(8).
- [237] Su H, Zhu M, Tsui CK, van der Lee H, Tehupeiory-Kooreman M, Zoll J, et al. Potency of olorofim (F901318) compared to contemporary antifungal agents against clinical *Aspergillus fumigatus* isolates, and review of azole resistance phenotype and genotype epidemiology in China. *Antimicrob Agents Chemother*. 2021.
- [238] Singh A, Singh P, Meis JF, Chowdhary A. In vitro activity of the novel antifungal olorofim against dermatophytes and opportunistic moulds including *Penicillium* and *Talaromyces* species. *J Antimicrob Chemother*. 2021;76(5):1229-33.
- [239] du Pre S, Beckmann N, Almeida MC, Sibley GEM, Law D, Brand AC, et al. Effect of the Novel Antifungal Drug F901318 (Olorofim) on Growth and Viability of *Aspergillus fumigatus*. *Antimicrob Agents Chemother*. 2018;62(8).
- [240] Wiederhold NP. Review of T-2307, an Investigational Agent That Causes Collapse of Fungal Mitochondrial Membrane Potential. *J Fungi (Basel)*. 2021;7(2).
- [241] Yamashita K, Miyazaki T, Fukuda Y, Mitsuyama J, Saijo T, Shimamura S, et al. The Novel Arylamidine T-2307 Selectively Disrupts Yeast Mitochondrial Function by Inhibiting Respiratory Chain Complexes. *Antimicrob Agents Chemother*. 2019;63(8).
- [242] Mitsuyama J, Nomura N, Hashimoto K, Yamada E, Nishikawa H, Kaeriyama M, et al. In vitro and in vivo antifungal activities of T-2307, a novel arylamidine. *Antimicrob Agents Chemother*. 2008;52(4):1318-24.
- [243] Mammen MP, Armas D, Hughes FH, Hopkins AM, Fisher CL, Resch PA, et al. First-in-Human Phase 1 Study To Assess Safety, Tolerability, and Pharmacokinetics of a Novel Antifungal Drug, VL-2397, in Healthy Adults. *Antimicrob Agents Chemother*. 2019;63(11).
- [244] Dietl AM, Misslinger M, Aguiar MM, Ivashov V, Teis D, Pfister J,

- et al. The Siderophore Transporter Sit1 Determines Susceptibility to the Antifungal VL-2397. *Antimicrob Agents Chemother.* 2019;63(10).
- [245] Kovanda LL, Sullivan SM, Smith LR, Desai AV, Bonate PL, Hope WW. Population Pharmacokinetic Modeling of VL-2397, a Novel Systemic Antifungal Agent: Analysis of a Single- and Multiple-Ascending-Dose Study in Healthy Subjects. *Antimicrob Agents Chemother.* 2019;63(6).
- [246] Mahmoudi S, Rezaie S, Daie Ghazvini R, Hashemi SJ, Badali H, Foroumadi A, et al. In Vitro Interaction of Geldanamycin with Triazoles and Echinocandins Against Common and Emerging *Candida* Species. *Mycopathologia.* 2019;184(5):607-13.
- [247] Ma C, Chen J, Li P. Geldanamycin induces apoptosis and inhibits inflammation in fibroblast-like synoviocytes isolated from rheumatoid arthritis patients. *J Cell Biochem.* 2019;120(9):16254-63.
- [248] Ochel HJ, Eichhorn K, Gademann G. Geldanamycin: the prototype of a class of antitumor drugs targeting the heat shock protein 90 family of molecular chaperones. *Cell Stress Chaperones.* 2001;6(2):105-12.
- [249] High KP. The antimicrobial activities of cyclosporine, FK506, and rapamycin. *Transplantation.* 1994;57(12):1689-700.
- [250] Gao L, Sun Y. In vitro interactions of antifungal agents and tacrolimus against *Aspergillus* biofilms. *Antimicrob Agents Chemother.* 2015;59(11):7097-9.
- [251] Lee Y, Lee KT, Lee SJ, Beom JY, Hwangbo A, Jung JA, et al. In Vitro and In Vivo Assessment of FK506 Analogs as Novel Antifungal Drug Candidates. *Antimicrob Agents Chemother.* 2018;62(11).
- [252] Pandit RT. Antifungal effects of cyclosporine A. *Cornea.* 2003;22(1):92-3.
- [253] Robbins N, Leach MD, Cowen LE. Lysine deacetylases Hda1 and Rpd3 regulate Hsp90 function thereby governing fungal drug resistance. *Cell Rep.* 2012;2(4):878-88.
- [254] Wurtele H, Tsao S, Lepine G, Mullick A, Tremblay J, Drogaris P, et al. Modulation of histone H3 lysine 56 acetylation as an antifungal therapeutic strategy. *Nat Med.* 2010;16(7):774-80.
- [255] Zhong W, Jeffries MW, Georgopapadakou NH. Inhibition of inositol phosphorylceramide synthase by aureobasidin A in *Candida* and *Aspergillus* species. *Antimicrob Agents Chemother.* 2000;44(3):651-3.
- [256] Teymuri M, Shams-Ghahfarokhi M, Razzaghi-Abyaneh M. Inhibitory effects and mechanism of antifungal action of the natural cyclic depsipeptide, aureobasidin A against *Cryptococcus neoformans*. *Bioorg Med Chem Lett.* 2021;41:128013.
- [257] Munusamy K, Vadivelu J, Tay ST. A study on *Candida* biofilm growth characteristics and its susceptibility to aureobasidin A. *Rev Iberoam Micol.* 2018;35(2):68-72.
- [258] Tan HW, Tay ST. The inhibitory effects of aureobasidin A on *Candida* planktonic and biofilm cells. *Mycoses.* 2013;56(2):150-6.
- [259] Larwood DJ. Nikkomycin Z-Ready to Meet the Promise? *J Fungi (Basel).* 2020;6(4).
- [260] Lazzarini C, Haranahalli K, Rieger R, Ananthula HK, Desai PB, Ashbaugh A, et al. Acylhydrazones as Antifungal Agents Targeting the Synthesis of Fungal Sphingolipids. *Antimicrob Agents Chemother.* 2018;62(5).

- [261] Lazzarini C, Haranahalli K, McCarthy JB, Mallamo J, Ojima I, Del Poeta M. Preclinical Evaluation of Acylhydrazones SB-AF-1002 as a Novel Broad-Spectrum Antifungal Agent. *Antimicrob Agents Chemother*. 2020;64(9).
- [262] Yu Y, Albrecht K, Groll J, Beilhack A. Innovative therapies for invasive fungal infections in preclinical and clinical development. *Expert Opin Investig Drugs*. 2020;29(9):961-71.
- [263] Svanstrom A, van Leeuwen MR, Dijksterhuis J, Melin P. Trehalose synthesis in *Aspergillus niger*: characterization of six homologous genes, all with conserved orthologs in related species. *BMC Microbiol*. 2014;14:90.
- [264] Al-Bader N, Vanier G, Liu H, Gravelat FN, Urb M, Hoareau CM, et al. Role of trehalose biosynthesis in *Aspergillus fumigatus* development, stress response, and virulence. *Infect Immun*. 2010;78(7):3007-18.
- [265] Puttikamonkul S, Willger SD, Grahl N, Perfect JR, Movahed N, Bothner B, et al. Trehalose 6-phosphate phosphatase is required for cell wall integrity and fungal virulence but not trehalose biosynthesis in the human fungal pathogen *Aspergillus fumigatus*. *Mol Microbiol*. 2010;77(4):891-911.
- [266] Shibata M, Mori K, Hamashima M. Inhibition of hyphal extension factor formation by validamycin in *Rhizoctonia solani*. *J Antibiot (Tokyo)*. 1982;35(10):1422-3.
- [267] Shibata M, Uyeda M, Mori K. Stimulation of the extension of validamycin-inhibited hyphae by the hyphal extension factor present in *Rhizoctonia solani*. *J Antibiot (Tokyo)*. 1981;34(4):447-51.
- [268] Shibata M, Uyeda M, Mori K. Reversal of validamycin inhibition by the hyphal extract of *Rhizoctonia solani*. *J Antibiot (Tokyo)*. 1980;33(6):679-81.
- [269] Suami T, Ogawa S, Chida N. The revised structure of validamycin A. *J Antibiot (Tokyo)*. 1980;33(1):98-9.
- [270] Guirao-Abad JP, Sanchez-Fresneda R, Valentin E, Martinez-Esparza M, Arguelles JC. Analysis of validamycin as a potential antifungal compound against *Candida albicans*. *Int Microbiol*. 2013;16(4):217-25.
- [271] Ji Y, Yang F, Ma D, Zhang J, Wan Z, Liu W, et al. HOG-MAPK signaling regulates the adaptive responses of *Aspergillus fumigatus* to thermal stress and other related stress. *Mycopathologia*. 2012;174(4):273-82.
- [272] Day AM, Quinn J. Stress-Activated Protein Kinases in Human Fungal Pathogens. *Front Cell Infect Microbiol*. 2019;9:261.
- [273] Hagiwara D, Takahashi H, Kusuya Y, Kawamoto S, Kamei K, Gono T. Comparative transcriptome analysis revealing dormant conidia and germination associated genes in *Aspergillus* species: an essential role for AtfA in conidial dormancy. *BMC genomics*. 2016;17:358.
- [274] Hagiwara D, Suzuki S, Kamei K, Gono T, Kawamoto S. The role of AtfA and HOG MAPK pathway in stress tolerance in conidia of *Aspergillus fumigatus*. *Fungal Genet Biol*. 2014;73:138-49.